THE SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *STREPTOCOCCUS PNEUMONIAE* IN THE CAPE PENINSULA.

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Thesis submitted in fulfillment of the requirements for the Masters Diploma in Technology in the Faculty of Applied Sciences at the Cape Technikon.

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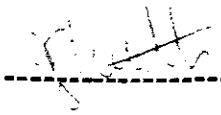
I, Janet Scholtz declare that the content of this thesis is representative of my own work. It has not been submitted for any degree or examination to any other Technikon or tertiary institution. This study was carried out at the SAIMR, Greenpoint complex, Cape Town. Serotyping of pneumococcal strains was carried out at the SAIMR, Central Institute, Johannesburg.

The opinions and conclusions drawn are my own and are not necessarily those of the Cape Technikon.



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SUMMARY

Streptococcus pneumoniae (S.pneumoniae) infections are an important cause of morbidity and mortality in adults and children worldwide. Mortality rates are highest amongst the very young and the elderly. *Streptococcus pneumoniae* is the most common form of community acquired bacterial pneumonia. Other diseases commonly caused by *Streptococcus pneumoniae* include meningitis, pericarditis, bacteraemia and septicaemia. Penicillin is today still considered the drug of choice when treating pneumococcal infections. The emergence of resistant pneumococcal strains has made it necessary to adapt antimicrobial regimens when treating pneumococcal infections. Hansman (1967) reported the first penicillin resistant strain, which was isolated from a woman in Australia in 1967. Since then penicillin and multi-resistant *Streptococcus pneumoniae* strains have been observed worldwide, including South Africa.

Streptococcus pneumoniae infections may be caused by any one of the 84 serotypes recognized to date. The distribution of serotypes varies, depending on geographical area, age and site of infection. High-level penicillin resistance and multiple resistant *Streptococcus pneumoniae* strains have been recognised worldwide in a few pneumococcal serotypes. Pneumococcal vaccines have been used since the seventies. These capsular polysaccharide vaccines are generally recommended for at risk population such as the elderly and immunocompromised patients. This vaccine is not effective in children under 2 years old. The current vaccine in South Africa (Pneumovax, MSD) consists of purified capsular polysaccharides of 23 pneumococcal serotypes. Conjugated polysaccharide vaccines have been developed to overcome the problems of efficacy in children < 2 years old. These vaccines consist of a capsular

polysaccharide linked to a protein carrier, which makes them immunogenic in infants. Clinical trials of these vaccines are currently under way to demonstrate safety, efficacy and immunogenicity.

Knowledge of serotype distribution and antimicrobial susceptibility patterns are important in relation to the treatment of pneumococcal diseases and vaccination programmes. This study was designed to define the serotypes and susceptibility patterns of *Streptococcus pneumoniae* isolated from infections in Cape Town where limited data were available. Susceptibility testing was performed using an agar dilution technique. Penicillin, chloramphenicol, tetracycline, erythromycin, clindamycin, rifampicin, ceftriaxone, cotrimoxazole and vancomycin were incorporated into agar in serial dilutions to determine the minimum inhibitory concentrations of *Streptococcus pneumoniae* isolates. Serotyping was performed using the Quellung reaction. The *Streptococcus pneumoniae* isolates were serotyped using type specific pneumococcal antisera from Staten's Seruminstitut (Copenhagen, Denmark).

During this study 564 *Streptococcus pneumoniae* isolates were tested, 49% constituted non-invasive isolates and 50,5% invasive isolates. These isolates were obtained from both children (<14years old) and adults (>14 years old). The most prevalent serotypes of *Streptococcus pneumoniae* isolated in this study were 6,19,14,1,3 and 18. The four most commonly isolated serotypes in this study from children were 6,19,14 and 23 which accounted for 71% of isolates. The distribution of serotypes varied significantly according to age and source of isolate. Serotypes 19, 1, 6,3 and 14 accounted for 59% of all adult serotypes. A significantly higher number of serotypes 6, 14 and 23 were found in children than adults and significantly higher numbers of serotypes 1, 3 and 18

were found in adults than in children. The common serotypes associated with invasive infections were 6,1,14,19 and 23 while serotypes 19,6,14,3,18 and 3 were associated with non-invasive infections.

The frequency of penicillin resistance among pneumococcal isolates in the Cape Peninsula was 21%. In children this rate was 33% compared to 8% in adults. There was a significant difference in the intermediate resistance rates for penicillin between the invasive (13%) and non-invasive groups (22%). The resistant rates for other antimicrobials tested were tetracycline 10%, erythromycin 5%, clindamycin 6,5%, rifampicin 5%, and cotrimoxazole 19% and no resistance was seen for ceftriaxone and vancomycin. A significantly higher number of resistance isolates were encountered from children than from adults to all antimicrobials tested except ceftriaxone and vancomycin. There was a significantly higher number of resistant isolates found in the non-invasive group for chloramphenicol and cotrimoxazole than in the invasive group of isolates. Serotypes 23,6,19 and 14 were the most commonly found resistant serotypes in this study.

The results of serotyping obtained in this study are comparable with results obtained in studies performed in South Africa and compared with both developing and developed countries. The 23 polyvalent pneumococcal vaccine will cover 78% of infections in adults and 89% of children.

A significant difference was seen in the resistant rates to penicillin from children and adults in this study. The results of the penicillin resistant rates in this study are consistent with published data in South Africa. It is of interest that there was a significant association of serotype 6 with intermediate and high resistant rates to

penicillin in this study. The resistant rates in this study for the various antimicrobials are consistent with reports in South Africa. There was only a significant difference between children and adult resistant rates for tetracycline and cotrimoxazole. The most common serotypes associated with resistance to the various antimicrobials were 6,19,14,23,1,4,5,and 3, which are the most commonly isolated serotypes.

Multi drug resistance was first reported in South Africa in 1977 and is now seen more commonly worldwide. In this study, of the 105 isolates resistant to penicillin 49% were resistant to one or more antimicrobials and 27% resistant to one or two and 21% resistant to three or four more antimicrobials.

Penicillin is today still considered the drug of choice when treating non-invasive infections and a cephalosporin when treating invasive infections.

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INTRODUCTION

LITERATURE REVIEW

1.1 HISTORY

Streptococcus pneumoniae was first visualised in pulmonary tissue in the early 1800's by Eberth (1880), Koch (1881) and Klebs (1875) and was isolated by Sternberg and Pasteur during the 1880's who recovered the organism from the saliva of human carriers. The organisms were injected into rabbits' who subsequently died and the organism was cultured from the rabbit's blood. Sternberg established that humans are carriers of pneumococcus by culturing pneumococci from his own saliva. During 1882, Friedlander showed the association of *Streptococcus pneumoniae* with lobar pneumonia and in the late 1880's *Streptococcus pneumoniae* was reported to cause other infections such as otitis, arthritis, sinusitis, purulent meningitis, conjunctivitis and infections at other non-pulmonary sites.

In 1902 Neufeld described the development of antibodies against pneumococcus following infection or vaccination, as well as the description of the Quellung reactions with specific antisera. In 1913, Lister in South Africa and Dochez and Gillipsie at the Rockerfeller Institute in New York segregated pneumococci into specific types that lead to the study of the immunologic and chemical properties of the capsular polysaccharides (Applebaum 1987; Baltimore and Shapiro1991).

These studies have proved to be most important in developing a specific antisera for therapy and in the use of the agglutination reaction and contributed to the development of a polyvalent polysaccharide vaccine. In 1977 the first polysaccharide pneumococcal vaccine was licensed in the USA (Rusen *et al* 1997; Joklik *et al* 1988; Baltimore and

Shapiro 1991). This vaccine contained purified capsular material of 14 serotypes and subsequently a 23 polyvalent vaccine was licensed in 1983 in the USA, which is currently in use as reported in the Pneumococcal Polysaccharide Vaccine Morbidity and Mortality Weekly Report of 1981. However, this vaccine is not effective in children under the age of two years. In the last decade, work on a conjugated pneumococcal vaccine has begun with promising results.

Penicillin has always been the drug of choice for the treatment of pneumococcal infections. In 1967 Hansman and Bullen reported the first penicillin resistant strain isolated from a woman in Australia. Subsequently, resistant strains were reported from Papua New Guinea in 1974. In 1977 drug resistant pneumococcal strains were reported by Jacobs *et al* (1978) and Appelbaum *et al* (1977) from both Johannesburg and Durban in South Africa. In July 1977, Jacobs *et al* detected the first multiple drug resistant pneumococci in Johannesburg.

Pneumococcal pneumoniae and bacteremia occur with greater frequency in HIV sero-positive patients than HIV sero-negative patients regardless of the age of the patient (Janoff *et al* 1993; Jones *et al* 1998). It has been suggested that this is due to low CD4 T cells and impaired humoral response to *Streptococcus pneumoniae* in HIV sero-positive individuals (Janoff *et al* 1993). HIV- positive adult patients have significantly more penicillin resistant strains than HIV-negative patients and that the most commonly isolated serotypes (6,14,19 and 23) usually isolated from children both HIV- positive and HIV – negative are found frequently in HIV-positive adults (Jones *et al* 1998).

1.2 EPIDEMIOLOGY

1.2.1 Incidence:

Streptococcus pneumoniae causing pneumonia is the most common form of community acquired bacterial pneumonia. Pneumococcal infections occur throughout the world. Incidence rates vary according to age with most cases occurring in young children (Table 1.1). Rates tend to be higher in developing countries compared to developed countries. The estimated attack rate in the USA is 2.1 cases per 1 000 population as reported by Joklik *et al* (1988).

Table 1.1: Incidence of pneumococcal disease

COUNTRY	REFERENCE	YEAR OF STUDY	POPULATION	RATE/100 000
ISRAEL	Dagan *et al* 1992, 1994	1988-1990	<1YEAR OLD < 5 YEARS OLD	104 42
FINLAND	Eskola *et al* 1992	1985-1989	<2 YEARS OLD <5 YEARS OLD	45 24
USA	Hofman *et al* 1995	1994	Adults and children	30
THE GAMBIA	Usen *et al* 1998	1993-1995	< 1 YEAR OLD 12 – 23 MONTHS 24 - 35 MONTHS	224 139 82
SOUTH AFRICA	Jones *et al* 1998	1996	< 5 YEARS OLD >16 YEARS OLD	106 42

1.2.2 Disease distribution:

Diseases commonly caused by *Streptococcus pneumoniae* include both upper and lower respiratory tract infections, meningitis, pericarditis, bacteraemia and septicaemia (Joklik *et al* 1988; Baltimore and Shapiro 19991). Jette *et al* (1989) reported on the surveillance of invasive *Streptococcus pneumoniae* infection in Quebec, Canada from 1984 to 1986 and revealed that in paediatric patients, infections such as otitis media, sinusitis, and orbital and periorbital cellulitis were the most frequent (28%), followed by bacteraemia without a primary focus of infection (23.4%), pneumonia (22.4%) and meningitis (15.1%) whereas in adults, pneumonia was most frequent (76.8%) followed by meningitis (7.2%) and bacteraemia (5.4%). Epidemiological reports from Israel (1988 to 1990) reported that pneumonia and bacteraemia account for 39% and 37% of infections respectively, with meningitis accounting for 17% and cellulitis 3% (Dagan *et al* 1992; 1994). In Finland (1985 to 1989), Eskola *et al* (1992) reported that bacteraemia constituted 69% of all infections, pneumonia 15% and meningitis 11 %. During 989 and 1991 in Johannesburg, South Africa, it was reported by Friedland and Klugman (1992) that meningitis accounts for 34% of infections, bacteraemia 9% and lower respiratory tract infections 51%.

1.2.3 Carriage:

Pneumococci are carried in the nasopharynx of healthy individuals and these individuals are the reservoir for pneumococcal infections. Carriage rates differ depending on environment and age. The highest carriage rates are in children between 2 and 4 years old. In adults with no contact with children the rate is only 5% (Joklik *et al* 1988;

Baltimore and Shapiro 1991). Carriage studies can provide an indication of antimicrobial resistance and serotypes that could be the cause of invasive diseases in the community. Carrier status of *Streptococcus pneumoniae* in children has been reported in Cape Town by Hussey *et al* (1993). The results show that the carriage rate in normal children is high (67,5%). Rusen *et al* (1997) reported on the results of a study performed in Kenya that determined the antimicrobial resistance and strain types isolated from the nasopharynx of Kenyan children. They found a 60% intermediate penicillin resistance and 34% resistance to tetracyclines and no resistance to clindamycin, chloramphenicol, erythromycin and rifampicin.

1.2.4 Transmission:

Pneumococci are usually spread via respiratory secretions and aerosols and only after spreading to other areas of the respiratory tract, or, if it penetrates the nasopharyngeal mucosa, leading to systemic circulation via the cervical lymphatics, will it give rise to serious disease. Individuals or groups at risk for pneumococcal disease include:

a) immunocompetent adults usually over 65 years old because of chronic illness such as pulmonary disease, cardiovascular disease or cirrhosis.

b) immunocompromised adults, which include those with splenic dysfunction, Hodgkins disease and lymphoma.

c) adults with asymptomatic or symtomatic HIV infection

d) Children with chronic illnesses such as HIV, cardiac infections and splenic dysfunction.

1.2.5 Mortality:

Pneumococcal infection is an important cause of morbidity and mortality in adults and children worldwide. Mortality rates tend to be higher amongst the very young and the elderly. The overall case-fatality rates reported from Israel in children up to 12 years old was 2.2% but 30% during the first month of life was reported by Dagan *et al* (1992; 1994) and in another study in Israel, Michel *et al* (1983) reported a case fatality rate of 2,6% in patients with invasive disease. In a similar study performed in Finland, the case-fatality rate was 1.3% in children up to 15 years of age as reported by Eskola *et al* (1992). The total mortality rate of 12,8% has been reported by Jette *et al* (1989) in a study of invasive *Streptococcus pneumoniae* infection in Quebec, Canada, from 1984 to 1986. Interestingly, the mortality rates increase with age: 1.6% in paediatric patients, 14,8% in patients 18-64 years and 31% in those >65 years. Pallares *et al* (1995) reported an overall mortality of 28% in a 10-year prospective study performed in Spain of adult patients with pneumococcal pneumonia. The overall mortality rate was reported by Levin *et al* (1996) in a study done in Sao Paulo to be 34 % for adults and children with pneumococcal bacteraemia. Friedland *et al* (1992) reported a case fatality rate of 31% in children with pneumococcal meningitis in Johannesburg, South Africa during 1989 and 1991.

1.3 TREATMENT

Penicillin is the drug of choice when treating pneumococcal infections. Patients allergic to penicillin may be given cephalosporin or erythromycin for pneumonia and chloramphenicol for meningitis (Joklik *et al*, 1988).

1.3.1 Penicillin and Multi drug resistant pneumococci.

The emergence of resistant pneumococcal strains has made it necessary to adapt antimicrobial regimens when treating pneumococcal infections. Since the 1960's penicillin and multi-resistant *Streptococcus pneumoniae* strains have been observed worldwide (Klugman, 1990) including South Africa, reported by Applebaum *et al* (1977), Jacobs *et al* (1978) and Friedland and Klugman (1992). This poses a problem and therefore pneumococcal susceptibility patterns to commonly used antimicrobial agents in various communities need to be monitored.

In 1977, Penicillin resistant strains were reported from both Johannesburg and Durban in South Africa and in July of 1977. Jacobs *et al* (1978) detected multiple resistant pneumococci in Johannesburg. The results were that carriers of Types 6A and 19A penicillin resistant pneumococci, resistant to antimicrobial concentrations ranging between 0.12 µg and 4 µg per milliliter were found in 29% of 543 paediatric patients and 2% of 434 hospital staff members. The South African surveillance continued when Friedland and Klugman (1992) reported antimicrobial resistant pneumococcal disease in South African children attending Baragwanath hospital. They found that 40% of community acquired isolates and 95% of hospital acquired isolates were resistant to penicillin. Resistance to chloramphenicol, tetracycline and erythromycin occurred in 9%, 12% and 4% of all isolates, respectively.

Multiple drug resistant type 19A strains, resistant to the ß-lactam antimicrobials, erythromycin, clindamycin, tetracycline and chloramphenicol. were isolated from 128 carriers, and were responsible for bacteraemia in four patients. Isolates from 40 other

carriers were resistant to penicillin alone or to penicillin and chloramphenicol or to penicillin, chloramphenicol and tetracycline. The multiple drug resistant pneumococci were found primarily in young children (<3 years), many of who had measles and pneumonia (Jacobs *et al,* 1978).

In Durban at the King Edward VIII Hospital, Applebaum *et al* (1987) reported *Streptococcus pneumoniae* resistance to penicillin and chloramphenicol. The strain of *Streptococcus pneumoniae* was serotype 19A and was isolated from five infants ranging from ages 4 months to 24 months. *Streptococcus pneumoniae* resistance to penicillin in children hospitalised at the Red Cross hospital in Cape Town is reported by Koornhof *et al* (1992) to have increased from 12.8% in 1979 to 22% in 1990.

A summary of studies of penicillin and other antimicrobial resistance patterns is illustrated in Table 1.2 and Table 1.3. Reported rates of penicillin resistance vary according to the criteria each investigator has adopted, but for this purpose MICs < 0,06 µg/ml will be considered sensitive, with MICs of 0.1 - 1.0 µg/ml intermediate resistant and greater than or equal to 2 µg/ml highly resistant.

Table 1 2: Penicillin resistance patterns from various centers around the world.

