upper respiratory tract of 30% - 60% of preschool children and nearly 20% of adults, in the form of nasopharyngeal carriers[8]. Its spread is enhanced by upper respiratory tract infection and crowding. Pneumococcal pneumonia develops when local or general immune defenses of the host are impaired. Most cases are endogenous following aspiration of oral secretions containing normal flora that includes *S pneumoniae*. Person-to-person transmission during epidemics occurs by droplet aerosols. The major virulence factor of *S pneumoniae* is its antiphagocytic polysaccharide capsule, and strains with a thick mucoid capsule such as type 3 are especially virulent[8].

To protect against infection by pneumococci, capsular polysaccharide type vaccines are now available, while conjugate polysaccharide vaccines are being developed for use in infants and young children[9].

The World Health Organization (WHO) recommends in addition to penicillin the use of cotrimoxazole (trimethoprim/ sulfamethoxazole) for the treatment of pneumonia in developing countries, mainly because of cost considerations and its spectrum of activity, but also because this combination is available in oral form. Unfortunately cotrimoxazole resistance in pneumococci has become very common in many countries, including South Africa[4].

S pneumoniae colonises the upper respiratory tract. In developing countries and elsewhere 30-60% of children are nasopharyngeal carriers at any one time[8]. Colonization is more common in childhood, in the elderly, and in those living in closed communities such as hospitals, nursing houses, day-care centres and other restricted communities[8].

S pneumoniae attaches itself to human pharyngeal cells through the specific interaction of bacterial surface adhesins and epithelial cell receptors. Epithelial cell glycoconjugates containing the disaccharide glucose-N-acetyl-β1-4-galactose (Glc NAc β1 - 4Gal) are thought to serve as the binding site of the bacterium[10]. The nature of the bacterial adhesin is yet to be characterized but cell wall components adhere to human endothelial cells[11].

CHAPTER 1 INTRODUCTION

1.1 World problem of pneumococcal disease and antibiotic resistance

Pneumococci were first described to be fully resistant to penicillin and resistant to multiple antibiotics in South Africa in Durban in 1977[1] and Johannesburg in 1978[2]. These resistant organisms have been shown to create major problems in the treatment of pneumococcal meningitis[3]. Studies over the last 15 years have shown that these strains are now also prevalent in many countries in Africa, Asia, Europe and America[4]. In pneumococcal pneumonia, penicillin or ß-lactam resistance is not of great significance as antimicrobial levels obtained in blood and lung tissues considerably exceed the minimal inhibitory concentrations (MIC's) of these agents. However, in the treatment of pneumococcal meningitis penicillin resistance is very important where both intermediate resistance with penicillin MICs ranging between 0.1 - 1.0 ug/ml and full resistance with MICs of > 1ug/ml are very likely to affect the outcome of pneumococcal meningitis if treated with penicillin, ampicillin or amoxycillin[4]. At the same time strains with high-level penicillin resistance may exhibit intermediate or high-level resistance to the third generation cephalosporins, ceftriaxone and cefotaxime[5]. As these cephalosporins are commonly used in the empiric treatment of meningitis, the prevalence of high-level penicillin resistance may also compromise the usefulness of these agents and treatment with vancomycin, ideally in combination with ceftriaxone or cefotaxime may have to be resorted to[6]

Very few data are available on antimicrobial resistance in the pneumococcus from Africa, with the exception of South Africa. Worldwide, approximately 10 million children under five years of age die from preventable diseases. The majority of these (96%) occur in developing countries. According to the World Health Organisation (WHO), 4 million (30%) of these are due to acute respiratory tract infections[7].