Location (ref)	Reference	Year(s) of study	Isolates	Pg (IR)	Pg (HR)
New Guinea	Hansman *et al* 1974	1968-1970	inv & niv	12	
New Zealand	Hefferman 1987	1981-1986	inv	1.3	
Australia	Hansman *et al* 1974	1968-1970	inv & niv	0.3	
Canada	Jette *et al* 1989	1984-1986	inv	1.3	0
Spain	Lataorre Ottin et *al* 1988	1984-1986	inv & niv	37	15
France	Gelsin *et al* 1995	1984-1986	inv & niv	<1.1	
Spain	Pallers *et al* 1995	1984-1988	inv	13	6
Uraguay	Mogdasy *et al* 1992	1985-1986	inv & niv	3	0
Saudi Arabia	El Mouzan *et al* 1988	1985-1986	inv	0	0
Pakistan	Mastro *et al* 1991	1986-1989	niv	9	
South Africa	Koornhof, Wasas, Klugman 1992	1986-1990	inv		11.8
Canada	Austrian 1986	1987-1995	inv & niv	43.1	6.7
Belgium	Verhaeggen *et al* 1995	1987-1993	inv & niv	2.2	3.2
UK	MacGowan *et al* 1993	1987-1991	inv & niv		0.5
France	Gelsin *et al* 1992	1988	inv & niv		13
Spain	Pallers *et al* 1995	1989-1993	inv	20	15
USA: Texas	Mason *et al* 1992	1989-1991	inv & niv	2.1	
South Africa	Friedland and Klugman 1992	1989-1990	inv	41,2	3,5
France	Gelsin *et al* 1992	1990	inv &niv	12	48
Kenya	Rusen *et al* 1997	1990	niv	60.8	0
Sao Paulo	Levin *et al* 1996	1991	inv & niv	24	0
Austria	Mittermayer *et al* 1996	1991-1994	inv & niv	4.9	0.3
Korea	Lee *et al* 1995	1991-1993	inv & niv	37	33
Egypt	Ostroff *et al* 1996	1991-1993	niv inv	29.1 22.4	0 0
USA 11 States	Butler *et al* 1996	1993-1994	inv	14.1	3.2
South Africa	Crewe Browne *et al* 1997	1993-1995	inv	12.2	1.9
USA: Atlanta	Hofman *et al* 1995	1994	inv	18	7

inv = invasive isolates ; niv = non invasive isolates: PG (IR) = Penicillin intermediate resistant; PG(HR)= Penicillin high resistance

Table1. 3: Tetracycline, Erythromycin, Cotrimoxazole and Chloramphenicol resistant patterns.

Location (ref)	Reference	Year(s) of study	Isolates	Tet	Eryth	Cotri	Chlor
Nigeria	Hansman 1978	1977	niv	20			14
USA: Oklahoma	Tarpay *et al* 1982	1979-1981	Niv		6.3	5	
New Zealand	Hefferman 1987	1981-1986	Inv	3.9	0.4	5.4	
Israel	Michel *et al* 1991	1981-1982	Inv	7.4	1.3	8.7	0
France	Gelsin *et al* 1992	1984-1990	Inv & niv	19	22	14.5	6
Canada	Jette *et al* 1989	1984-1986	inv	1.7			
Spain	Lataorre Ottin *et al* 1988	1984-1986	inv & niv	72.5	5.5	67	47.3
Spain	Pallers *et al* 1995	1984-1988	inv	44	7	41	
Uraguay	Mogdasy *et al* 1992	1985-1986	inv & niv			21	
Saudi Arabia	El Mouzan *et al* 1988	1985-1986	inv	25	4.1	65	3
Pakistan	Mastro *et al* 1991	1986-1989	niv	9	0	31	39
Belgium	Verhaegen *et al* 1995	1987-1993	inv & niv	15	13.8		3.3
UK	MacGowan *et al* 1993	1987-1991	inv & niv		1.6		
Spain	Pallers *et al* 1995	1989-1993	inv	33	13	37	
South Africa	Freidland and Klugman 1992	1989-1990	niv	11.5	4.4		8.8
Kenya	Rusen *et al* 1997	1990	niv	34	0		0.4
Austria	Mittermauer *et al* 1996	1991-1994	inv & niv	8.5	2.3	3.3	
Sao Paulo	Levin *et al* 1996	1991	inv & niv	32	0	32	0
Korea	Lee *et al* 1995	1991-1993	inv & niv		19		24
Egypt	Ostroff *et al* 1996	1991-1993	niv inv		2.5 0	0.6 0	21.5 29.7
USA: 11 States	Butler *et al* 1996	1993-1994	inv	5.4	3.5	5	1.6
South Africa	Crewe Browne *et al* 1997	1993-1995	inv	9.4	3.7	5.8	3.1
USA: Atlanta	Hofman *et al* 1995	1994	inv	8	15	26	3

inv = invasive isolates

niv = non-invasive isolates

PG(IR) = Penicillin intermediate resistance

PG(HR) = Penicillin high resistance

France showed an increasing resistance to penicillin from 13% in 1988 to 48% in 1990 and fairly high resistance to tetracycline, erythromycin and cotrimoxazole. Spain also showed a high percentage resistance to penicillin, as well as resistance to tetracycline, cotrimoxazole and chloramphenicol when compared to the rest of Europe reported by Gelsin *et al* (1992) and Klugman in 1990. High rates (33%) of penicillin resistance had also been reported in Korea by Lee *et al* (1995). Resistance rates of penicillin had increased in Canada from 3.6% in 1987 to 13.6% in 1995, reported by Austrian (1981).

A high percentage resistance was seen in Pakistan where 31% of isolates were resistant to cotrimoxazole and 39% were resistant to chloramphenicol. In Saudi Arabia pneumococci showed a high percentage resistance to cotrimoxazole (65%) and in Nigeria there was a high percentage resistance to tetracycline (20%) and chloramphenicol (14%). Egypt showed high resistant rates to chloramphenicol while Kenya showed high resistant rates to tetracyclines (34%) and a report from Sao Paulo (1996) stated that there was a 32% resistance rate to tetracycline and cotrimoxazole of pneumococci isolated from both invasive and non-invasive sites.

Multi drug resistance has been reported worldwide. In Canada from 1991-1995, an overall 8.5% of penicillin resistant isolates were resistant to one other antimicrobial agent and 25,2% were resistant to two or more. Butler *et al* (1996) reported from 11 states in the USA, of an overall 12,6% multi drug resistant strains with 18.8% in children and 9.3% in adults. Levin *et al* (1996) reported from Sao Paulo that 26% of the isolates were resistant to two drugs tested, which included penicillin, tetracycline and cotrimoxazole. A 52,1 % multi drug resistant strains with 2 or more antimicrobials was reported from Belgium by Verhaegen (1995). Multi drug resistant strains involving three or four different antimicrobials were reported by Mittermauer *et al* (1996) from Austria. A serotype 6B, multi resistant pneumococcus (penicillin and chloramphenicol) causing meningitis in a day care centre was reported from Denver, Colorado by Radetsky *et al* (1981).

1.4 PNEUMOCOCCAL SEROTYPE DISTRIBUTION.

Streptococcus pneumoniae is a gram-positive organism with a cell wall composed of peptidoglycan and teichoic acid. The teichoic acid contains the determinant for C polysaccharide antigenic activity. The capsule contains polysaccharides that form the basis of the separation of pneumococci into 84 different serotypes (Joklik *et al* 1988). *Streptococcus pneumoniae* infections may be caused by any one of the 84 serotypes recognized to date. Serotyping of *Streptococcus pneumoniae* is important as the distribution of serotypes vary with

a) geographical areas: developing and developed countries.

b) age: adults and children , and

c) site of infection: invasive and non- invasive.

1.4.1 Geographical areas:

The most frequently encountered serotypes worldwide include 4,6,9,11,14,19,23 which have been reported by Eskola *et al* (1992), Hansman (1974), Jette *et al* (1989) and Orange and Grag (1993). Serotype distribution from developing countries differ from those in developed countries. Sniadack *et al* (1995) reported on reviewed published and unpublished data from developed and developing countries to determine the geographical and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children. Sniadack included USA, Belgium, Denmark, Finland and Spain in the group of developed countries, Brazil, Uruguay, Rwanda, The Gambia, Egypt, Pakistan and Papua New Guinea in the group of developing countries, and South Africa, Israel and Australia into an intermediate group, as these countries

studies had characteristics of developed and developing countries. The most common serotypes from developed countries were 14,6,19,18,9,23,7,4,1 and 15. In developing countries the order was 6,14,8,5,1,19,9,23,18,15 and 7 and in the intermediate group the most commonly found serotypes were 14,1,6,18,19,9,4,5 and 23. The most commonly found serotypes found in South Africa were 6,14,19 and 23.

1.4.2 Age:

A variety of serotypes are frequently isolated from children and adults. In Finland, serotypes 14,6 and 19 constitute 54% of serotypes isolated from children < 15 years old (Eskola *et al,* 1992). The most commonly isolated serotypes from Quebec, Canada, reported by Jette *et al* (1989), were serotypes 14,19,18,6,9 and 4 from children under 18 years of age and serotypes 4,3,9,6,8 and 19 isolated from adults >18 years old. In Spain, the most commonly isolated serotypes isolated from children were 23,6,19,15 and 3 (Lataorre Ottin *et al* 1988). In Texas, USA, reported by Mason *et al* (1992), the most commonly encountered serotypes from children are 6,14,18 and 23. Whereas in Alabama, USA the most commonly encountered serotypes in children were 6,9,14,19 and 23 reported by Orange and Grag (1993) and in Atlanta, USA serotypes 14,6,19,9,4 and 23 were the most frequently isolated serotypes isolated from children and adults, reported by Hofman *et al* (1995).

Reports of uncommon serotypes causing disease such as a serotype 12F strain which caused invasive disease in 4 of 6 children in a day care centre in Baltimore, Maryland was reported by Cherian *et al* (1994).

El Mouzan *et al* (1988) reported from Saudia Arabia, that the most commonly encountered serotypes in children were 19 and 6 and serotypes 19,31,16 and 9 dominated the serotypes. Mogdasy *et al* (1992) reported from Uruguay that serotypes 14,5,9,3 and 1 were the most common serotypes causing invasive infections in children. Serotypes 7,12,14 and 15 constituted 93,3% of isolates reported from Bangladesh by Saha *et al* (1997) causing invasive infections in children during 1992-1995. Levin *et al* (1996) reported from Brazil that serotypes 14,5,23,3 and 6 were the most commonly isolated serotypes found in children and adults.

A study in Israel, reported by Michel *et al* (1991), that the most commonly isolated serotypes were 1,6,5,14,23 and 19, which were cultured from children and adults.

The results obtained reported in the studies above are confirmed by a study carried out by the World Health Organisation over the period 1982-1987 as reported by Nielsen and Hendrichsen (1997) which found that the most frequently encountered serotypes among children were 3,1,14,7,4,6 and 8 and adults serotypes 23,9 and 19. This study included data from Europe excluding Denmark and the Middle East, but mainly, isolates from Israel, Asia, Canada, New Zealand, Africa and South America. Sniadack *et al* (1995) reported that there was no difference in serotypes isolated from children < 2 years versus children 2-4 years old.

1.4.3 Site of infection:

A report by Shapiro and Austrian (1994) of pneumococcal isolates from children from usually sterile body sites shows that serotypes 4,6,9,14,18,19 and 23 were responsible for 84% of invasive infections overall and 86% of infections among children <2 years old

in this study. These results are confirmed by a study carried out by the Center for Disease Control from 1978-1994, where pneumococcal isolates from patients <6 years old were serotyped. The following serotypes where responsible for 84% of invasive infections, 14,6,19,18,23,4 and 9. Verhaegen *et al* (1995) reported from Belgium that serotypes 19,14,6,3,1,9,23,7 and 18 constituted 73% of stains isolated from pleural fluid, CSF, middle ear aspirates or other sites during 1980-1993.

The most frequently encountered serotypes found in children <12years old with invasive disease reported from Israel were 1,5,14,6,7 and 23 reported by Dagan *et al* (1992).

Friedland and Klugman (1992) reported that the most common serotypes found in children with invasive disease in a study conducted at Baragwanath Hospital, South Africa, were 6,14,19 and 23 and these accounted for 70% of all the isolates and 99% of all resistant isolates.

1.4.4. Serotype and penicillin resistance:

High-level penicillin resistance and multiple drug resistant *Streptococcus pneumoniae* strains have been recognised worldwide in a few pneumococcal serotypes, viz. serotypes 6,14,19,24 (Apllebaum *et al* 1987, Gelsin *et al* 1992, Mason *et al* 1997, Koornhof, Wasas, Klugman 1992, Michel *et al* 1983 and Hansman *et al* 1974).

Results reported from Paupau New Guinea and Australia by Hansman et al (1974) show that serotypes 4,6,11,14,15,16,19,23,34 are associated with penicillin resistance.

A report by Appelbaum *et al* (1977) described an isolate that was a serotype 19a resistant to penicillin and chloramphenicol, which was responsible for three fatal cases in Durban, South Africa. Multi drug resistant strains have been reported in South Africa

by Koomhof, Wasas and Klugman (1992) belonging to serotypes 6,19,4 and 23. Penicillin intermediate and high resistance was most commonly found in serotypes 6,14,19 and 15 in children and adults in a study performed in Israel (Michel *et al* 1983). In a study carried out by the National Reference Center for Pneumococci in Paris, France from 1984-1990, Geslin *et al* (1992) reported that of the pneumococcal strains that had intermediate or high resistance to penicillin, 80% belonged to serotypes 23,19,6,14 and 9 with more than 80% of strains with high resistance to penicillin being serotype 23F. Serotype 23 has also been associated with penicillin resistance in Spain (Lataorre Ottin *et al* 1988) where 50% of isolates from children where intermediately resistant to penicillin.

Results obtained from various studies in the USA show that serotype 6 was associated with penicillin resistance in isolates from Texas children (Mason *et al* 1997), serotypes 19,6 and 14 accounted for two thirds of resistant strains from children in Alabama (Orange and Grag 1993) and serotypes 6,19,23,14 and 9 was associated with penicillin resistance in isolates from children and adults in Atlanta during 1994 (Hofman *et al* 1995).

1.5 VACCINES

1.5.1 Capsular polysaccharide vaccines:

The first vaccine was produced in 1911, but licensed for use in 1977. The current vaccine (Pneumovax, MSD) consists of purified capsular polysaccharides of 23 pneumococcal serotypes, viz. 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 16B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. This vaccine is effective in adults and older

children. However it is poorly immunogenic in children, especially in children less than 2 years of age, the age at which the disease most often occurs (Dagan *et al* 1992 and Eskola *et al* 1992). Gable *et al* (1990) performed an efficacy study of the pneumococcal vaccine. Their results showed that vaccination significantly reduced pneumonia incidence, with overall efficacy of 69% and higher efficacy in women (86%) than in men (33%). They concluded that the pneumococcal pneumonia vaccine is efficacious in persons having had pneumonia and persons "at risk" of developing pneumonia or persons over 50 years of age.

1.5.2 Conjugate vaccines:

Conjugated polysaccharide vaccines have been developed to overcome the problems of efficacy in children < 2 years old and to reduce mucosal carriage of *Streptococcus pneumoniae*. These vaccines consist of a capsular polysaccharide linked to a protein carrier, which makes them immunogenic in infants. Clinical trials of these vaccines are currently under way to demonstrate safety, efficacy and immunogenicity (Shann 1990, Eskola 1999). Pneumococcal conjugate vaccines appear to be safe. Rennels *et al* (1998) concluded from their study of heptavalent pneumococcal vaccine conjugated to CRM_{197} that it was safe to administer four consecutive doses of the vaccine to children from 2-15 months of age which resulted in a significant rise in antibodies to all 7 serotypes. There are however questions to be answered around their efficacy, sustained immunological response, herd immunity and cost-effectiveness (Eskola 1999; Cole, Sniffen and Nadler 1999).

This study proposed to define the serotypes and susceptibility patterns of *Streptococcus pneumoniae* isolated in clinical infections in Cape Town where limited data was available.

The objectives of this study were

1. To determine the serotypes of clinical isolates of *Streptococcus pneumoniae* causing invasive and non-invasive disease in the Cape Peninsula.

2. To determine the susceptibility patterns of the clinical isolates of *Streptococcus pneumoniae* causing invasive and non-invasive disease in the Cape Peninsula.

3. To determine the relationship between serotypes and antimicrobial agent resistance of *Streptococcus pneumoniae* causing invasive and non-invasive disease in the Cape Peninsula in various age groups and different types of clinical syndromes.

The results of this study will add to the body of knowledge of pneumococcal disease and be of value to physicians having to treat cases before susceptibility study results become available. It will also benefit health authorities in their decision-making with respect to the use of pneumococcal vaccines.

CHAPTER 2

METHODS

METHODS

This prospective descriptive study was performed and the data analysed from the clinical isolates of *Streptococcus pneumoniae* cultures obtained from patients hospitalised in the greater Cape Town area. Participating laboratories included the Cape Provincial Administration laboratories at Groote Schuur Hospital (GSH), Red Cross Children's Hospital (RCH) and Tygerberg Hospital (TBH), the laboratories of the South African Institute for Medical Research (SAIMR) and the Dietrich, Street and Partners (DSP), Cape Town laboratory.

All the pneumococcal isolates obtained from infections causing invasive disease (cerebrospinal fluid, blood cultures, pleural fluid and joint aspirates) were investigated. A sample of the non-invasive isolates (ear, nose, throat, sputum, pus from wounds and vaginal swabs etc.) was investigated. This convenience sample comprised of the first 20 isolates from children and the first 10 adult patients per month. All *Streptococcus pneumoniae* isolates from laboratories in Cape Town (GSH, TBH, RCH, DSP, SAIMR) were received at the SAIMR laboratory, Cape Town.

1. The source of the specimen, the age, the clinical details and the disc sensitivity test results of the patients from which the *Streptococcus pneumoniae* was isolated was recorded and given a laboratory number. Only one isolate per patient was included in the study. In the case of one patient having a blood and cerebrospinal fluid isolate, the cerebrospinal fluid isolate was included in the

study. In some instances the cerebrospinal fluid isolate lost viability before it was freeze dried and in these cases the blood culture isolate was included in the study.

2. All isolates were subcultured onto blood agar plates and confirmed to be *Streptococcus pneumoniae* by colonial morphology - typical Alpha-hemolytic, draughtsman colonies, Gram stain - gram positive lanceolate diplococci and susceptibility assay to optochin (ethylhydrocuprein).