Oropharyngeal carriage of pneumococci is common and contributes to the difficulty in interpreting the significance of pneumococci in cultures of expectorated sputum. *S pneumoniae* is present in the

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evidence of pneumonia, indicating that a positive pneumococcal culture from the nasopharynx in this study group had a 92% predictive of radiologically confirmed pneumonia. Of the 128 nasopharyngeal carriers, 33.6% yielded strains that were fully susceptible to all antibiotics tested, leaving 85 (66.4%) isolates that were resistant to at least one antibiotic. Sixty-seven strains (52%) were resistant to penicillin while 28%, 18% and 9.4% of isolates were resistant to cotrimoxozole, tetracycline and chloramphenicol, respectively. Approximately ⅓ of strains were mono-resistant, i e, resistant to only one of the tested antibiotics, while the same proportion of isolates were resistant to more than 1 of the antibiotics tested. Antibiotic-resistant pneumococci were significantly more common in children under 3 years of age as well as in children from lower socio-economic surroundings as evidenced by domicile in straw huts as opposed to cement houses. The most prevalent serotype was 19F (47%), followed by serotype 14 (13%) and then by serotype 23F (7%).

The largest numbers of children with pneumonia came from Polana Canico, an area of very low socio-economic status, and Matola suburbs. Of the 51 children from the former suburb, 36 (71%) were carriers and of these 31 (86%) were resistant to one or more antibiotics. The vast majority of these resistant strains belonged to serotype 19F.

There was an excellent correlation with regard to the serotype and antibiotic resistance patterns of nasopharyngeal pneumococcal cultures compared with blood culture isolates from the same patients. Seventeen of the 22 available blood culture isolates (72%) were resistant to penicillin. Of these 13 belonged to serotype 19F including 5 strains that were, in addition, resistant to cotrimoxazole. Many of the children examined 6 weeks after discharge from hospital have lost their nasopharyngeal pneumococci and 67% of those that remained were resistant to antibiotics.

The high rate of penicillin resistance in Maputo children suggests that penicillin and chloramphenicol treatment of meningitis caused by *S pneumoniae* would be inappropriate but that penicillin should still be highly efficient for the treatment of pneumococcal pneumonia, because of high levels of penicillin achieved in the lungs of patients. The relatively high rate (28%) of cotrimoxazole resistance is a matter of concern as this antimicrobial combination is recommended for the treatment of pneumonia in developing countries.

ABSTRACT

This research report represents a study to determine the prevalence of antibiotic-resistance of pneumococci and their serotypes in Maputo, Mozambique in children under 5 years of age, who were admitted with pneumonia in the Paediatric Unit at the Hospital Central de Maputo. The children included in the study were from suburbs surrounding central Maputo.

Pneumonia remains a common and important problem worldwide; acute respiratory infections (ARI) are extremely common, particularly in children. ARI constitute the only group of infectious diseases ranking as causes of death among the top 3 in developing countries, as well as the top 10 in First World countries.

For the study, medical and nursing staff were trained in the appropriate collection of specimens. A technologist spent 2 weeks at The South African Institute for Medical Research (SAIMR) to acquire experience in modern laboratory methodologies relating to the proposed study. There was constant communication between the Maputo and SAIMR laboratories.

Objectives of the study included an assessment of the nasopharyngeal carriage rate of *Streptococcus pneumoniae* in children under 5 years of age hospitalized for pneumonia at the Hospital Central de Maputo. Secondly the study was geared to determine the antimicrobial resistance patterns of pneumococcal isolates by the disc diffusion and minimum inhibitory concentration (MIC) methods and finally to establish the serotypes of all the strains isolated in the study. Blood cultures were also performed on all the children admitted to the study and the serotypes and antibiotic resistance patterns of these isolates were compared with those from the nasopharynx of the same children. Nasopharyngeal carriers were, where possible, followed up 6 weeks after discharge from hospital to assess possible effects of hospitalization and antibiotic treatment on nasopharyngeal pneumococci. Four hundred children under five years of age who presented with pneumonia and other entry criteria were included in the study.

Streptococcus pneumoniae was isolated from the nasopharynx of 128 (32%) of the children. An X-ray examination was performed on 122 of the 128 nasopharyngeal culture positive children. One-hundred-and-twelve of the 122 culture-positive children who were X-rayed showed radiological

ACKNOWLEDGEMENTS

I would like to thank Professor Keith Klugman, Director of the South African Institute for Medical Research, Johannesburg, for initiating the project and for allowing me to study and complete my MSc (Med) degree during my study and training at the SAIMR. I also appreciate greatly all the ities he made available to me during the period of my study.