3. All isolates were then freeze-dried before serotyping and antimicrobial susceptibility tests were performed.

4. All isolates included in the study were sent to the SAIMR, Central Institute, Johannesburg for serotyping. All *Streptococcus pneumoniae* cultures were serotyped using type specific antipneumococcal sera from Statens Seruminstitut (Copenhagen, Denmark). Typing with factor sera was not performed. Serotyping was performed using the Quellung reaction. The test was performed by mixing a loopful of emulsified isolate with a loopful of antipneumococcal serum and methylene blue and examined microscopically. In a positive reaction, which occurs when pneumococci are brought into contact with homologous capsular antiserum, the capsule becomes more retractile and greatly swollen in appearance.

5. Disc sensitivity testing was performed by the Johannesburg laboratory using the Kirby Bauer disc sensitivity test method. All isolates were inoculated into trypticase soy broth. The concentration of the inoculum conformed to a 0.5 Macfarlane standard. These organisms were then inoculated onto cation adjusted Meullller Hinton agar plates. The following discs were tested and the criteria for resistance is as follows:

ANTIMICROBIAL DISC CONTENT	RESISTANT ZONE SIZE
1µg oxacillin	<20mm
30µg chloramphenicol	<19mm
30µg tetracycline	<19mm
15µg erythromycin	<20mm
2µg clindamycin	<20mm
5µg rifampicin	<20mm

The plates were incubated at 35 C at 5% CO_2 overnight. The results of these tests were recorded and were compared to the MIC values.

6 MIC (Minimum inhibitory concentration) is the lowest concentration at which no bacterial growth occurs for a given bacterial strain. The agar dilution method was performed, which is a MIC method recognised by the National committee for clinical laboratory standards (NCCLS) (Jorgensen *et al* 1991). This method is the routine method of performing MIC in our laboratory, as it both time saving and cost effective. The reasons for this, is that the inoculation is semi-automated and

the reading of the MIC is automated using the Masterscan 600 instrument. Table 2.1 shows the choice of antimicrobials that were used including the range of serial dilutions for each antimicrobial.

Table 2.1: Antimicrobials and the range of serial dilutions.

Antimicrobial	Dilutions in mg/l							
Penicillin	0.03	0.06	0.12	0.25	0.5	1	2	4
Chloramphenicol			2	4	8			
Tetracycline	0.5	1	2	4	8	16	32	
Erythromycin	0.12	0.25	0.5	1	2	4	8	16
Clindamycin	0.06	0.12	0.25	0.5	1	2	4	8
Rifampicin	0.06	0.12	0.25	0.5	1	2	4	8
Ceftriaxone	0.25	0.5	1	2	4	8	16	
Cotrimoxazole	0.06	0.12	0.25	0.5	1	2	4	8
Vancomycin	0.5	1	2	4	8	16	32	64

The method employed for performing the antimicrobial dilutions is as follows:

2.1 PREPARATION OF ANTIMICROBIAL SOLUTIONS:

The antimicrobial powders for penicillin (Novonordisk), erythromycin (Abbot laboratories), clindamycin (Upjohn), rifampicin (Rolab) and vancomycin (Eli Lilly) were obtained from the pharmaceutical companies. Adatab tablet forms of chloramphenicol, tetracycline, ceftriaxone, cotrimoxazole, coamoxyclav and ampicillin were obtained from Davies Diagnostics (MAST, UK) to prepare the antimicrobial stock solutions.

Table 2.2 : Preparation of penicillin stock solutions:

ANTIMICROBIAL *	DILUENT	CONCENTRATION	SOLUTION *
1ml Novocillin	2ml distilled water	100 000µg/ml	A
0,64ml soln A	9,36ml distilled water	6 400 µg/ml	B
1ml soln B	4ml distilled water	1 280 µg/ml	C
1ml soln C	4ml distilled water	640 µg/ml	D
1ml soln D	4ml distilled water	320 µg/ml	E
1ml soln E	4ml distilled water	160 µg/ml	F
1ml soln F	4ml distilled water	80 µg/ml	G
1ml soln G	4ml distilled water	40 µg/ml	H

Preparation of penicillin agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,5ml of each solution to 17,5ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:40 (0,5ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to H ranged from 0,03 mg/l to 4 mg/l.

* Please note that solutions in column 1 marked ANTIMICROBIAL are the same as solutions in column 4 marked SOLUTIONS. The solutions in column 4 were used to make the final concentrations of the media. The above information applies to Tables 2.1 to 2.12.

Table 2.3: Preparation of chloramphenicol stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
4^X 0,8mg tablets	4ml distilled water	800 µg/ml	A
1ml soln A	1ml distilled water	400 µg/ml	B
1ml soln B	1ml distilled water	200 µg/ml	C

Preparation of chloramphenicol agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to C ranged from 2mg/l to 8mg/l.

Table 2.4: Preparation of tetracycline stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
4^{X} 1,6mg tablets	4ml distilled water	1600 µg/ml	A
1ml soln A	1ml distilled water	800 µg/ml	B
1ml soln B	1ml distilled water	400 µg/ml	C
1ml soln C	1ml distilled water	200 µg/ml	D
1ml soln D	1ml distilled water	100 µg/ml	E
1ml soln E	1ml distilled water	50 µg/ml	F
1ml soln F	1ml distilled water	25 µg/ml	G

Preparation of tetracycline agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to G ranged from 0,5mg/l to 32mg/l.

Table 2.5: Preparation of erythromycin stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
0,008g	10ml distilled water	800 µg/ml	A
1ml soln A	1ml distilled water	400 µg/ml	B
1ml soln B	1ml distilled water	200 µg/ml	C
1ml soln C	1ml distilled water	100 µg/ml	D
1ml soln D	1ml distilled water	50 µg/ml	E
1ml soln E	1ml distilled water	25 µg/ml	F
1ml soln F	1ml distilled water	12,5 µg/ml	G
1ml soln G	1ml distilled water	6,25 µg/ml	H

Preparation of erythromycin agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to G ranged from 0,12mg/l to 16mg/l.

Table 2.6: Preparation of clindamycin stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
0,0016mg	4ml distilled water	400 µg/ml	A
1ml soln A	1ml distilled water	200 µg/ml	B
1ml soln B	1ml distilled water	100 µg/ml	C
1ml soln C	1ml distilled water	50 µg/ml	D
1ml soln D	1ml distilled water	25 µg/ml	E
1ml soln E	1ml distilled water	12,5 µg/ml	F
1ml soln F	1ml distilled water	6,25 µg/ml	G
1ml soln G	1ml distilled water	3,125 µg/ml	H

Preparation of agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to H ranged from 0,06mg/l to 8mg/l.

Table 2.7: Preparation of rifampicin stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
0,008mg	1ml methanol added to 9ml distilled water	800 µg/ml	A
1ml soln A	1ml distilled water	400 µg/ml	B
1ml soln B	1ml distilled water	200 µg/ml	C
1ml soln C	1ml distilled water	100 µg/ml	D
1ml soln D	1ml distilled water	50 µg/ml	E
1ml soln E	1ml distilled water	25 µg/ml	F
1ml soln F	1ml distilled water	12,5 µg/ml	G
1ml soln G	1ml distilled water	6,25 µg/ml	H

Preparation of agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to H ranged from 0,06mg/l to 8mg/l.

Table 2.8: Preparation of ceftriaxone stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
4^X 0,8mg tablets	4ml distilled water	800 µg/ml	A
1ml soln A	1ml distilled water	400 µg/ml	B
1ml soln B	1ml distilled water	200 µg/ml	C
1ml soln C	1ml distilled water	100 µg/ml	D
1ml soln D	1ml distilled water	50 µg/ml	E
1ml soln E	1ml distilled water	25 µg/ml	F
1ml soln F	1ml distilled water	12,5 µg/ml	G
1ml soln G	1ml distilled water	6,25 µg/ml	H
1ml soln H	1ml distilled water	3,125 µg/ml	I

Preparation of agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to I ranged from 0,25mg/l to 16mg/l.

Table 2.9: Preparation of cotrimoxazole stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
5^X 6,4/0,32mg tablets	4ml distilled water	8000/400 µg/ml	A
1ml soln A	1ml distilled water	4000/200 µg/ml	B
1ml soln B	1ml distilled water	2000/100 µg/ml	C
1ml soln C	1ml distilled water	1000/50 µg/ml	D
1ml soln D	1ml distilled water	500/25 µg/ml	E
1ml soln E	1ml distilled water	250/12,5 µg/ml	F
1ml soln F	1ml distilled water	125/6,25 µg/ml	G
1ml soln G	1ml distilled water	62,5/3,125 µg/ml	H

Preparation of agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to H ranged from 0,06mg/l to 8mg/l.

Table 2.12: Preparation of vancomycin stock solutions:

ANTIMICROBIAL	DILUENT	CONCENTRATION	SOLUTION
0,0064 g powder	4ml distilled water	1600 µg/ml	A
1ml soln A	1ml distilled water	800 µg/ml	B
1ml soln B	1ml distilled water	400 µg/ml	C
1ml soln C	1ml distilled water	200 µg/ml	D
1ml soln D	1ml distilled water	100 µg/ml	E
1ml soln E	1ml distilled water	50 µg/ml	F
1ml soln F	1ml distilled water	25 µg/ml	G

Preparation of agar-dilution plates:

Medium: Cation adjusted Meuller Hinton agar supplemented with 5% laked horse blood, was used as an alternative to the NCCLS recommendation of broth dilution.

The final concentration obtained by adding 0,4ml of each solution to 17,6ml Meuller Hinton agar and 2ml laked blood. The dilution factor of 1:50 (0,4ml antimicrobial solution in 20ml media) was taken into account for the final concentration. The final concentration of solutions A to H ranged from 0,5mg/l to 64mg/l.

2.2 PREPARATION OF INOCULUM:

Inoculum: Before MIC tests were performed all freeze-dried *Streptococcus pneumoniae* cultures were reconstituted using nutrient broth. All isolates were sub-cultured twice onto blood agar plates before minimum inhibitory concentrations (MIC) were performed. A standard inoculum of $5^{x}10^{4}$ colony forming units was prepared in a trypticase soy broth from a few colonies of an overnight growth of *Streptococcus pneumoniae* on horse blood agar. Using a multipoint inoculator (Mast Diagnostics,UK), 0.3 µl of inoculum was transferred from the inoculum pin to the antimicrobial plates.

Staphylococcus aureus ATCC 25923 was included as a control for all MIC assays. All plates were incubated at 35° C CO_2 in a humid atmosphere for 24hrs.

2.3 READING AND RECORDING OF RESULTS:

A growth control plate of cation adjusted Meuller Hinton agar with 5% horse blood containing no antimicrobials was read. This plate served as a control to ensure that the strains tested provided adequate growth on the medium. All tests were read using the Mastascan 600 instrument (MAST, UK) and recorded on a worksheet.

2.4 INTERPRETATION OF RESULTS:

The MIC is considered to be the lowest concentration of the antimicrobial that inhibits visible growth after 18-hour incubation in a 5% CO_2 environment at a temperature of 35C.

Specific criteria are defined for the resistance of *Streptococcus pneumoniae* isolates by the NCCLS vol14 no. 16 1994 (Jorgensen *et al* 1994). The data was analysed

according to that tabulated in Table 2.13 with the exception of penicillin which was divided into highly resistant if MIC value = 2ug/ml, intermediate resistance if MIC value lies between 0,1 and 1ug/ml and sensitive if MIC value is 0.06.

Table 2.13: Antimicrobial resistant criteria.

Antimicrobial	NCCLS
Penicillin	0.1ug/ml
Chloramphenicol	8
Tetracycline	8
Erythromycin	4
Clindamycin	1
Rifampicin	4
Ceftriaxone	-
Cotrimoxazole	4/76
Vancomycin	-

CHAPTER 3

RESULTS

CHAPTER 3

RESULTS

RESULTS

During this study a total of 564 *Streptococcus pneumoniae* isolates were received.

The *Streptococcus pneumoniae* organisms were classified into two groups, invasive, if organisms were isolated from CSF, blood culture, pleural fluid or joint aspirates (277,49,1%) and non-invasive if organisms were isolated from ears, eyes, noses, sputum, tracheal aspirates and other sites (285, 50,5%). There was no information available for the source of two specimens (2, 0,4%). The distribution of the 564 *Streptococcus pneumoniae* isolates according to source is shown in Table 3.1.

Table 3.1: Distribution of *Streptococcus pneumoniae* isolates according to source.

	SPECIMEN TYPE	NO.	%
INVASIVE	CEREBROSPINAL FLUID	62	11.0
	BLOOD CULTURE	207	36.7
	PLEURAL FLUID	6	1.1
	JOINT ASPIRATE	2	0.4
	TOTAL INVASIVE	277	49.1
NON INVASIVE	SPUTUM	123	21.8
	EAR	62	11.0
	NOSE	25	4.4
	EYE	25	4.4
	TRACHEAL ASPIRATES	22	3.9
	OTHER	14	2.5
	SINUS	7	1.2
	VAGINAL	5	0.9
	THROAT	2	0.4
	TOTAL NON INVASIVE	285	50.5
	NO SITE	2	0.4
	TOTAL	564	100.0

The patients were divided into two age groups, children <14 years of age and adults > or = 14 years of age. Table 3.2 shows the age distribution of all isolates. The patient's ages ranged from 1 month to 92 years. 18% were isolated from children less than 1 year, 45 % from children less than 5 years old. The exact age of 14 patients was not available.

Table 3.2: Age distribution in years of *Streptococcus pneumoniae* isolates

AGE IN YEARS	TOTAL NO.	PERCENTAGE
<1	100	18
1-5	146	27
6-15	39	7
16-30	61	11
31-60	147	27
>60	57	10
TOTAL	550	100

The age distribution of all isolates in number and percentage is illustrated in figures 1 and 2.

Figure 1
Age distribution of *Streptococcus pneumoniae* isolates

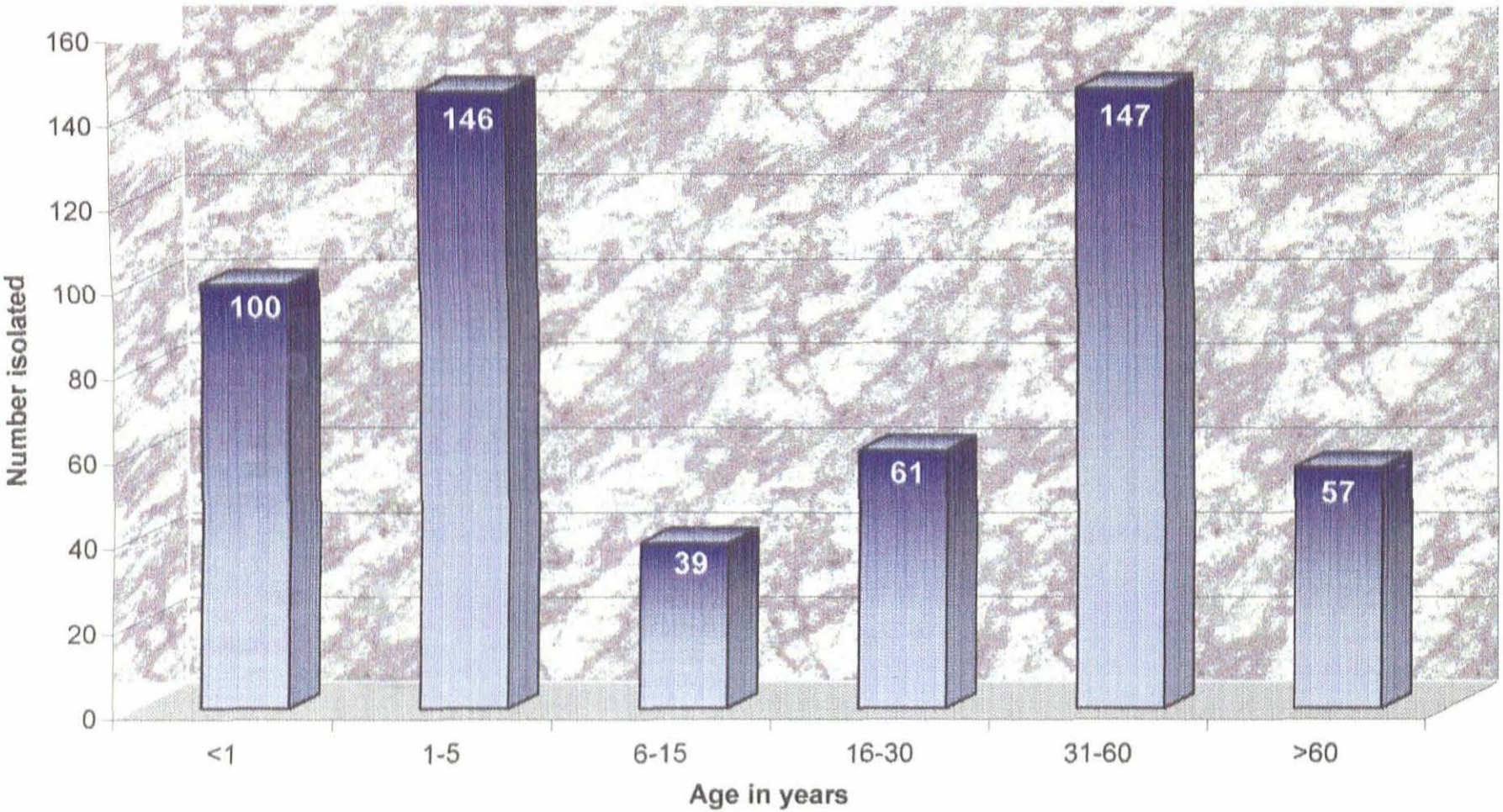
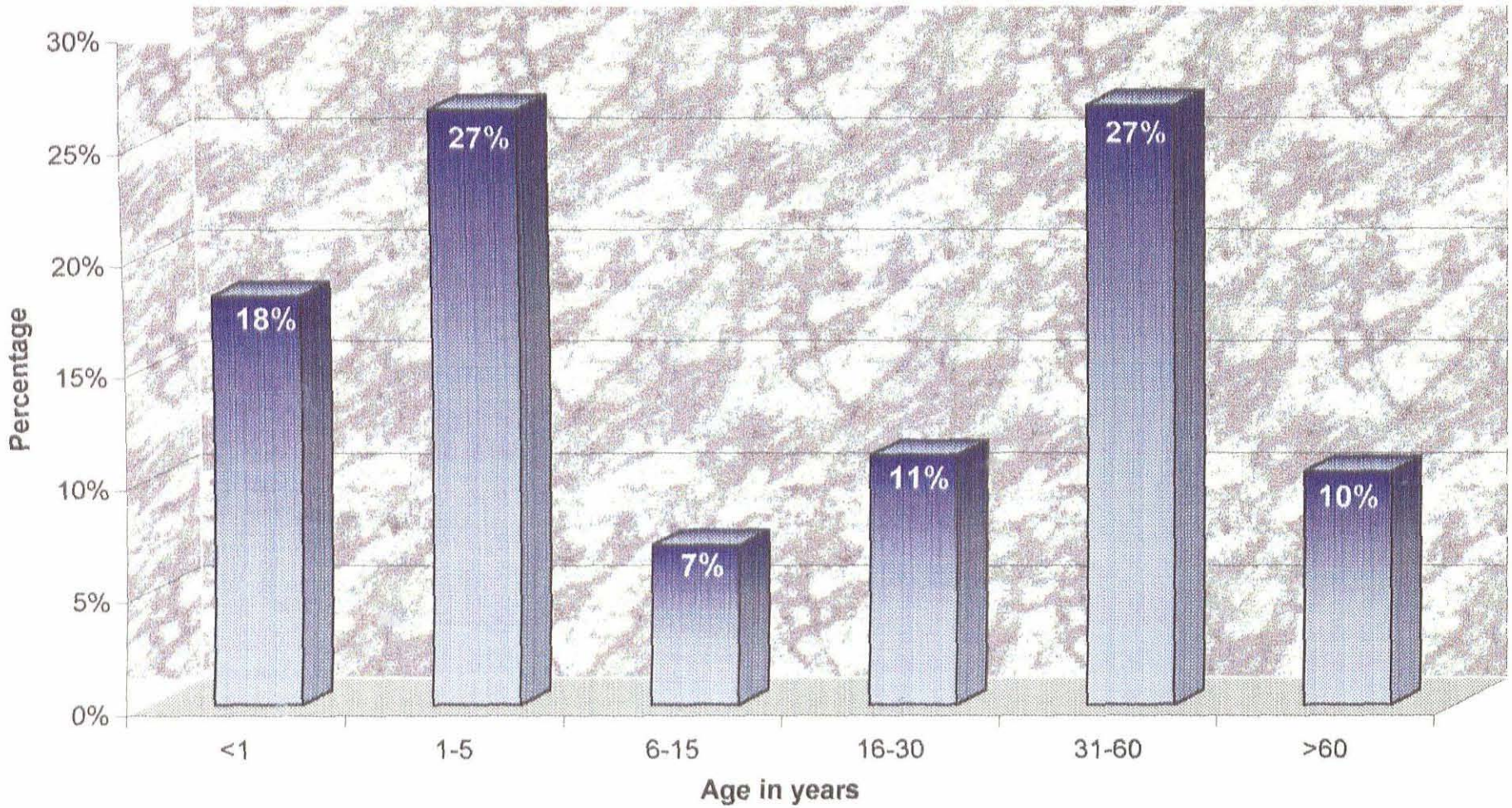