In particular I would like to express my greatest thanks to Professor H J Koornhof, Head of the Department of Medical Microbiology at the SAIMR, my tutor. He has the greatest patience, devout dedication and spent hours with me on my study, constantly revising my Report and advising me. The benefits that I have gained were all due to his great experience, insight and knowledge, which have been an inspiration to me.

I would also like to thank and appreciate the help given to me by Mrs Olinda de Gouveia, Senior Microbiology technologist, Department Microbiology SAIMR. I have enjoyed and appreciated the time, patience, effort she has spent with me and her assistance with translating and typing.

I would like to thank Mrs Avril Wasas for her collaboration and assistance with laboratory investigations. I am also grateful to my colleagues at the SAIMR for their cooperation and in particular, Mrs L Battaglia (Chief Librarian) and her staff.

My greatest thanks to Mr Andre Manhica, Principal Technologist of the Microbiology Sector of the Hospital Central de Maputo, for his great laboratory skills and the endless hours he spent assisting me with the laboratory investigations.

Also, a special thanks to all the staff in the Paediatric Intensive Care Unit, especially Dr Helena Ferreira, Paediatrician at the Hospital Central de Maputo.

Finally to my husband Armindo and my children for their understanding and patience. Also to my mother for her understanding and for looking after my family during the many periods of absence which I spent away from home, in Johannesburg.

ETHICS

Ethical approval for the study was obtained from the Human Ethics Committee from the University of the Witwatersrand and the appropriate local committee in Maputo.

FUNDING

Funding for this project was allocated from the research funds of the MRC Pneumococcal Research Unit, Department of the Medical Microbiology, South African Institute for Medical Research (SAIMR) and University of the Witwatersrand.

DECLARATION

I declare that this research report is my own, unaided work. It is being submitted for the degree of MSc (Med) in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____

H Elizabeth M R Coelho Hamene

February 1998

February 1998

Horacia Elizabeth Maria Rodrigues Coelho Hamene

STUDY TO DETERMINE PREVALENCE OF ANTIBIOTIC-RESISTANT PNEUMOCOCCI IN MAPUTO, MOZAMBIQUE

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MSc (MED) RESEARCH REPORT
UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
FEBRUARY 1998

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STUDY TO DETERMINE PREVALENCE OF ANTIBIOTIC-RESISTANT PNEUMOCOCCI IN MAPUTO, MOZAMBIQUE

Horacia Elizabeth M R Coelho Hamene

Supervisor: Professor H J Koornhof

The study was done in collaboration with Professor K P Klugman[1], Dr M Julien[2] and Mr M Manhiça[3]

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree MSc (Med) by coursework.

JOHANNESBURG

HOSPITAL CENTRAL DE MAPUTO

A. DEMOGRAPHIC DATA

PATIENT NAME_____ PATIENT NO _____ ORDER NO_____

AGE____ SEX____ HEIGHT_____ WEIGHT_____ TRIBE_____ REGION_____

NO. OF CHILDREN IN FAMILY UNDER 5 YEARS OF AGE _____

B. HOSPITALIZATION DATA

	DATE	NASO PHARYNGEAL SWAB TAKEN	
ADMISSION TO HOSPITAL	_____	YES	NO
ADMISSION TO WARD	_____		
DISCHARGE FROM HOSPITAL	_____	YES	NO
DURATION OF HOSPITAL STAY	_____	DAYS	

C. ANTIBIOTIC DATA

	ANTIBIOTIC	DURATION
BEFORE ADMISSION	_____	_____DAYS
IN HOSPITAL	_____	_____DAYS
		_____DAYS
		_____DAYS
		_____DAYS

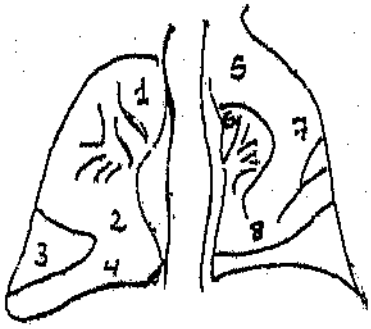
D. CLINICAL DATA

CLINICAL DATA	DAY 1 REPORT (Admission)	DAY 2 REPORT (Discharge)	DAY 3 REPORT (6 week)
WHITE CELL COUNT			
CHEST X - RAY			
BLOOD CULTURE			
NASOPARYNGEAL SWAB CULTURE			

Lung localization 1 - 8

Type

1 = infiltrated
2 = opaque
3 = abscess



X-ray result:

.....