Figure 2
Age distribution of *Streptococcus pneumoniae* isolates



Of the 550 isolates, 277 were isolated from children and 273 from adults. The age distribution of isolates according to invasive and non-invasive isolates is shown in Table 3. 3 and the distribution of isolates according to source and age are shown in Table 3.4.

Table 3.3: Age distributions of patients of invasive and non-invasive isolates

AGE (YEARS)	AGE (MONTHS)	NO. INVASIVE	% INVASIVE	NO. NON-INVASIVE	% NON-INVASIVE
0-5	<=6	27	10	26	9
	7-12	32	12	46	17
	13-18	19	7	19	7
	19-24	10	4	16	6
	25-36	10	4	18	7
	37-48	7	2	12	4
	49-60	2	1	2	1
6-10		11	4	16	6
11-14		6	2	4	1
15-25		21	7	18	7
26-35		36	13	24	9
36-45		30	11	19	7
46-55		20	7	25	9
56-65		24	9	14	5
66-75		16	6	13	5
>75		4	1	3	1
TOTAL		275	100	275	100

Of the 550 isolates, 277 were isolated from children and 273 from adults. The age distribution of isolates according to invasive and non-invasive isolates is shown in Table 3. 3 and the distribution of isolates according to source and age are shown in Table 3.4.

Table 3.3: Age distributions of patients of invasive and non-invasive isolates

AGE (YEARS)	AGE (MONTHS)	NO. INVASIVE	% INVASIVE	NO. NON-INVASIVE	% NON-INVASIVE
0-5	<=6	27	10	26	9
	7-12	32	12	46	17
	13-18	19	7	19	7
	19-24	10	4	16	6
	25-36	10	4	18	7
	37-48	7	2	12	4
	49-60	2	1	2	1
6-10		11	4	16	6
11-14		6	2	4	1
15-25		21	7	18	7
26-35		36	13	24	9
36-45		30	11	19	7
46-55		20	7	25	9
56-65		24	9	14	5
66-75		16	6	13	5
>75		4	1	3	1
TOTAL		275	100	275	100

Table 3.4: Distribution of isolates according to source and age group.

	SPECIMEN TYPE	CHILDREN		ADULT		TOTAL
		NO	*%	NO	*%	
INVASIVE	CEREBROSPINAL FLUID	26	42	34		60
	BLOOD CULTURE	88	43	119	57	207
	PLEURAL FLUID	3	50	3	50	6
	JOINT ASPIRATE	0	0	2	100	2
	TOTAL INVASIVE	117	43	158	57	275
NON INVASIVE	SPUTUM	34	29	83	71	117
	EAR	53	91	5	9	58
	NOSE	20	80	5	20	25
	EYE	19	83	4	17	23
	TRACHEAL ASPIRATES	15	68	7	32	22
	OTHER	8	57	6	43	14
	SINUS	5	71	2	29	7
	VAGINAL	3	60	2	40	5
	THROAT	1	50	1	50	2
	TOTAL NON INVASIVE	158	55	115		273
	NO SITE	2	100	0	0	2
	TOTAL	277	50	273	50	550

* % OF TOTAL SPECIMEN TYPE

3.1 SEROTYPES

All isolates were sent to the Central SAIMR in Johannesburg for serotyping. Of the 564 *Streptococcus pneumoniae* isolates, 540 were serotyped. Serotypes for 24 isolates were not available, as these isolates had lost viability before typing could be completed. A total of 32 of the 83 known serotypes were encountered in this study as shown in Table 3.5. Table 3.5 lists the serotypes in order of frequency. Figure 3 and Figure 4 illustrate the most commonly isolated serotypes in number and percentage respectively.

Table 3.5: Serotype distribution of 540 *Streptococcus pneumoniae* isolates.

SEROTYPE	NO.	%	CUM %
6	104	19.3	19.3
19	99	18.3	37.6
14	71	13.1	50.7
1	53	9.8	60.6
3	28	5.2	65.7
18	25	4.6	70.4
23	23	4.3	74.6
4	19	3.5	78.1
7	15	2.8	80.9
15	14	2.6	83.5
5	12	2.2	85.7
9	8	1.5	87.2
8	8	1.5	88.7
28	6	1.1	89.8
39	6	1.1	90.9
41	5	0.9	91.9
10	5	0.9	92.8
2	4	0.7	93.5
13	4	0.7	94.3

SEROTYPE	NO.	%	CUM %
11	3	0.6	94.8
17	3	0.6	95.4
31	3	0.6	95.9
37	3	0.6	96.5
42	3	0.6	97.0
20	2	0.4	97.4
24	2	0.4	97.8
34	2	0.4	98.1
40	2	0.4	98.5
16	1	0.2	98.7
22	1	0.2	98.9
29	1	0.2	99.1
32	1	0.2	99.3
33	1	0.2	99.4
36	1	0.2	99.6
TOTAL	540	100.0	100.0

The predominant serotypes included serotypes 6, 19, and 14, these constituted (50%) of all types followed by 1, 3,18, and 23. These seven serotypes constituted 74,6% of all isolates. Serotypes 4,7,15 and 5 were found in less than 4% of patients and serotypes 9,8,28,39 and 41 were found in less than 2% of cases.

Figure 3 Serotype distribution of 540 *Streptococcus pneumoniae* isolates

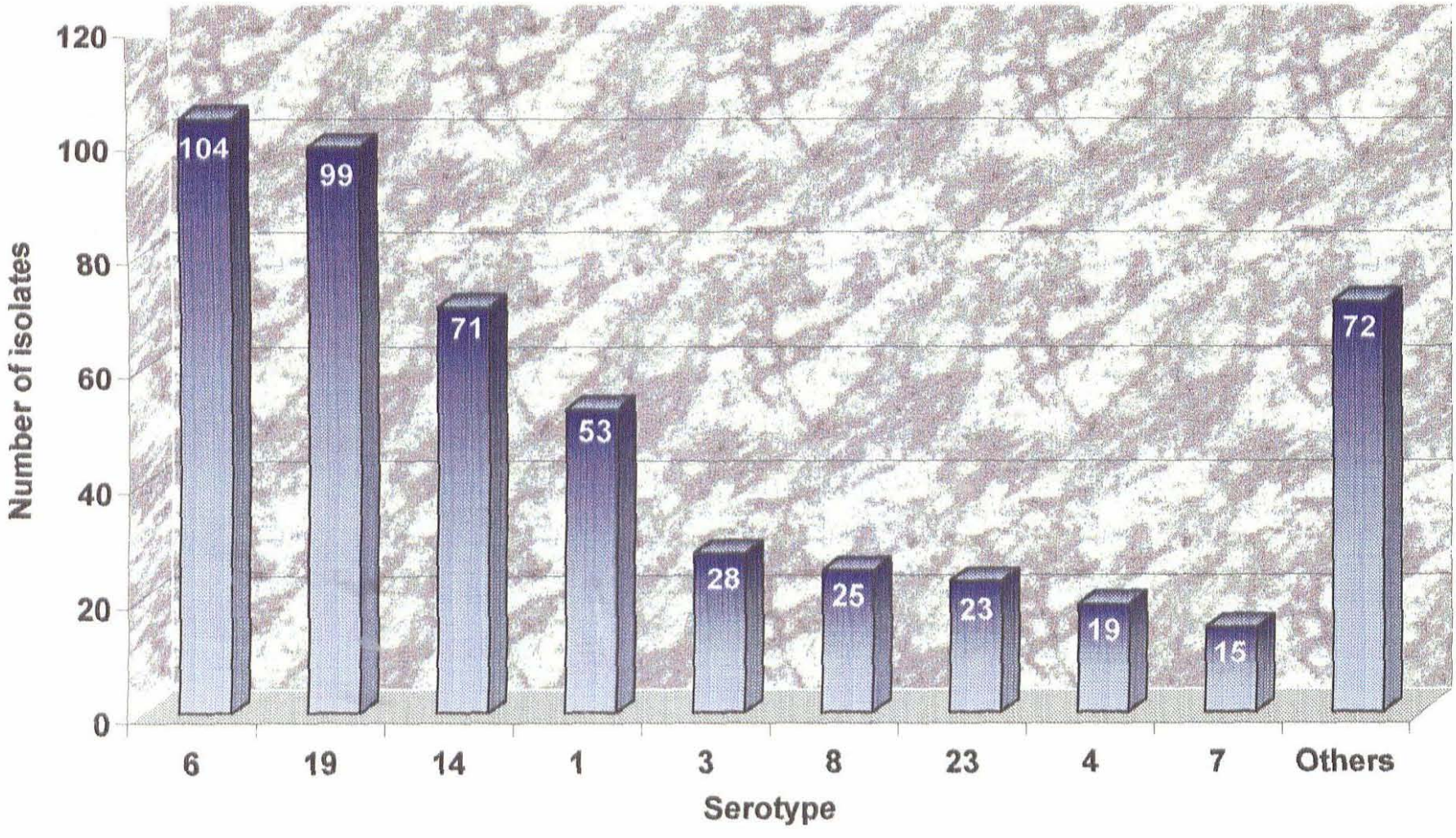


Figure 4 Serotype distribution of 540 *Streptococcus pneumoniae* isolates

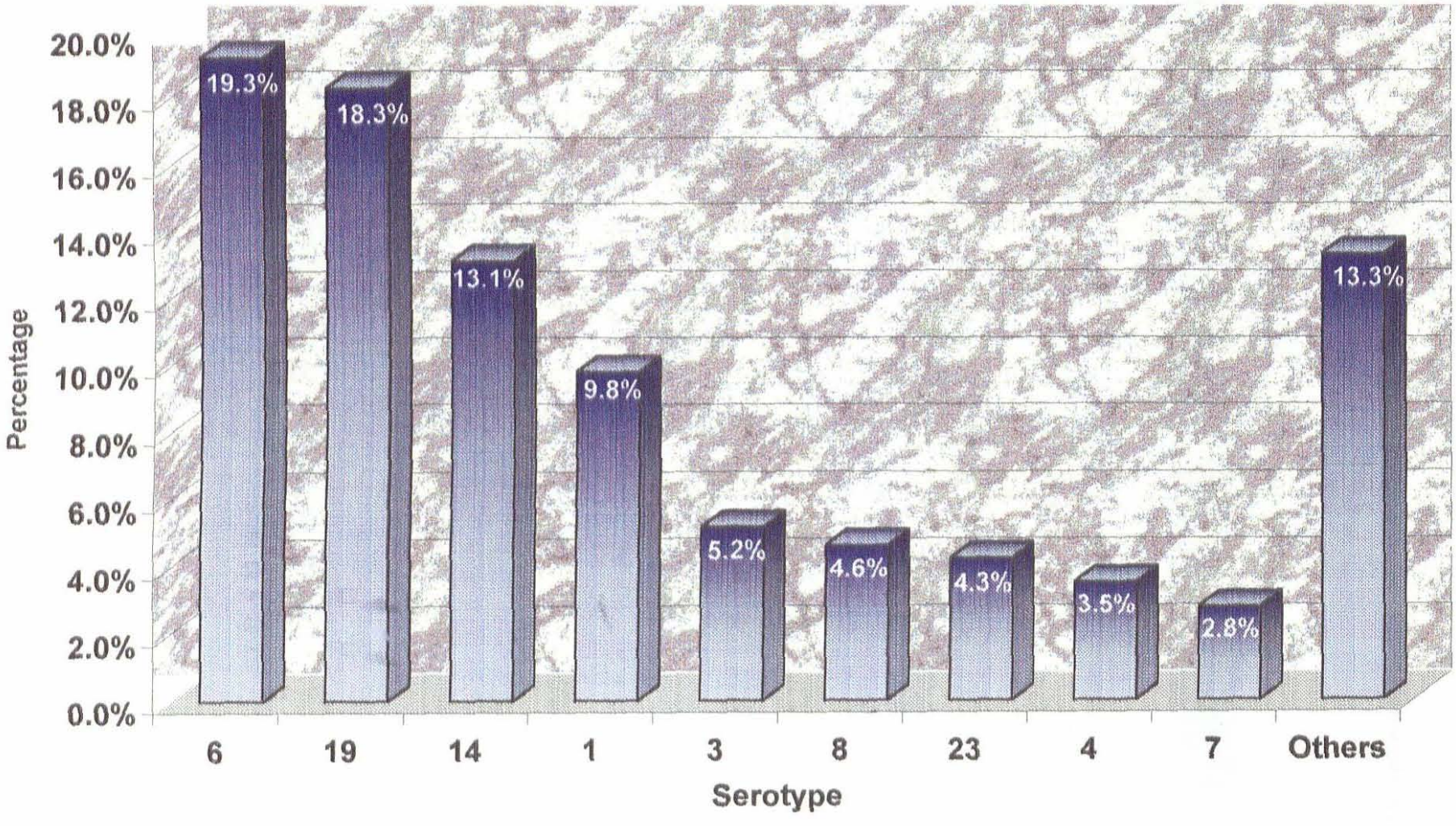


Table 3.6 shows significant differences in the distribution of pneumococcal serotypes with respect to the ages of the patients.

Table 3.6: Serotype distributions according to age groups.

SEROTYPE	CHILDREN		ADULTS		P-VALUE
	NO.	%	NO.	%	
6	72	27	32	12	<0.001
19	57	21	42	15	0.080
14	47	18	24	9	0.002
23	18	7	5	2	0.005
1	15	6	38	14	0.001
3	3	1	25	9	<0.001
18	7	3	18	7	0.026
4	6	2	13	5	0.109
5	6	2	6	2	0.979
7	6	2	9	3	0.449
15	6	2	8	3	0.607
OTHER*	25	9	47	17	0.007
TOTAL	268	100	272	100	

*Other includes <5 of the following types for children: 2,8,9,10,28,29,33,34,39,40,41,42,46 and none <5 of the following types for adults: 2,8,9,10,11,13,16,17,20,22,24,28,31,32,34,36,37,39,41,42 and 43

Serotypes 6,19, 14 and 23 constituted 73 % of all serotypes found in children and serotypes 19,1,6,3 and 14 constituted 59% of all serotypes found in adult patients. Serotypes 6,14 and 23 were found more frequently in children than adults, whereas serotypes 1,3 and 18 were isolated more often from adult patients. Serotype 1,3 and 18 constituted 20% of all adult types but only 10% of all children isolates (Figure 5). Vaccine types constitute 96% of both children and adult isolates.