E. MICROBIOLOGICAL DATA (SUMMARY)

SPECIMEN	PNEUMOCOCCI PRESENT YES/NO	SEROTYPE	RESISTANCE DATA DISC	MIC ug/ml	E-TEST ug/ml
Swab 1					
Swab 2					
Swab 3					
Blood culture					

S = susceptible R = resistant I = intermediate
P = penicillin C = chloramphenicol T = tetracycline E = erythromycin Cl = clindamycin
R = rifampicin Cx = cotrimoxazole

2.5 Assessment of clinical presentations of children with pneumonia

On arrival in the hospital, the accompanying parent/guardian was asked the following questions:

Patient's name, age, sex, tribe, region in which the child resides, the type of habitation and the number of children under the age of 5 years in the household and whether the child was on any medication. The accompanying forms (page 13 & 14) were completed for each new patient. Informed consent was obtained for the child's entry into the study.

The clinical examination consisted of:

- Measuring of the childs' temperature axillary or oral
- chest auscultation was established on each child to determine the respiratory rate and respiratory sounds (fine crepitus not cleared by coughing and/or bronchial breathing).
- the child was examined for the presence or absence of intercostal retraction
- the childs pulse rate was determined
- an X - Ray examination of the chest was performed on each of the children
- the child was then weighed and its height was determined.

Monitoring of patients in hospital comprised:

- Temperature (axillary), 6- ourly
- pulse and respiratory rate, 6-hourly
- chest auscultation morning and afternoon
- presence or absence of chest (intercostal) retraction morning and afternoon.

children) and Alto Mae (30 children).

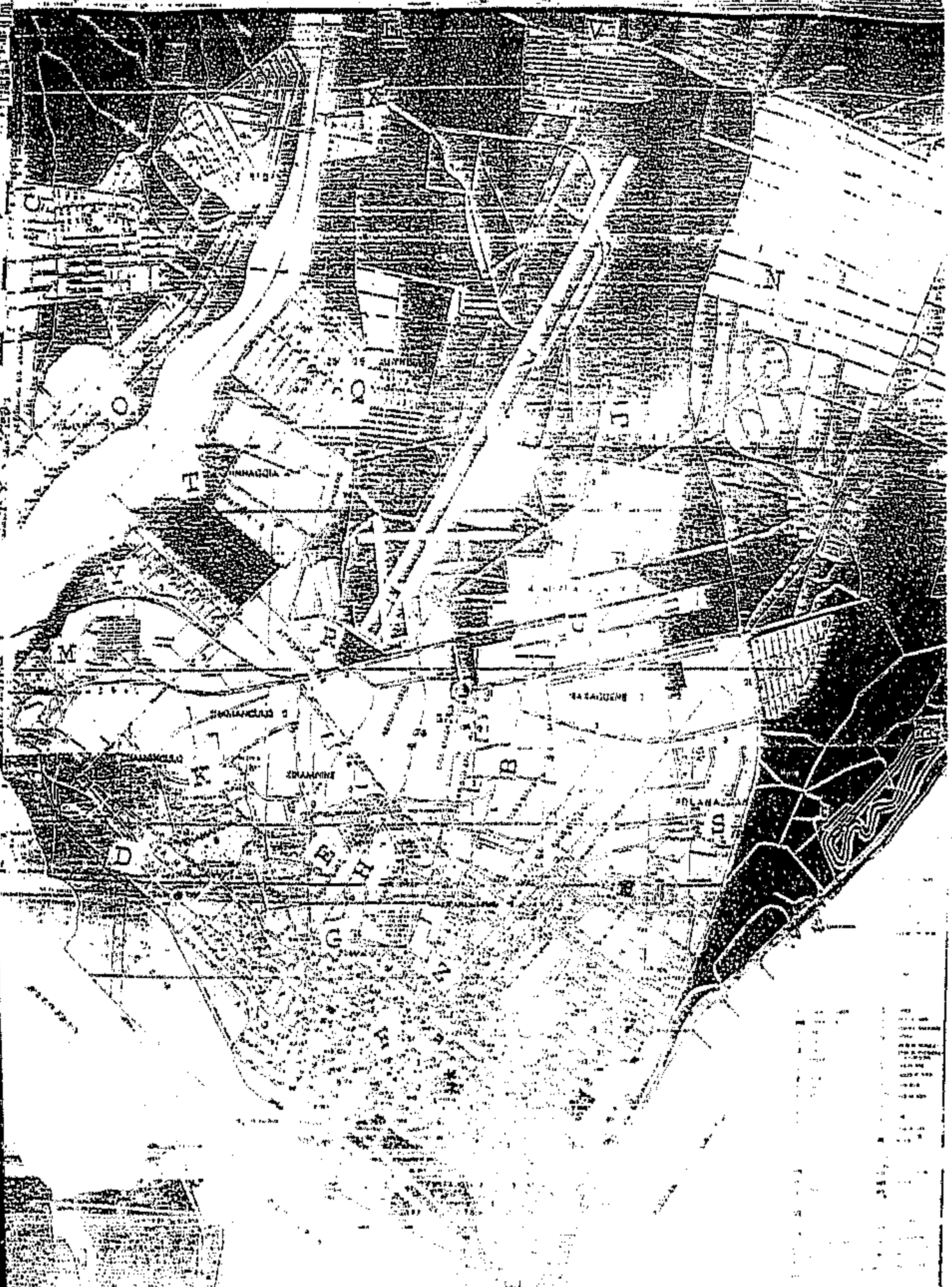
TABLE 2.1 Numbers Of Patients Residing In The Different Suburbs Of Maputo

Zone	*Suburb*	*Number of patients*
A	BAIRRO FERROVIARIO	7
B	MAXAQUENE	15
C	MATOLA	45
D	MALANGA	17
E	MKADGUINE	9
F	BAIRRO CENTRAL	12
G	ALTO MAE	30
H	MAFALALA	4
I	MATUTUINE	3
J	HULENE	7
K	CHAMANCULO	15
L	MICHAFUTINE	7
M	PATRICE LUMUMBA	10
N	LAULANE	5
O	INFULENE	7
P	COSTA DO SOL	9
Q	25 DE JUNHO	5
R	AERPORTO	6
S	POLANA CANICO	51
T	NHAGOIA	3
U	MAVALANE	11
V	DJAVELA	2
W	POLANA CIMENTO*	5
X	BENFICA	4
Y	JARDIM	5
Z	MALHANGALENE	7
Unknown or outside of Maputo		99
TOTAL		400

* Hospital Central de Maputo in this suburb

From Table 2.1 it is clear that most cases came from Polana Canico (51 children), Matola (45

MAP



Respiratory sounds - Fine crepitus not cleared by coughing and/or bronchial breathing.
Chest X-ray on admission
Routine investigations on admission

Total white cell count (WCC)
Blood cultures
Nasopharyngeal swabs for culture

Age, sex, antibiotic exposure, reason for hospitalization and duration of hospitalization of each individual child were determined and recorded.

2.3 Discharge criteria

Absence of fever for at least 24 hours
Ability to feed by mouth
Absence of chest intercostal retraction
Respiratory rate normalised

2.4 Geographical distribution of children in study

The Hospital Central de Maputo is an urban general hospital situated in the centre of the suburb Polana Cimento. This busy hospital has 1500 beds, and serves a very large community.

On the following page is a map of Maputo, where the study was undertaken, indicating the different suburbs within Maputo (A-Z). The location of the hospital is denoted by a "**".

Table 2.1 lists the suburbs of Maputo and indicates where the children in this study came from. Accurate population figures are not available. Results of a census which ended on the 15 August 1997 have not yet been released. The previous estimate was a population of 15 000 000 countrywide[16].

2. To determine the antimicrobial resistance patterns of both nasopharyngeal and blood culture isolates of *Streptococcus pneumoniae* by disc diffusion methods with confirmation of resistance by means of minimal inhibitory concentration (MIC) determinations.