Figure 5 **Serotype distributions according to age groups**

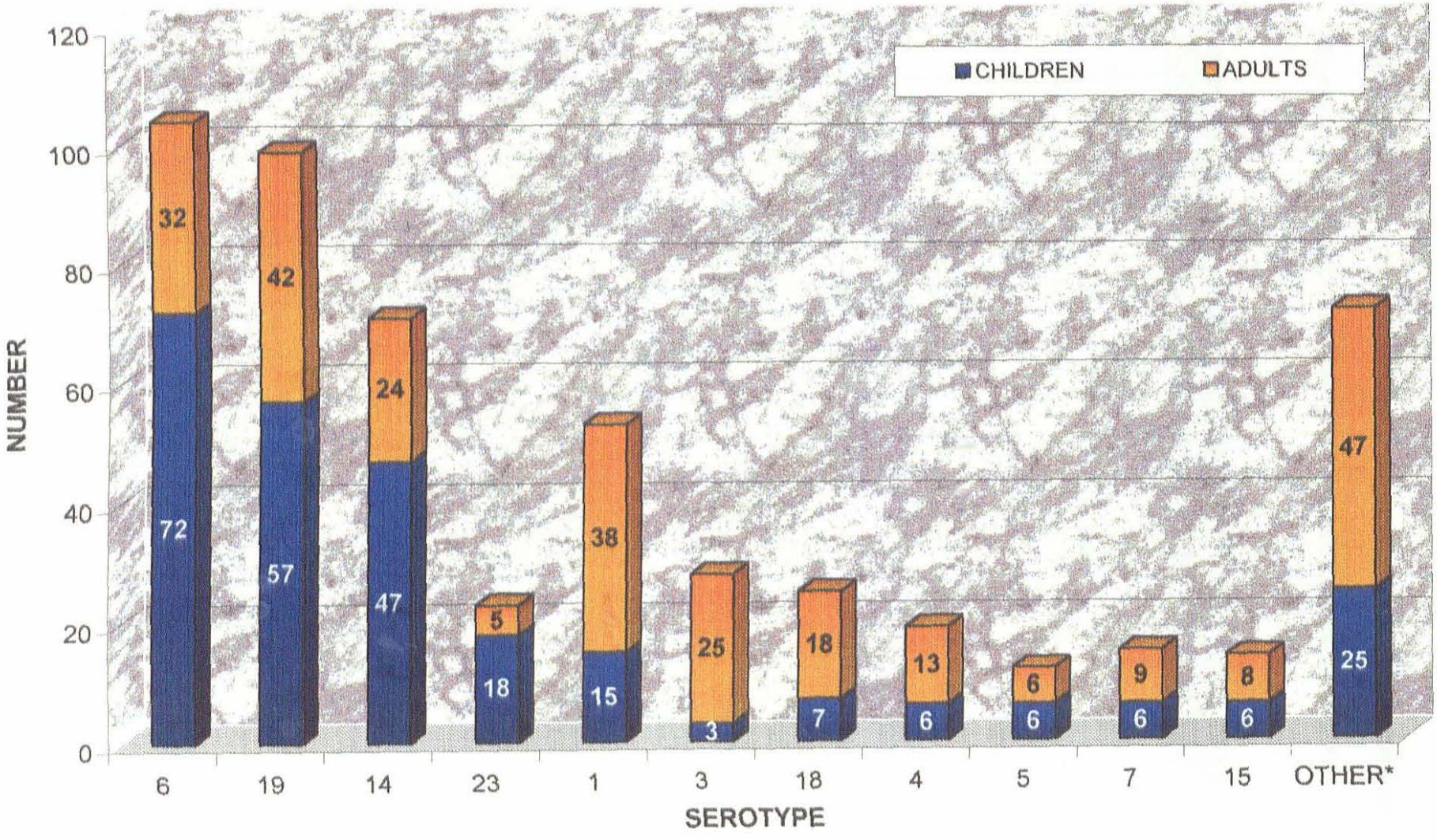


Table 3.7 shows serotype distribution according to invasive and non-invasive isolates.

Table 3.7: Serotype distribution according to invasive and non-invasive isolates.

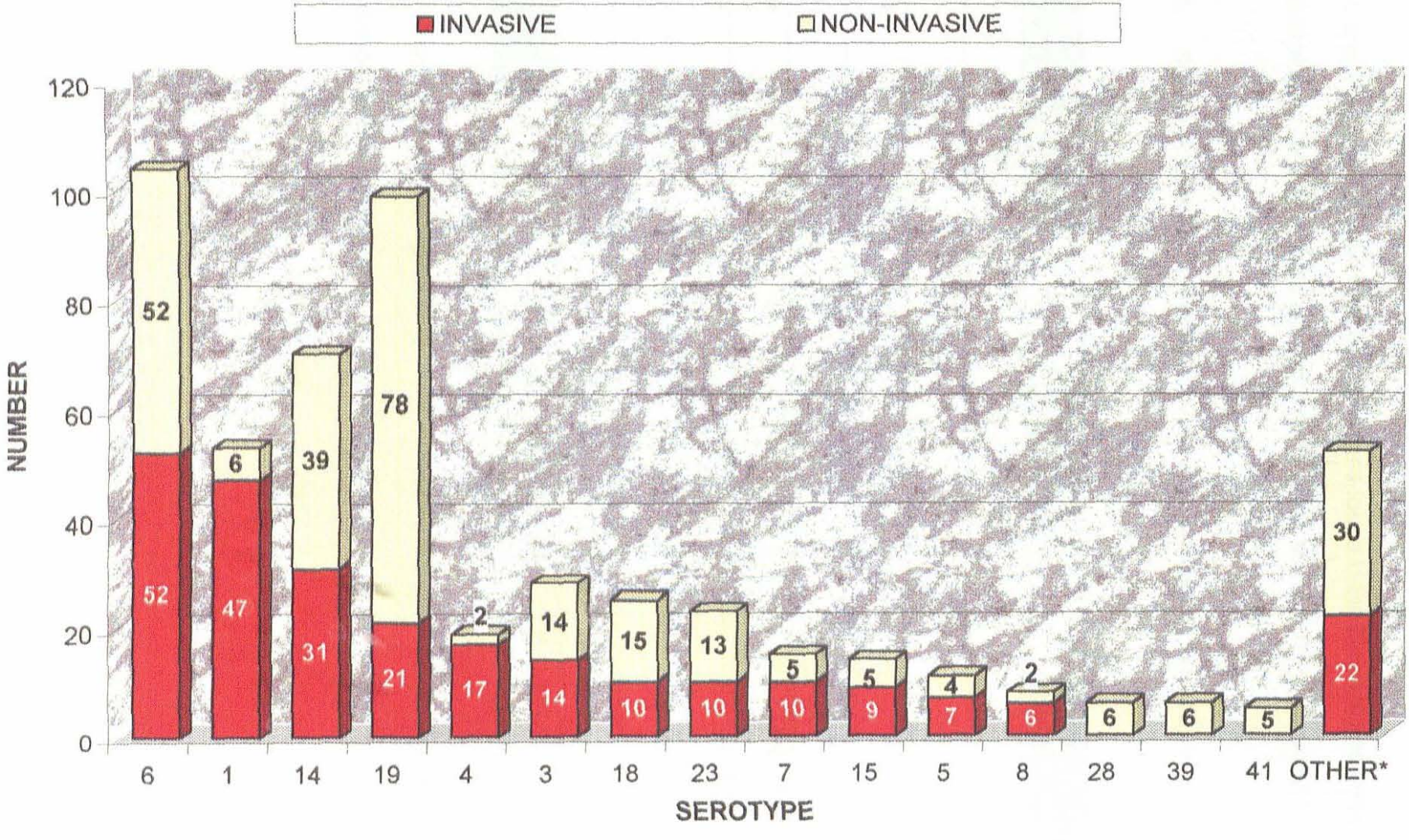
SEROTYPE	INVASIVE		NON-INVASIVE		P –VALUE
	NO.	%	NO.	%	
6	52	20	52	18	0.583
1	47	18	6	2	<0.001
14	31	12	39	14	0.553
19	21	8	78	28	<0.001
4	17	7	2	1	<0.001
3	14	5	14	5	0.792
18	10	4	15	5	0.437
23	10	4	13	5	0.687
7	10	4	5	2	0.133
15	9	4	5	2	0.205
5	7	3	4	1	0.281
8	6	2	2	1	0.118
28	0	0	6	2	0.017
39	0	0	6	2	0.017
41	0	0	5	2	0.032
OTHER*	22	9	30	11	0.423
TOTAL	256	100	282	100	

*Other includes <5 of the following types for the invasive group: 2,9,10,11,13,16,17,20,24,50

<5 of the following types for the non-invasive group: 9,10,11,13,17,22,24,31,32,33,34,36,37,40,42,43 and 46.

Pneumococcal serotypes 6,1,14,19 and 4 were most common in the invasive group of isolates and serotypes 19,6,14,18,3 and 23 most common in the non-invasive group. Serotypes 1 and 4 were isolated more often from the invasive group than non-invasive group whereas serotype 19 was most common in the non-invasive group of isolates (Figure 6). Vaccine types included in the polyvalent polysaccharide vaccine constituted 98% of all invasive isolates but only 87% of the non-invasive isolates.

Figure 6 **Serotype distribution according to invasive and non-invasive isolates**



Tables 3.8 and 3.9 show the most common serotypes found in the invasive and non-invasive *Streptococcus pneumoniae* isolates from children and adults respectively.

Table 3.8: Serotype distribution of invasive and non-invasive isolates in children.

SEROTYPE	INVASIVE		NON-INVASIVE		P-Value
	No.	%	No.	%	
6	35	31	37	24	0.170
14	20	18	27	17	0.910
1	11	10	3	2	0.004
19	9	8	48	31	<0.001
23	8	7	10	6	0.990
Other	29	26	31	20	0.886
TOTAL	112	100	156	100	

Serotype 19 was found to be significantly more commonly isolated from children in the non-invasive group and serotype 1 in the invasive group.

Table 3.9: Serotype distribution of invasive and non-invasive isolates in adults.

SEROTYPE	INVASIVE		NON-INVASIVE		P-Value
	No.	%	No.	%	
1	36	26	3	3	<0.001
6	17	12	15	13	0.880
3	14	10	10	9	0.680
19	12	9	30	26	<0.001
14	12	9	12	10	0.650
18	12	9	6	5	0.280
4	12	9	1	1	0.005
Other	23	17	39	34	0.002
Total	138	100	116	100	

Serotype 1 was most commonly isolated from the invasive group and serotype 19 from the non-invasive isolates respectively from the adult patients.

Table 3.10 and Table 3.11 show the most common serotypes isolated from the invasive and non-invasive groups respectively from both children and adults.

Table 3.10: Serotype distribution of invasive isolates in children and adults.

SEROTYPE	CHILDREN	ADULTS	P-Value
6	35	17	<0.001
14	20	12	0.031
1	11	36	0.001
19	9	12	0.850
23	8	2	0.020
3	0	14	<0.001
18	4	12	0.100
4	5	12	0.187
Other	20	21	0.570
Total	112	138	

Serotypes 6, 14 and 23 were isolated from invasive group significantly more often from children than from adults while serotype 1 and 3 occurred more frequently in adults.

Table 3.11: Serotype distribution of non-invasive isolates in children and adults.

SEROTYPE	CHILDREN	ADULTS	P-value
6	37	15	0.025
14	27	12	0.105
1	3	3	0.713
19	48	30	0.370
23	10	3	0.144
3	4	10	0.025
18	3	6	0.158
4	1	1	0.833
Other	23	36	0.001
Total	156	116	

Serotype 3 was isolated from adults and serotype 6 from children most frequently in the non-invasive group. Table 3.12 shows the serotype distribution of the cerebrospinal fluid and blood culture isolates from children and adults.

Table 3.12: Serotype distribution of CSF and blood culture isolates.

SEROTYPE	CHILDREN	ADULTS		CHILDREN	ADULTS	
	CSF	CSF	P-value	BC	BC	P-value
1	3	8	0.272	7	28	0.002
6	5	7	0.973	30	10	<0.001
14	5	0	0.006	14	12	0.254
3	0	2	0.223	0	11	0.002
19	2	3	0.921	7	9	0.984
23	3	2	0.400	5	0	0.010
Other	6	11	0.500	21	39	0.109
TOTAL	24	33		84	109	

Serotype 14 was found in significantly higher numbers in cerebrospinal fluid of children and serotypes 6 and 23 were isolated more often from blood cultures of children. Serotypes 1 and 3 were found in significantly higher numbers in blood cultures from adults.

3.2 ANTIMICROBIALS

MIC determinations were performed on 506 *Streptococcus pneumoniae* isolates using the agar dilution method. MIC's were not performed on 58 isolates as they had lost viability before MIC could be completed.

Disc sensitivity test results were obtained for 53 of the 58 isolates that had no MIC performed. Of these only three isolates were resistant to penicillin, one isolate resistant to tetracycline, and one resistant to erythromycin and five resistant to cotrimoxazole. Five isolates had no disc sensitivity test results as these organisms had lost viability before the tests could be completed. The overall percentage resistant rates are not affected by these results. The resistance patterns and range of MIC of the 506 isolates to various antimicrobials is summarized in Table 3.13.

Table 3.13: Resistance patterns of 506 *Streptococcus pneumoniae* isolates.

ANTIMICROBIAL	MIC RANGE	CUTTOFF CONC	RESISTANT	
	mg/l	Mg/l	NO.	%
PENICILLIN	0.12-2	0.1	105	21
CHLORAMPHENICOL	2-8	8	23	4.5
TETRACYCLINE	0.5-32	8	52	10
ERYTHROMYCIN	0.125-16	4	24	5
CLINDAMYCIN	0.06-8	1	33	6.5
RIFAMPICIN	0.06-8	4	26	5
CEFTRIAXONE	0.25-16	-	0	0
COTRIMOXAZOLE	0.06-8	4	95	19
VANCOMYCIN	0.5-64	2	0	0

Figure 7 shows the number of sensitive and resistant *Streptococcus pneumoniae* strains to antimicrobials tested in this study. High percentages of resistance was seen for penicillin (21%), cotrimoxazole (19%) and tetracycline (10%).

Figure 7 Susceptibility patterns of 506 *Streptococcus pneumoniae* isolates

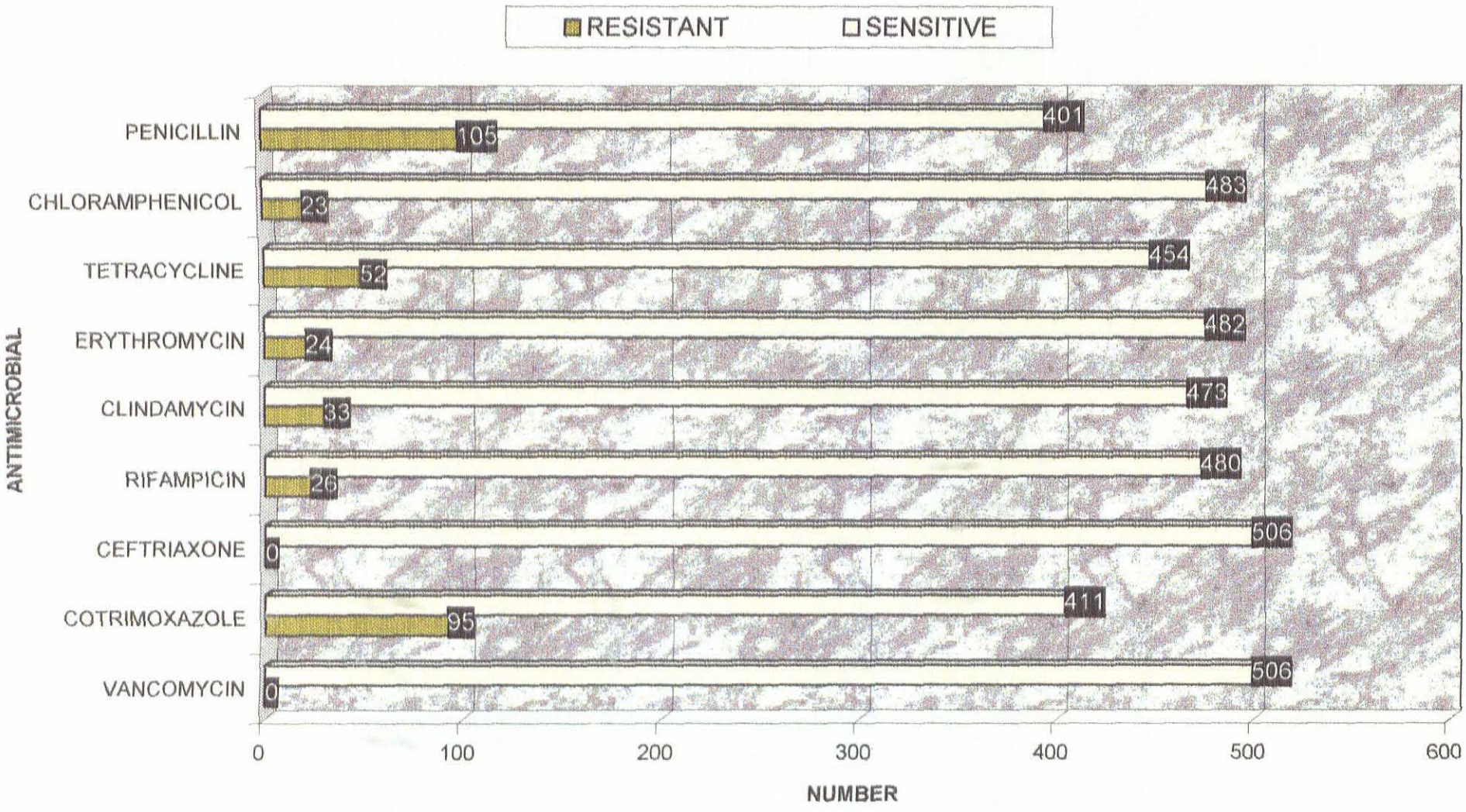


Table 3.14 shows the distribution of *Streptococcus pneumoniae* strains MIC's and Table 3.15 shows the MIC for 50% (MIC50) and 90%(MIC90) of strains.