3. To correlate blood culture and nasopharyngeal isolates from individual patients in order to assess the predictive value of nasopharyngeal isolates as causes of the patients' pneumonia.

4. To determine the pneumococcal serotypes with the view of possible incorporation into polyvalent polysaccharide vaccines in the future.

5. To retest carriers 6 weeks after discharge from hospital to detect changes in carriage rates and antibiotic resistance patterns following hospitalization and treatment for pneumonia.

6. Transfer of technology learnt at the SAIMR to Mozambique in order to improve laboratory techniques used in Maputo.

Although the study was not specifically designed to assess the clinical manifestations of pneumonia in Maputo children, these were recorded. Some potential risk factors for nasopharyngeal carriage of penicillin-resistant *S pneumoniae* and the development of pneumonia in these patient population such as age, nutritional status, family size and socio-economic conditions exposure were also assessed.

2.2 Inclusion criteria and tests on admission

Diagnostic criteria for inclusion into the study

Aged more than one month of age but less than 5 years old
Pyrexia
Confirmed diagnosis of acute respiratory infection:
Respiratory rate abnormal

CHAPTER 2 DESIGN OF MAPUTO STUDY

In order to gain an indication of antibiotic-resistant *Streptococcus pneumoniae* in a community, resistance patterns of nasopharyngeal pneumococci in children under 5 years of age, who are known to have a high carrier rate in this anatomical site, could be determined periodically in order to detect changes in antimicrobial resistance[1-4].

Pneumococcal disease can be prevented by immunization in adults using a polyvalent polysaccharide vaccine and conjugate vaccines are being developed and evaluated for efficacy in infants and children[9].

It would therefore also be useful to determine the serotypes of these nasopharyngeal pneumococci in order to ascertain whether the serotypes present in the vaccines currently being developed would be relevant to Mozambique. With this background, it was decided, following discussions with Professor K P Klugman of the South African Institute for Medical Research (SAIMR), Johannesburg, to conduct a study in Maputo, examining the nasopharyngeal isolates of children with pneumonia being admitted to the Maputo Hospital with regards to their antibiotic resistance patterns and serotype profiles.

2.1 Aims and objectives of the study

The two main aims of the study were to determine the antibiotic resistance patterns of nasopharyngeal isolates of *Streptococcus pneumoniae* in Maputo children, as well as the serotypes prevalent in that paediatric population.

Within these aims, the following objectives of the study were identified:

1. To determine the nasopharyngeal carriage prevalence rate of *Streptococcus pneumoniae* in children with pneumonia hospitalized at the Maputo Hospital.

present with severe malnutrition associated with other pathologies. Malnutrition is manifested as kwashiorkor, marasmus, and very low weight. Of the vitamin deficiencies, pellagra is the most prevalent followed by xerophthalmia, beri-beri and scurvy, the latter being the least common. Anaemia due to iron and folic acid deficiencies is also very common. Parasitaemias and other infections occur frequently and are all interrelated in this vicious circle[15, 16].

In the Paediatric Unit at the Hospital Central de Maputo, 598 children admitted during 1996 presented with severe malnutrition - kwashiorkor and marasmus. Of these 101 cases died, giving a case fatality rate 16,8%. (These statistics were kindly provided by the Hospital Central de Maputo administration). Deaths were mainly caused by gastroenteritis or respiratory infections, including tuberculosis while malaria was also responsible for considerable mortality.

As antimicrobial therapy is life saving in severe pneumococcal infections it was decided to establish resistance patterns in nasopharyngeal isolates of *S pneumoniae* to commonly used antimicrobial agents in Maputo in children under 5 years of age. A study was therefore initiated involving 400 of the children admitted to paediatric wards at the Hospital Central de Maputo over a period of 20 months (August 1995 - April 1997) to investigate the incidence of antibiotic resistance in *S pneumoniae* in Mozambique and to determine pneumococcal serotypes prevalent in this country.

The pneumococcal equivalent to the C-polysaccharide found in strains of different species of the *Streptococcus* genus is a teichoic acid constituent that contains choline phosphate and is covalently linked to peptidoglycan on the surface of the cell wall. This antigen is common to all *S.pneumoniae* strains[8].

The most common illnesses caused by *S pneumoniae* are pneumonia, meningitis, bacteraemia, otitis media and sinusitis. These infections are more prevalent during the cold winter months and in patients with predisposing factors such as viral infections, bronchitis or other chest infections, heart diseases, liver diseases, a history of heavy drinking, head injuries, malignancy or spleen abnormalities[8].

The diagnosis of pneumococcal disease is confirmed by isolating *S.pneumoniae* from specimens from normally sterile sites such as blood, cerebrospinal fluid (CSF) and the middle ear. They may also be isolated from carriers using nasopharyngeal swabs and the sputum and nasal sinus secretions in patients with pneumonia and sinusitis respectively[14].

1.2 Background to pneumococcal disease in Maputo and Mozambique

Childhood mortality in Maputo is one of the highest in the world. The major causes of morbidity and mortality in this age group are malnutrition and infectious diseases such as diarrhoeal diseases, acute respiratory infection and cerebral malaria. The mean monthly admission rate to the Hospital Central de Maputo of patients with acute respiratory infections is 338 cases per month with a case fatality rate of 2.6%. (These statistics were kindly provided by the Hospital Central de Maputo administration).

Mozambique is one of the poorest countries in the world, still currently suffering the consequences of a long war that tragically affected the most vulnerable, ie. children under 5 years of age.

In Maputo many of the children admitted to paediatric wards at the Hospital Central De Maputo

Those persons who have a diminished capacity to form antibodies eg young infants, patients infected with the human immunodeficiency virus (HIV) or individuals lacking specific antibodies to the colonizing strains, remain susceptible as long as they are colonized and have subminimal levels of the specific antibodies. The exact duration of colonization with *S.pneumoniae* is unknown, but is estimated at approximately 1 - 2 months in children 12 - 18 months-of-age, being longer in people with low or non-existent levels of antibodies against the *S pneumoniae* strain concerned, such as in infants and young children[8].

Pneumococci produce a haemolytic toxin, called haemolysin, which is related immunologically to the oxygen-labile haemolysins of beta-haemolytic streptococci. The pneumococcus also produces a toxic neuraminidase which is released during autolysis[8].

The bacterial capsule acts as a virulence factor of the organism. This capsule may prevent or delay ingestion of pneumococcal cells by phagocytes. The capsule is made up of repeating oligosaccharides that are synthesized within the cytoplasm and polymerized by transferase that are bound to the cell membrane[12].

S.pneumoniae has the capacity to cause disease because it is able to escape ingestion and killing by host phagocytic cells in the absence of anticapsular antibody and complement[13].

The mechanism that allows the capsule to prevent phagocytosis is uncertain. It is still uncertain what levels of anticapsular antibody protect a human subject against the ordinary pneumococcal challenge.

Capsular polysaccharides permit the classification of *S.pneumoniae* into 90 different serotypes. Some serogroups/types eg. 1, 2, 3, 4, 6, 7, 8, 12, 14, 19, 23 are more prevalent than others and are associated with increased virulence. The serotypes 6A, 6B, 14, 19A, 19F and 23F are common in children and also exhibit a tendency to acquire penicillin resistance.

members may have experienced problems in collecting swabs from infants and the younger children. In the process, they may have failed to reach the nasopharynx via the pernasal route, resulting in suboptimal specimens.

The geographical distribution of children entered into the study is given in Table 4.2.

CHAPTER 4 RESULTS AND ANALYSIS OF DATA

A total number of 400 children were included in the survey. These children were admitted to the Hospital Central de Maputo between August 1995 and April 1997.

All the admissions presented with the manifestations of pneumonia discussed earlier.

4.1 Nasopharyngeal carriage of *S pneumoniae*

The numbers of patients carrying pneumococci were divided into the following age groups in Table 4.1.