Table 3.14: Susceptibility of 506 strains of *Streptococcus pneumoniae* to 9 various antimicrobial agents.

ANTIMICROBIAL	Number of strains with MIC (mg/l)											
	Total	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
PENICILLIN	506	342	59	34	33	19	8	6	5			
CHLORAMPHENICOL	506					99	0	200	184	21	1	1
TETRACYCLINE	506					452	1	0	1	2	10	40
ERYTHROMYCIN	506			418	61	0	0	3	5	10	9	
CLINDAMYCIN	506		187	19	16	251	13	0	0	20		
RIFAMPICIN	506		477	1	0	1	1	0	2	24		
CETFTRIAXONE	506				489	7	10					
COTRIMOXAZOLE	506		79	137	42	45	65	43	45	50		
VANCOMYCIN	506					476	30					

Table 3.15: *Streptococcal pneumoniae* MIC for 50% and 90% of strains.

ANTIMICROBIAL	MIC mg/l			RESISTANCE
	RANGE	MIC50	MIC90	% TOTAL
PENICILLIN	0.03 - 4	0.03	0.25	21
CHLORAMPHENICOL	0.5 - 32	2	4	4.5
TETRACYCLINE	0.5 - 32	0.5	2	10
ERYTHROMYCIN	0.125 - 16	0.125	0.25	5
CLINDAMYCIN	0.06 - 8	0.5	1	6.5
RIFAMPICIN	0.06 - 8	0.06	0.13	5
CETFTRIAXONE	0.25 - 4	0.25	0.25	0
COTRIMOXAZOLE	0.06 - 8	0.25	4	19
VANCOMYCIN	0.125 - 16		0.5	0

3.2.1 PENICILLIN

The overall resistance rate to penicillin was 21% which includes the intermediate resistant (IR) with an MIC of between 0,06-1mg/l and high resistance (HR) ranges with an MIC of 2mg/l or greater. A significantly higher percentage resistance was seen in pneumococci isolated from children (5% HR and 28% IR) than adults (0%HR and 8% IR) (Figure 8) and significantly higher intermediate resistance (22% IR) was seen in the non-invasive group than invasive group (Figure 9). These results are shown in Table 3.16.

Table 3.16: Penicillin resistance pattern according to age groups, invasive and non-invasive groups.

PENICILLIN	CHILDREN	ADULT	P-VALUE	INVASIVE	NON-INVASIVE	P-VALUE
	NO. (%)	NO. (%)		NO. (%)	NO. (%)	
HR _2 mg/l	13(5)	0(0)	<0.001	3(1)	10(4)	0.110
IR 0.12-1 mg/l	74(28)	18(8)	<0.001	30(13)	62(22)	0.009
S _0.06 mg/l	177(67)	224(92)	<0.001	193(86)	206(74)	0.002
TOTAL	264(100)	242(100)		226(100)	278(100)	

Figure 8 **Penicillin resistance pattern according to age groups**

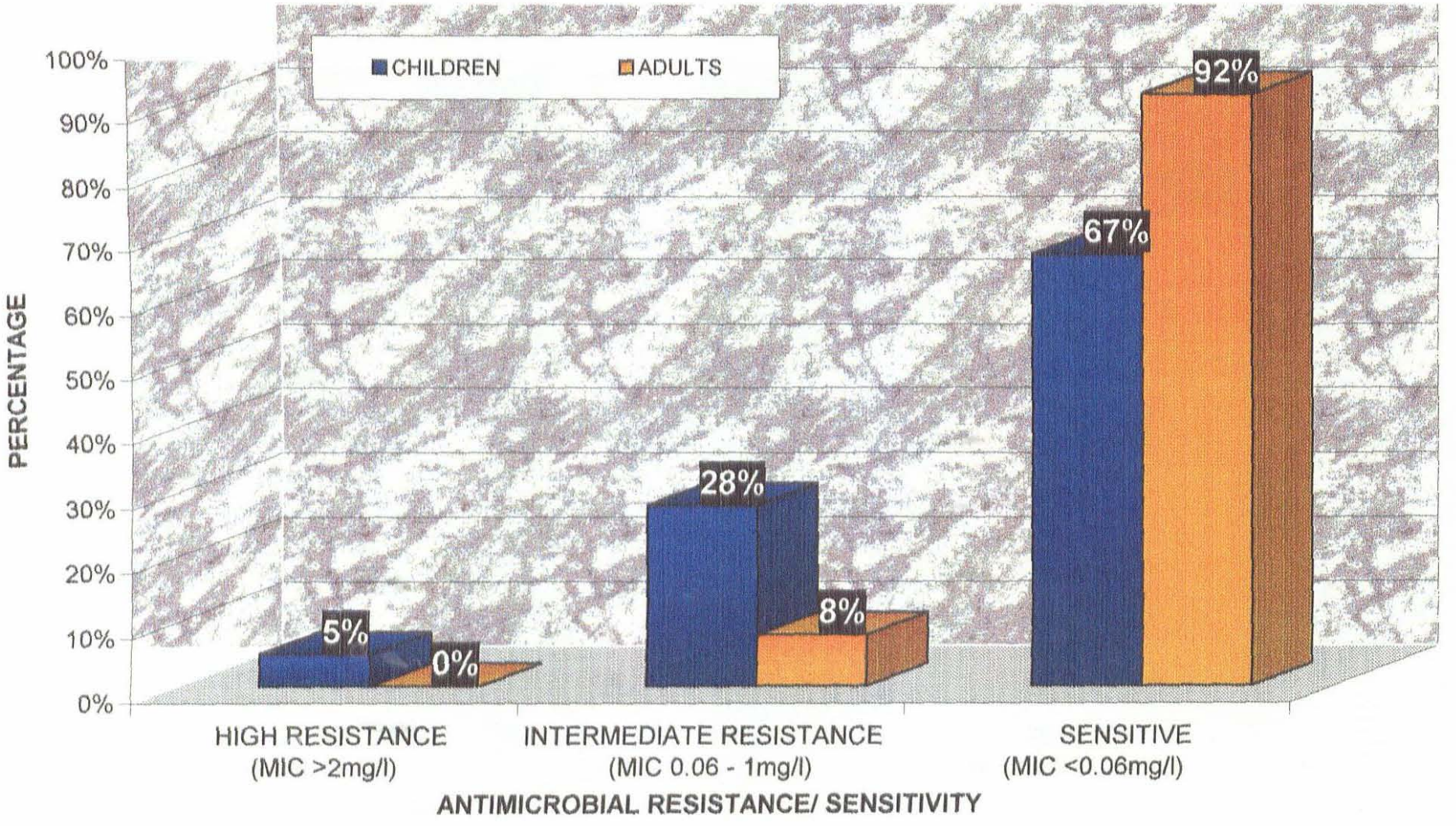
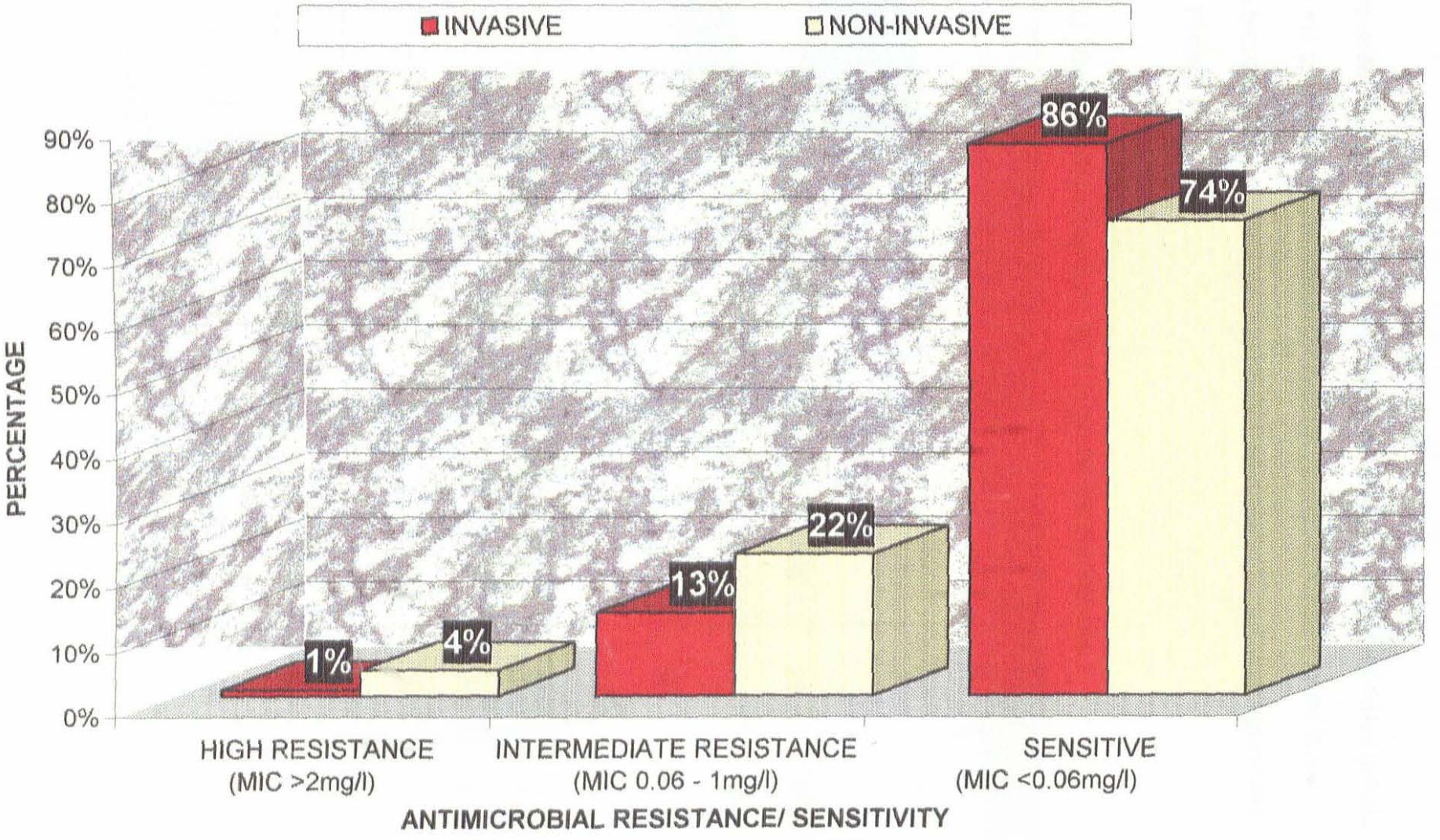


Figure 9 Penicillin resistance patterns according to invasive / non-invasive groups



Although overall there was a higher resistance to penicillin in the non-invasive group, looking at the specific source of the isolates, penicillin resistance was found to be highest in blood culture isolates 27% and sputum specimens 23%.These figures are shown in Table 3.17.

Table 3.17: Source of isolate according to penicillin resistance.

	SPECIMEN	PENICILLIN			
		HR	IR	TR	%
INVASIVE	CEREBROSPINAL FLUID	1	4	5	5
	BLOOD CULTURE	2	26	28	27
NON- INVASIVE	SPUTUM	4	19	23	22
	EAR	3	15	18	17
	TRACHEAL ASPIRATE	0	13	13	12
	NOSE	3	7	10	9
	EYE	0	4	4	4
	OTHER	0	3	3	3
	SINUS	0	1	1	1
	TOTAL	13	92	105	100

TR = Total resistant.

Penicillin resistance was particularly associated with serotypes 6,19,23,14,4 and 28 , which accounted for 35% of the 302 isolates in these serotypes. 73% of all serotype 23 isolates and 49% of serotype 6 isolates were penicillin resistant strains. The serotype distribution according to penicillin resistance is shown in Table 3.18.

Table 3.18: Serotype distribution of penicillin resistant pneumococci.

SEROTYPE		PENICILLIN			
	TS	HR	IR	TR	%TR
6	98	2	46	48	49
19	95	4	23	27	28
23	22	4	12	16	73
14	62	3	6	9	14.5
4	19	0	2	2	10.5
28	6	0	1	1	2
NONE		0	2		
TOTAL	302	13	92	105	35

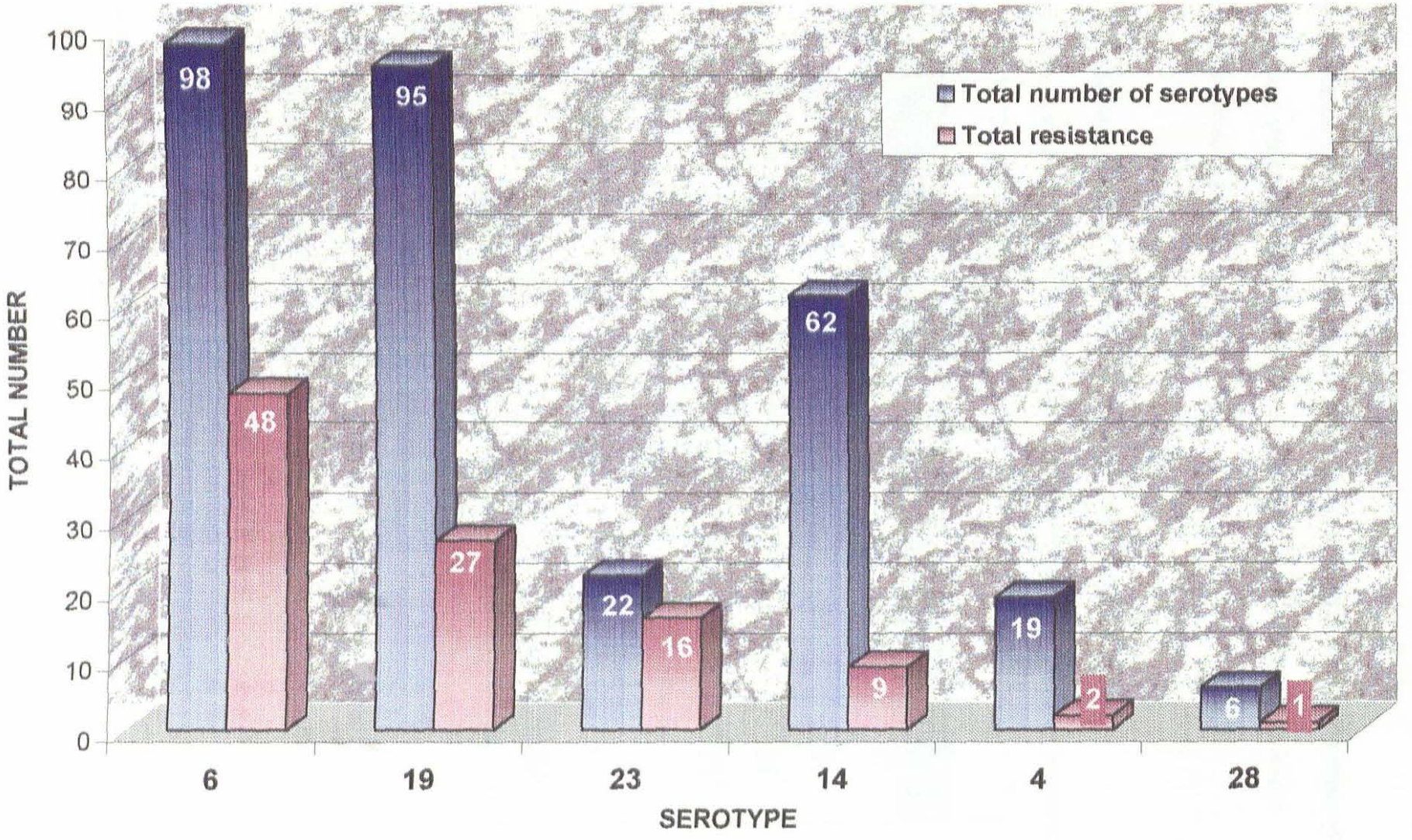
TS = Total no. of serotypes

TR= Total resistance

%TR = Total % resistance for all serotypes

When comparing each serotype against the others there is no significant difference in the resistance to penicillin except when comparing serotype 6 against all the other serotypes then there is a significantly higher resistance to penicillin in serotype 6 than 14 (Figure 10).

Figure 10 Serotype distribution of penicillin resistant pneumococci



Multi drug resistance was seen in this study. Table 3.19 shows the resistance rates of the pneumococcal strains to various antimicrobials that were resistant, intermediate resistant and sensitive penicillin.

Table 3.19 Resistant rates to various antimicrobials of *Streptococcus pneumoniae* that were resistant, intermediate resistant and sensitive to penicillin.

	PENICILLIN			
ANTIMICROBIAL	HR	IR	S	TOTAL
CHLORAMPHENICOL	9	12	2	23
TETRACYCLINES	9	15	28	52
ERYTHROMYCIN	1	6	17	24
CLINDAMYCIN	1	6	26	33
RIFAMPICIN	5	10	11	26
COTRIMOXAZOLE	12	23	60	95
TOTAL	37	72	144	253

3.2.2. VARIOUS ANTIMICROBIALS

A summary of the interpretation of the MIC for chloramphenicol, tetracycline, erythromycin, clindamycin, rifampicn, ceftriaxone, cotrimoxazole and vancomycin according to age groups are shown in Table 3.20. Overall there is a higher number of resistance isolates in children than adults to the various antimicrobials, with cotrimoxazole, chloramphenicol and tetracycline significantly higher.

Table 3.20: Susceptibility patterns for various antimicrobials except penicillin according to age groups.

ANTIMICROBIAL		CHILDREN		ADULTS		P-VALUE
		NO.	%	NO.	%	
CHLORAMPHENICOL	R	22	8	1	0.4	<0.001
	S	242	92	241	99.6	
TETRACYCLINE	R	41	15.5	11	4.5	<0.001
	S	223	85.5	231	95.5	
ERYTHROMYCIN	R	18	7	6	2	0.017
	S	236	93	236	98	
CLINDAMYCIN	R	24	9	9	4	0.014
	S	240	91	233	96	
RIFAMPICIN	R	21	8	5	2	0.002
	S	243	92	237	98	
CEFTRIAXONE	R	0	0	0	0	-
	S	264	100	242	100	
COTRIMOXAZOLE	R	71	27	24	10	<0.001
	S	193	73	218	90	
VANCOMYCIN	R	0	0	0	0	-
	S	264	100	242	100	

A significantly higher resistance rate occurs in children for all the agents except for ceftriaxone and vancomycin.

Table 3.21 shows the susceptibility patterns for various antimicrobials according to invasive and non-invasive groups. There is a significantly higher resistance to chloramphenicol and cotrimoxazole in the non- invasive group.

Table 3.21: Susceptibility patterns for various antimicrobials except penicillin according to invasive and non-invasive groups except penicillin.

ANTIMICROBIAL		INVASIVE		NON- INVASIVE		P-VALUE
		NO.	%	NO.	%	
CHLORAMPHENICOL	R	5	2	18	6	0.023
	S	221	98	260	94	
TETRACYCLINE	R	19	8	33	12	0.204
	S	207	92	245	88	
ERYTHROMYCIN	R	7	3	17	6	0.114
	S	219	97	261	94	
CLINDAMYCIN	R	11	5	22	8	0.176
	S	215	95	256	92	
RIFAMPICIN	R	7	3	19	7	0.059
	S	219	97	259	93	
CEFTRIAXONE	R	0	0	0	0	-
	S	226	100	278	100	
COTRIMOXAZOLE	R	32	14	62	22	0.014
	S	194	86	216	78	
VANCOMYCIN	R	0	0	0	0	-
	S	226	100	278	100	

The number and percentage resistance to various antimicrobials according to serotype is shown in Tables 3.22 and 3.23

Table 3.22: Number of serotypes resistant to various antimicrobials.

SEROTYPE	PG	C	T	E	CD	RIF	TS	TOTAL
6	48	4	11	9	9	4	24	98
19	27	4	5	3	6	7	26	95
14	9	2	13	8	10	1	18	62
1	0	0	4	0	4	2	4	45
3	0	0	1	0	0	1	0	24
18	0	0	0	0	0	0	1	25
23	16	13	15	2	0	8	10	22
4	2	0	1	1	1	0	1	19
7	0	0	0	0	0	1	1	14
15	0	0	0	0	0	0	2	14
5	0	0	1	1	1	0	3	11
9	0	0	0	0	1	0	1	8
43	0	0	0	0	0	1	1	1

Total= total number of serotypes with MIC results.

Table 3.23: Percentage of serotypes resistant to various antimicrobials.

SEROTYPE	PG	C	T	E	CD	RIF	TS	TOTAL
6	49	4	11	9	9	4	24	98
19	28	4	5	3	6	7	27	95
14	15	3	21	13	16	2	29	62
1	0	0	9	0	9	4	9	45
3	0	0	4	0	0	4	0	24
18	0	0	0	0	0	0	4	25
23	73	59	68	9	0	36	45	22
4	11	0	5	5	5	0	5	19
7	0	0	0	0	0	7	7	14
15	0	0	0	0	0	0	14	14
5	0	0	9	9	9	0	27	11
9	0	0	0	0	13	0	13	8
43	0	0	0	0	0	100	100	1

Serotype 23,6,19 and 14 are the most commonly resistant types found in this study. When comparing serotype 23 to serotype 6,19 and 14 to the resistant rates of all the antimicrobials, there is a significant increase of resistance in serotype 23 compared to serotype 6 in the resistant rates to chloramphenicol and significantly an increase in the resistance to clindamycin in serotype 14 compared to serotype 23.

Table 3.24 shows all the penicillin resistant isolates that were all resistant to more than one other antimicrobial. Of the 105 isolates resistant to penicillin, 49% were resistant to one or more and 27% resistant to one or two and 21% resistant to three or four antimicrobials.

Table 3.24: Patterns of antimicrobials of 105 penicillin resistant pneumococcal strains.

ANTIMICROBIAL COMBINATIONS	NUMBER
PG,C	1
PG,T	1
PG,E	2
PG,CD	1
PG,RIF	3
PG,TS	13
PG,C,T	1
PG,RIF,TS	2
PG,CD,TS	1
PG,E,CD	2
PG,T,TS	1
PG,C,T,E	2
PG,C,T,TS	7
PG,C,T,RIF	2
PG,T,CD,TS	1
PG,C,T,RIF	1
PG,T,RIF,TS	1
PG,E,CD,TS	1
PG,C,T,RIF,TS	6
PG,C,T,E,TS	1
	51

CHAPTER 4

DISCUSSION

DISCUSSION

During this study 564 *Streptococcus pneumoniae* isolates were tested, 49% constituted non-invasive isolates and 50,5% invasive isolates. These isolates were obtained from both children (<14years old) and adults (>14 years old). The majority of isolates from children were isolated from children <1 year old (24%). Children under 5 constituted 45% of al isolates and in the adult age group the majority were isolated from patients between 31 and 60 years old (27%).

Of the 564 isolates obtained during this study, 502 isolates were serotyped. The most prevalent serotypes of *Streptococcus pneumoniae* isolated in this study were found to be 6,19,14,1,3 and 18. These serotypes constituted 74.6% of all isolates. The current 23 valent pneumococcal vaccine covers all these serotypes. As with other published studies world-wide serotypes differed in children and adults (Jette *et al* 1989; Hofman *et al* 1995; Michel *et al* 1983). The four most commonly isolated serotypes in this study from children were 6,19,14 and 23, which accounted for 71% of isolates and the five most frequently from adults was 19, 1, 6,3 and 14, which accounted for 59% of all adult serotypes. Significantly higher numbers of serotypes 6,14 and 23 were found in children than adults and significantly higher numbers of serotypes 1, 3 and 18 were found in adults than in children.

Serotype distribution may vary according to the source of the pneumococcus. In this study the most commonly isolated serotypes, in order of prevalence were 6,1.14,19 and 23 in the invasive group and serotypes 19.6,14.3,18 and 3 in the non - invasive group. The frequency of penicillin resistance among pneumococcal isolates in this study was 21%, only 3% HR strains. From the children, the rate was 33% resistance to

penicillin compared to 8% in the adults. There were no highly resistance strains found in the isolates from the adults whereas 13 strains were found in children. A higher rate of penicillin resistance was seen in the non-invasive group of isolates than the invasive group. When comparing the specific source of the isolates, penicillin resistance was found to be highest in blood culture isolates (27%).

4.1 SEROTYPE DISTRIBUTION:

The results obtained in this study are comparable with results obtained in prior studies from Johannesburg and Cape Town (Jacobs *et al* 1978; Applebaum *et al* 1977; Friedland and Klugman 1992) and elsewhere (El Mouzan *et al* 1988; Hansman *et al* 1974; Jette *et al* 1989; Michel *et al* 1991; Lee *et al* 1995). Serotype distribution varies geographically. A recent article (1995) by Sniadack *et al*, who reported that the most commonly found serotypes in developed countries was 14,16,19,18,9,23,7,4,1 and 15 and in developing countries the order was 6,14,8.5,1,19,9,23,18,15 and 7. All the serotypes above were found in this study. In addition serotype 3 was found in 9% of adults in this study but not reported by Sniadack. This is illustrated by the difference in serotypes seen in Africa, where serotype 1,5 and 12 was seen commonly in a study performed in West Africa (El Mouzan *et al* 1988) whereas serotypes 5 and 12 were not encountered in large numbers in this study.

The most commonly found serotypes isolated from children in this study were the same as those isolated in Johannesburg where serotypes 6,14,19 and 23 constituted 70% of isolates (Friedland and Klugman 1992:Crewe Browne et al 1997). The prevalence of serotype 1 in children (6%) is consistent with the results obtained from a study performed in Johannesburg where 7% of isolates from invasive isolates from children

were also serotype 1 (Friedland and Klugman 1992). The predominant serotypes found in this study in adults were 19,1,6,3,14 and 8. Serotype distribution in children and adults when compared geographically world wide vary. For instance in Saudi Arabia serotypes 6 and 19 were the most commonly found in children (El Mouzan *et al* 1988) and in Alabama, USA the most common serotypes were 6, 14,19 and 23 (Orange and Grag 1993), which is consistent with results obtained in this study whereas in other countries such as Bangladesh and Uraguay there are distinct differences in serotypes isolated from children. Serotypes 7,12,114,15,4,23,18,5 and 22 constituted 70% of isolates reported by Sasha *et al* (1997) from children in Bangladesh and Mogdasy *et al* (1992) reported that serotypes 14,5,9,3 and 1 were most commonly found in Uruguayan children. The serotypes most commonly found in adults were similar to studies performed in Quebec, Canada (Jette *et al* 1989). The difference in the distribution between children and adults is probably due to the ability to respond to different polysaccharide antigens, which varies with age (Nielsen and Hendrichsen 1992). Serotypes vary according to the source from which they are isolated. Serotype 6 constituted 21% of all invasive isolates and 18% of all non-invasive types. Serotype 19 constituted 28% of all non-invasive disease and only 8% of invasive disease. Serotype 1 was seen in only 2% of non-invasive disease whereas it made up 17% of isolates in the invasive group. Serotype 14 was evenly spread between the two groups, 11% invasive and 14% non-invasive. Invasive infections are caused by relatively small numbers of serotypes. Of interest is that in children, serotype 1 was found more commonly in the invasive group of isolates and serotype 19 in the non-invasive isolates, whereas in the adult population, serotypes 1 and 4 were more commonly isolated from

the invasive group and serotype 19 more prevalent in the non-invasive group of isolates. In the invasive group of isolates serotypes 6,14 and 23 were more prevalent in children than adults and serotype 3 more prevalent in adults than children. There was no significant difference in the serotypes in the non-invasive group of isolates from children and adults.

4.2 ANTIMICROBIALS:

4.2.1 Penicillin

Results obtained in this study are consistent with prior reports by Friedland and Klugman (1992) where the HR to penicillin was 3,5% and IR 41,2% in South African children, as well as with results obtained from 1986-1990 at Red Cross Childrens Hospital, where the incidence of penicillin resistance was 22% in children (Koornhof, Wasas and Klugman 1992) as well as consistent with results reported by Crewe-Brown *et al* (1997) from Johannesburg of 15,3% resistance to penicillin which included HR and IR from both children and adults. Recent unpublished data (1998) from the National Reference Centre (SAIMR, Johannesburg) showed an increase in resistance to penicillin from 17,8% in 1993 to 27% in 1997 from blood culture isolates and an increase from 24.8% in 1993 to 34.2% from pneumococci isolated from cerebrospinal fluid. Unpublished data received from the Red Cross Children's Hospital shows an increase from 34% in 1997 to 26% in 1999 from blood isolates and an increase from 40% to 52% for the same years from CSF isolates.

Penicillin resistance patterns vary throughout the world with high numbers of penicillin resistant strains seen in Spain where HR rates are reported to be 15% (Lataorre Ottin *et*

al 1988) and Korea where HR rates are 33% (Lee *et al* 1995), low numbers (1.3% IR) of resistant strains seen in Canada (Jette et al 1989), with resistant rates to HR penicillin of 7% and intermediate resistance is 18% been reported from Atlanta, USA (Hofman *et al* 1995), HR penicillin of 7,4% and IR to penicillin of 21% seen in Israel (Michel *et al* 1983) and in Austria 5,2% resistance and 0,3% HR is seen (Mittermayer *et al* 1996).

A significant difference was seen in the resistant rates to penicillin from children and adults in this study. This has been the case in a studies performed in Israel (Michel *et al* 1983), Spain (Lataorre Ottin *et al* 1988) and Korea (Lee *et al* 1995).

Although overall there was a higher resistance to penicillin in the non-invasive group compared to the invasive group of isolates, penicillin resistant rates in the invasive group would be clinically significant and this constituted 33% of all invasive isolates. Results obtained in this study coincide with reports from France where higher resistance rates to penicillin in non-invasive isolates were seen (Gelsin *et al* 1992). Penicillin resistance has been associated with 22 pneumococcal serotypes throughout the world (Lataorre Ottin *et al* 1988; Lee *et al* 1995; Michel *et al* 1983 ; Koornhof, Wasas and Klugman 1992). In this study, the serotype distribution shows that penicillin resistant isolates were associated with 6 different serotypes. It is of interest that there was a significant association of serotype 6 with intermediate and high resistant rates to penicillin in this study. Serotypes 6,19,23,14,4 and 28 included intermediate and high resistance, but serotypes 4 and 28 had intermediate resistance only. These serotypes associated with penicillin in this study are consistent with reports from South Africa and worldwide. Serotypes 6,9,14,19 and 23 were reported by Friedland and Klugman (1992) to be the most common serotypes resistant to penicillin in South African children. In

other countries worldwide such as France, serotypes 23,19,6,14 and 9 are associated with penicillin resistance (Gelsin *et al* 1992). In Canada, serotypes 14,6,9,23 are associated with IR penicillin (Jette *et al* 1989). In Israel, serotypes 6,14,3,19,15 are associated with resistant to HR penicillin (Michel *et al* 1983). In Australia and New Guinea serotypes 4,6,11,14,15,16,19,23,34 and 35 are associated with penicillin resistance (Hansman *et al* 1974). In Spain serotypes 6,15,19,23 are associated with penicillin resistance (Lataorre Ottin *et al* 1988). In Atlanta, USA, serotypes 14,6,9,4,23,19 are associated with penicillin resistance (Hofman *et al* 1995). In Pakistan serotype 19 is associated with IR to penicillin. In Uruguay serotypes 11,4,9 and 6 are associated with penicillin resistance (Mogdasy *et al* 1992). In Sao Paulo serotypes 14,23,3,6 and 19 are associated with penicillin resistance (Levin *et al* 1996) and Belgium, serotypes 23,19,14,9 and 6 are associated with penicillin resistance. Serotypes 9,3 and 15 have been reported to be associated with penicillin resistant from various countries worldwide such as Canada (Jette *et al* 1989), Israel (Michel *et al* 1983), Spain (Lataorre Ottin *et al* 1988), Atlanta, USA (Hofman *et al* 1995), Soa Paulo (Levin *et al* 1996) and Belgium (Verhaegen *et al* 1995). These three serotypes were not found to be resistant to penicillin in this study.

4.2.2 Chloramphenicol

The overall resistance rate for chloramphenicol in this study was 4.5%, with resistance rate in children 8% and adults 0,4%, which is statistically significant. It has been previously reported in South African children in Johannesburg that chloramphenicol resistance occurred in 8,8% of children (Klugman 1990) and in another report by Crewe

Brown *et al* 1997, which included isolates from both children and adults, where resistance to chloramphenicol was 3,1%, which is consistent with results obtained in this study. Chloramphenicol resistance rates are varied throughout the world with a high incidence of chloramphenicol resistance of 47.3 % reported in Spanish children (Lataorre Ottin *et al* 1988), 39% reported in invasive disease in Pakistani children (Mastro *et al* 1991), 29,7% resistance from invasive isolates in Eqypt (Ostroff *et al* 1996), 3% resistance was reported in children and adults both in Atlanta, USA (Hofman *et al* 1995) and Saudi Arabia (El Mouzan *et al* 1988), 1% reported in Austria (Mittermayer *et al* 1996) and no resistance has been reported in a number of studies from Australia, New Guinea (Hansman *et al* 1974), New Zealand (Hefferman 1987) and Israel (Michel *et al* 1983). Resistance to chloramphenicol and tetracycline in South Africa is less common than resistance to penicillin. In this study serotypes 6,14,19 and 23 were associated with chloramphenicol resistance, with 59% of serotype 23 strains being the most resistant.

4.2.3 Tetracyclines

The resistance rate for tetracyclines was 10% in this study which was the third highest rate of resistance compared to the other antimicrobials but is consistent with a report by Crewe-Brown *et al* 1997 from Johannesburg who found 9,4% resistance to tetracycline. The overall rate which includes all isolates, compares well with other studies performed world wide for instance in Atlanta, USA the resistance rate has been reported to be 8% (Hofman *et al* 1995). In Austria in a predominantly adult population the resistance rate was reported to be 8,5% (Mittermayer *et al* 1996). In Britain resistance rate is 13% (Ad-

hoc Study Group on Antimicrobial Resistance 1977). In New Zealand a low resistance rate of 3,9% (Hefferman 1987) was reported as well as in Quebec, Canada a low resistance of 1,7% to tetracyclines was seen (Jette *et al* 1989). Higher resistance rates of 25% have been reported in Saudi Arabia (El Mouzan *et al* 1988) and a high resistance rate of 32% was reported from Soa Paulo (Levin *et al* 1991). The resistance rate in children was 15,5% and adults 4,5% in this study. This is a significant difference. The resistance rates in children from this study compares well with reported resistance of 11,5% in South African children (Friedland and Klugman 1992). Higher resistance rates of 72,5% were reported from Spanish children (Lataorre Ottin *et al* 1988) and 80% from Pakistani children (Mastro *et al* 1991) compared with the results obtained in this study. Tetracycline resistant strains are usually associated with resistance to other antimicrobials. In this study of the tetracycline resistant strains, 9 strains were highly resistant to penicillin and 15 strains intermediately resistant to penicillin. 21 of the 22 multi drug resistant strains (4 or more resistant antimicrobials) were also resistant to tetracyclines. A number of serotypes were associated with tetracycline resistance in this study namely serotype 6, 19, 4, 23, 3, 4 and 5, with only one of each strain 3, 4 and 5 being resistant which correlates with some of the serotypes associated with low tetracycline resistance in Quebec, Canada (Jette *et al* 1989).

4.2.4 Erythromycin

The resistance rate for erythromycin was 5% in this study. The children showed a higher resistance rate (7%) than the adults (2%) . These results are similar to those reported by Crewe-Brown (1997) from Johannesburg, where resistance to erythromycin

was found to be 3,7% from isolates from children and adults.

The resistance rates worldwide vary, although generally a low resistance rate is seen. The resistance rates vary from no resistance seen in Pakistan (Mastro *et al* 1991), 0,4% in New Zealand (Hefferman 1987), 1,3% in Israel (Michel *et al* 1983), 2% in Austria, 2,5% in Eqypt (Ostroff *et al* 1996), 4% in Saudi Arabia (El Mouzan *et al* 1988) to higher resistant rates in France of 29% (Gelsin *et al* 1992). When comparing isolates from children the resistance rates are similar to those found in this study for instance 4,4% resistance is reported in South African children (Friedlanc' and Klugman1992) and 5,5% in Spanish children (Lataorre Ottin *et al* 1988). In this study serotypes 6,19,4,23,1 and 5 were associated with resistance to erythromycin. Serotypes 19,14 and 6 were reported by Verhaegen *et al* (1995) from Belgium to be associated with erythromycin resistance.

4.2.5 Clindamycin

Information concerning resistance rates to clindamycin is limited as resistance to erythromycin implies resistance to clindamycin. The resistance rate for clindamycin was 6.5% in this study. A higher resistance rate was found in children (9%) than in adults (4%). References show that in South African children the rate is 4,4% (Friedland and Klugman 1992), 0,9% in Israel which includes children and adults (Mic' .el *et al* 1991) and no resistance in Pakistani children (Mastro *et al* 1991). Serotypes associated with clindamycin in this study include types 6,19,14,1,4,5 and 9.

4.2.6 Rifampicin

The resistance rate for rifampicin was 5% in this study with children showing 8% resistance rate and adults 2%. These rates are similar to resistance rates reported by Crewe-Brown *et al* 1997 from Johannesburg of 3,1%. The resistance rate in children compares with a report of 7,1% resistance in South African children (Friedland and Klugman 1992) although no resistance in seen to rifampicin in Spanish (Lataorre Ottin *et al* 1988) or Pakistani (Mastro et al 1991) children. Serotypes associated with rifampicin resistance in this study were types 6,19,14,1,3,23,7 and 43.

4.2.7 Cotrimoxazole

The resistance rate for cotrimoxazole was 19% in this study with children showing resistant rate of 27% and adults 10%. Resistance rates vary throughout the world with reported rates of 67% in Spanish children (Lataorre Ottin *et al* 1988), 26% in Atlanta, USA (Hofman *et al* 1995), 65% in Saudi Arabia (El Mouzan *et al* 1988) 31% in Pakistani children (Mastro et al 1991), 8,7% in Israel (Michel *et al* 1983), 6,4% in New Zealand (Hefferman 1987), 3,3% in Austria (Mittermayer *et al* 1996) and 0,6% in Eqypt (Ostroff *et al* 1996). A number of serotypes were found to be associated with cotrimoxazole resistance with serotypes 6,19,14 and 23 being the most predominant.

4.2.8 Ceftriaxone

Although there are recent reports of cephalosporin resistant strains in children in Spain (Lataorre Ottin *et al* 1988) and South Africa, where an intermediate resistance rate of 1,5% to ceftriaxone was reported (Crewe Brown *et al* 1997) there was no resistance to ceftriaxone found in this study.

4.2.9 Vancomycin

There was no vancomycin recorded in this study, which is consistent with reports from Spain (Lataorre Ottin *et al* 1988) and Texas, USA (Mason *et al* 1992).

4.2.10 Multi drug resistance

Multi drug resistance was first reported in South Africa and is seen more commonly worldwide. In this study, of the 105 isolates resistant to penicillin 49% were resistant to one or more antimicrobials and 27% resistant to one or two and 21% resistant to three or four more antimicrobials. Crewe-Brown et al 1997 reported from Johannesburg that 5% of isolates are multi-drug resistant (3 different classes of antimicrobials) with serotypes 19, 23 and 14 associated with multi-drug resistance. Reports from countries such as Belgium show 52% of penicillin resistant strains resistant to one or more other drugs (Verhaegen *et al* 1995), Soa Paulo where 26% of isolates were resistant to two antimicrobials simultaneously (Levin *et al* 1996) and in USA where 12,6% of isolates were multi drug resistant with 5,3% resistant to three or more classes of antimicrobials and 1,4% to 4 or more classes.

CHAPTER 5.

CONCLUSIONS

CONCLUSIONS

The most commonly isolated serotypes found in this study were 6,19,14,1,3 and 18. The four most commonly isolated serotypes in this study from children were 6,19,14 and 23 and from adults 19, 1, 6,3 and 14. Serotype 6 was seen in significantly higher numbers from invasive isolates from children and this is a concern as serotype 6 is also associated with penicillin resistance. Serotype 1 was seen most frequently in the adult population from invasive isolates. The current vaccine (Pneumovax, MSD) consists of purified capsular polysaccharides of 23 pneumococcal serotypes that include the most commonly isolated serotypes in this study. Administration of this vaccine is usually restricted to high-risk groups such as adults > 65 years old and patients with disease such as sickle cell disease and Hodgkin's disease. This polysaccharide vaccine has not been promoted for use in children < 2 years since this vaccine is not immunogenic in this age group. The new conjugate vaccines, have been shown to be safe, immunogenic and effective and should be available to all children in a few years time, provided costs are reasonable.

Penicillin is still considered the drug of choice when treating pneumococcal infections. Since the 1960's resistance to penicillin and other antimicrobial agents has spread rapidly. The first reported case was in Australia in 1967, then cases were seen in New Guinea in 1969 and South Africa in 1977. With the increased rate of penicillin resistance seen both in South Africa and word wide. concerns have been raised regarding the treatment of diseases caused by *Streptococcus pneumoniae*. In this study we found that the rate of penicillin resistance among pneumococcal isolates in the Cape Peninsula was 21%, with a wide variation between the rates from children and adults,

33% and 8% respectively. There was no significant difference in the resistant rates when comparing non-invasive and invasive isolates. Although there are differences of opinion with regard to treatment of penicillin resistant isolates, most authors recognise that treatment with penicillin is effective unless the organism has a high level of resistance. Penicillin MIC's should therefore be performed on all pneumococci isolated from invasive sites to facilitate the clinician to determine treatment. In patients with meningitides it is recommended that a third-generation cephalosporin be used.

Penicillin resistance both HR and IR was most commonly associated with serotypes 6,19,23,14,4 and 28.

The resistant rates for the various antimicrobials tested in this study were tetracycline 10%, erythromycin 5%, clindamycin 6,5%, rifampicin 5%, cotrimoxazole19% and no resistance seen for ceftriaxone and vancomycin. A larger number of resistance isolates were encountered from children than from adults to the various antimicrobials with a significant difference seen with regard to resistance rates of chloramphenicol, tetracyclines and cotrimoxazole, which were higher in children than adults. Serotypes 6,19,14,1,3,18,23,4,7,5,9,53 were the types found to be resistant to the various antimicrobials tested.

As this study showed no resistance to ceftriaxone, this antimicrobial would be useful for treatment, but as there have been reported cases of cephalosporin resistance in Spain and South Africa, resistance of ceftriaxone should to be monitored. It is recommended that ceftriaxone MIC's are performed on all invasive isolates.

CHAPTER 6

REFERENCES

REFERENCES

Ad-hoc Study Group on Antimicrobial Resistance. Tetracycline resistance in pneumococci and Group A Streptococci. Br. Med. J.1977; 1:31-133.

Applebaum PC, Scragg JN, Bowen AJ, *et al.* *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. Lancet 1977; 995-997.

Appelbaum PC. World wide development of Antimicrobial resistance in pneumococci. Eur.J.Clin. Microbiol. 1987; 367-377.

Austrian R . Some aspects of the pneumococal carrier state. J. Am. Chem. 1986; 18, suppl. A. 35-45.

Austrian R. Pneumococcus: The Frist One Hundred Years. Rev. Infect. Dis.1981;Vol3:2:183-189.

Balitomore RS and Shapiro ED. Bacterial infections of Humans 2nd edition. Edited by AF Evans and PS Brachman. Plenum Medical book company, New York. 1991.

Butler J, Hofmann J, Cetron M *et al.* The continued emergence of drug resistant *Streptococcus pneumoniae* in the United States: an Update from the Centre of Disease Control and prevention's pneumococcal sentinel surveillance system. J. Infect. Dis. 1996;174:986-93.

Centers for Disease control, Recommendations of the Immunization Practices AdvisoryCommittee (ACIP): Pnuemococcal polysaccharide vaccine, Morbid.Mortal.Weekly Rep. 1981; 30:410-419.

Cherian T, Steinhoff MC, Harrison LH, *et al.* A cluster of invasive pneumococcal disease in young children in child care. JAMA.1994; 271:9: 695-967.

Cole K , Sniffen J and Nadler P. Prevention and Treatment of Pneumococcal Disease: Resistance and Vaccines. 39th Interscience conference on Antimicrobial Agents and Chemotherapy. September 29, 1999.

Crewe Browne H, Karstaedt A, Saunders G *et al.* *Streptococcus pneumoniae* Blood Culture Isolates from patients with and without Human Immmunodeficiency Virus Infection: Alterations in Penicillin Susceptibilities and in Serogroups or Serotypes. Clin. Infect. Dis.1997;25:1165-72.

Dagan R, Englehard D, Piccard E, *et al.* Epidemiology of invasive childhood pneumococcal infections in Israel. JAMA. 1992;268:3328-3332.

Dagan R, Isaachson M, Lang R *et al.* Epidemiology of pediatric meningitis caused by *Haemophilus influenze type b, Streptococcus pneumoniae*, and *Neisseria meningitidis* in Israel: A 3-year nationwide prospective study. J. Infec. Dis. 1994;169:912-6.

El-Mouzan MI, Twan-Danso K, Al-Awamy BH, *et al.* Pneumococcal infections in Eastern Saudi Arabia: serotypes and antimicrobial sensitivity patterns. Trop. Geogr. Med. 1988;40:213-17.

Eskola J, Takala AT, Kela E, *et al.* Epidemiology of invasive pneumococcal infectionsin children in Finland. JAMA. 1992;268:3323-3327.

Eskola J. Pneumococcal conjugate vaccines. Pediatr. Infect. Dis.1999,18: 543-51.

Friedland IR and Klugman K. Antimicrobial-resistant pneumococcal disease in South African Children. AJDC. 1992;Vol-146:920-923.

Gable CB, Holzer SS, Engelhart L *et al.* Pneumococcal Vaccine. Efficacy and Associated Cost Savings. JAMA.1990;Vol2264, No.22: 2910-2915.

Gelsin P, Buu-Hoi A, Fremaux A and Acar JF. Antimicrobial resistance to *Streptococcus pneumoniae*: An epidemiological survey in France, 1970-1990. Clin. Infect. Dis.1992;15:95-98.

Hansman D and Bullen MM. A resistant pneumococcus. Lancet ii.1967;264-265.

Hansman D. Chloramphenicol-resistant pneumococci in West Africa. Lancet . 1978;1:1102-1103.

Hansman D. Devitt L, Miles H and Riley I. Pneumococci relatively insensitive to penicillin in Australia and New Guinea. Med. J. Aust. 1974;2:353-356.

Hefferman H. Antimicrobial susceptibility of clinically-significant *Streptococcus pneumoniae* isolates. N. Z. Med. J. 1987;100:327.

Hofman JO, Cetron MS, Farley MM, *et al.* The prevelence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N. Engl. J. Med. 1995; 333: 481-486.

Hussey G, Coetzee G, Hitchcock J, Van Schalkwyk E, *et al.* Carriage of *Streptococcus pneumoniae* in Cape Town Children. S. A. J. Epidemiol. Infect. 1993;8:106-108.

Jacobs MR, Koornhof HJ, Robins Browne RM, *et al.* Emergence of multiple resistant pneumococci. N. Engl. J. Med. 1978;299:735-740.

Janoff EN, O Brien J, Thompson PP *et al.* *Streptococcus pneumoniae* Colonization, Bacteremia, and Immune Response among Persons with Human Immunodeficiency Virus Infection. J. Infect. Dis. 1993;167:49-56.

Jette LP, Lamothe F and the Pneumococcus study group. Surveillance of invasive *Streptococcus pneumoniae* infections in Quebec, Canada, from 1984 to 1986: serotype distribution, antimicrobial susceptibility, and clinical characteristics. J. Clin. Microbiol. 989;27:1-5.

Joklik , Willet, Amos and Wilfert: Zinser Microbiology. 1988. 19 th Ed. Published by Prentice Hall International.

Jones N, Heubner R, Khoosal M *et al.* The impact of HIV on *Streptococcus pneumoniae* bacteraemia in a South African population. AIDS 1998, 12:2177-2184.

Jorgensen J, Cleeland R, Craig W, *et al.* National Committee for Clinical Laboratory Standards. 1991;11: Table 2 and Table 7.

Jorgensen J, Cleeland R, Craig W, *et al.* National Committee for Clinical Laboratory Standards. 1994;Vol 15 No 16.

Klugman K. Pneumococcal resistance to antimicrobials. Clin. Microbiol. Rev. 1990;3:171-196.

Koornhof J, Wasas A and Klugman K. Antimicrobial resistance in *Streptococcus pneumoniae:* A South African perspective. Clin Infect. Dis 1992;15:84-94.

Lataorre Ottin C, Juncosa-Marros T and Sanfeliu Sala I. Antimicrob al susceptibility of *Streptococcus pneumoniae* isolates from paediatric patients. J. Am. Chem. 1988;22:659-665.

Lee HJ, Park JY, Jang SH *et al.* High incidence of resistance to multiple antimicrobials in clinical isolates of *Streptococcus pneumoniae* from a University hospital in Korea. Clin. Infect. Dis.1995;20:826-35.

Levin A, Teiseira L, Sessegolo and Barone A. Resistance of *Streptococcus pneumoniae* to antimicrobials in Sao Paulo, Brazil: Clinical features and serotypes. Rev. Inst.Med. Trop. S.Paulo 1996;38 (3):187-192.

MacGowan A, Brown N, Holt H *et al.* An eight year survey of the antimicrobial susceptibility patterns of 85,971 bacteria isolated from patients in a district general hospital and the local community. J Am. Chem.1993;31:543-557.

Mason EOJR, Kaplan SL, Lamberth L, *et al.* Increased rate of isolation of penicillin-resistant *Streptococcus pneumoniae* in a children's hospital and in vitro susceptibilities to antimicrobials of potential therapeutic use. Antimicro. Agents Chemother.1992;36:1703-7.

Mastro T, Ghafoor A, Nomani MK, *et al.* Antimicrobial resistance of pneumococci in children with acute lower respiratory tract infections in Pakistan. Lancet.1991;337:156-159.

Michel J, Dickman D, Greenberg Z and Bergner-Rabinowitz S. Serotype distribution of penicillin-resistant pneumococci and their susceptibilities to seven antimicrobial agents. Antimicro. Agents Chemother. 1983;23:397-401.

Mittermayer H, Jebelean C, Binder *et al.* Antimicrobial susceptibility of pneumococcal isolated in Austria over a four year period. Eur.J.Clin Microbiol. Infect. Dis. 1996; Vol.15: 817-820.

Mogdasy M, Camou T, Fajardo C and Hortal M. Colonizing and invasive strains of *Streptococcus pneumoniae* in Uruguayan children: type distribution and patterns of antimicrobial resistance. Pediatr. Infect. Dis. 1992;11:648-52.

Nielsen SV and Hendrichsen J. Capsular Types of *Streptococcus pneumoniae* isolated from blood and CSF during 1982-1987. Clin. Infect. Dis. 1992;15:794-8.

Orange M and Grag BM. Pneumococcal serotypes causing disease in children in Alabama. Ped. Infect. Dis. J. 1993;12:244-245.

Ostroff S, Harrison L, Khallaf N *et al.* Resistance Patterns of *Streptococcus pneumoniae* and Haemophilus influenzae isolates recovered in Egypt from children with pneumonia. Clin. Infect. Dis. 1996;23:1069-74.

Pallers R, Linares J and Vadillo M. Resistance to Penicillin and Cephalosporin and mortality form severe pneumococcal pneumonia in Barcelona, Spain. N. Engl. J. Med. 1995; Vol 333 No 8, 474-480.

Pneumovax 23. Pneumococal Vaccine Polyvalent. Merck, Sharp and Dohme.

Radetsky M, Johansen T, Lauer B *et al*, Multi resistant pneumococcus causing meningitis: Its epidemiology within a day care centre. The Lancet.1981;771-773.

Rennels M, Edwards K, Keyserling H *et al.* Safety and Immunogenicity of Heptavalent Pneumococcal Vaccine conjugated to CRM_{197} in United States Infants. Pediatrics Vol . 101 April 1998: 604-611.

Rusen ID, Fraser-Roberts L, Slaney L *et al.* Nasopharyngeal pneumococcal colonization among Kenyan children: antimicrobial resistance, strain types and associations with human immunodeficiency virus type 1 infection. Pediatr. Infect. Dis. 1997;16:656-62.

Saha S, Rikitomi N, Biswas D, *et al.* Serotypes of *Streptococcus pneumoniae* causing invasive childhood infections in Bangladesh, 1992-1995. J. Clin. Microbiol. 1997;785-787.

Shann F. Modern Vaccines: pneumococcus and influenza. Lancet 1990; 335:898-901.

Shapiro E and Austrian R. Serotypes responsible for invasive *Streptococcus pneumoniae* infections amoung children in Connecticut. J. Infect. Dis. 1994;169:212-4.

Sniadack DH, Schwartz B, Lipman H, *et al.* Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children-implications for vaccine strategies. Pediatr. Infect. Dis. J.1995;14:503-10

Tarpay MM, Welch DF, Salari H and Marks MI. In vitro activity of antimicrobials commonly used in the treatment of otitis media against *Streptococcus pneumoniae* isolates with different susceptibilities to penicillin. Antimicrob. Agents. Chemother. 1982;22:145-147.

Usen S, Adegbola R, Mulholland K *et al.* Epidemiology of invasive pneumococcal disease in the Western Region, The Gambia. Pediatr. Infect. Dis.1998;17:23-28.

Verhaegen J, Glupczynski Y, Verbist L *et al.* Capsular types and antimicrobial susceptibility of pneumococci isolated from patients in Belgium with serious infections, 1980-1993.Clin. Infect. Dis. 1995;20:1339-1345.