TABLE 4.1 *Distribution of pneumococcal carriers according to age*

Age group number (months)	Nasopharyngeal carriers	Percentage (%)	Total No of children
≤ 18	63	(28.1)	224
19 - 36	19	(24.7)	77
37 - 60	46	(46.5)	99
All ≤ 60	128	(32.0)	400

The carriage rates in the two ≤ 36 months age groups were lower than in the older children. These lower-than-expected rates may have been as a result of poor technique employed by the nursing staff who took the swabs. Although trained in the correct procedures, these staff

LABORATORY FORM USED IN MAPUTO

Date:	Guardian:
Hospital:	Region:
Patient number:	Diagnosis:
Patient name:	TB:
Age:	HIV:
Sex:	Height:
Tribe:	Weight:

Antibiotics in past month (type/duration): ____________________

Hospitalization (duration/diagnosis): ____________________

Reason for current attendance: ____________________

Day care center attendance: ____________________

Children in family (under 5 yrs): ____________________

Traditional medicine: ____________________

	Pneumo		Non-Pneumo			
Growth:	Y	N	[illegible]	Y	N	O
Number of clones picked:	1	2	[illegible]	Y	N	
Stored Robertson's	Y	N	[illegible]	Y	N	
Optochin	S	R	[illegible]	Y	N	
Bile solubility	Not done	Y	[illegible]	Y	N	
Stored -70°C	Y	N	[illegible]	Y	N	

Interpretation

	Disc Content	Pneumo S	I	R	Zone Diam. mm	(Susceptible ≥ ? Intermediate ≤ ? Resistant ≤ ?)		E TEST MIC
Oxacillin	1ug	≥ 20	12 - 19	≤ 12		(Penicillin)		
Chloramphenicol	30ug	≥ 19	14 - 19	≤ 14				
Tetracycline	30ug	≥ 19	14 - 19	≤ 13				
Erythromycin	15ug	≥ 20	16 - 20	≤ 16				
Clindamycin	2ug	≥ 27	13 - 27	≤ 13				
Rifampicin	5ug	≥ 20		≤ 20				
Other (Specify)								
[illegible]								
[illegible]								

☐ Fully Sensitive.

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

☐ Serotype: .... Serotype / Serogroup / Not typed

3.3 At the South African Institute for Medical Research (SAIMR)

After the specimens were collected and processed in Maputo and the pneumococcal isolates tested by disc diffusion for antibiotic susceptibility as described above, all isolates were transported in duplicate on Dorset egg and Charcoal Transport media to the Microbiology Laboratory of the Department of Medical Microbiology, South African Institute for Medical Research (SAIMR). Here the isolates were subjected to confirmatory MIC testing and serotyping using pneumococcal typing sera from the Statens Seruminstitut, Copenhagen.

The laboratory form used in Maputo (page 24) was completed for each new case, and was submitted to the SAIMR together with each isolate which required confirmation as well as additional laboratory work.

TABLE 3.2 NCCLS reference MIC values indicating degrees of resistance[17]

ANTIBIOTIC	MIC range in µg/ml denoting		
	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
Penicillin	≤ 0.06	≥ 0.1 - 1	≥ 1
Chloramphenicol	≤ 4		≥ 8
Tetracycline	≤ 2		≥ 8
Erythromycin	≤ 0.25	0.25 - 0.5	≥ 1
Clindamycin	≤ 0.25	0.25 - 0.5	≥ 1
Rifampicin	≤ 1	1 - 2	≥ 4
Cotrimoxazole	≤ 1[1]	1 - 2	≥ 4

(1) Expressed in terms of trimethoprim component

3.2.3 Blood cultures

After 18 - 24 hours incubation at 37°C the blood cultures were subcultured onto chocolate agar plates which were also incubated at 37°C.

Isolation, identification and susceptibility testing were carried out in the same way as mentioned above for nasopharyngeal swabs.

After the required period of incubation, ±18 hours, when bacterial growth became distinctly visible, the minimum inhibitory concentration (MIC) value at the point of intersection between the zone edge and the Etest strip was read.

Minimal Inhibitory Concentration (MIC) determinations

All isolates were retested at the SAIMR by the broth dilution method to determine their MICs. The NCCLS guidelines were followed in the performance of these assays[17]. This test determines the lowest concentration of antibiotics measured in µg/ml that would prevent the in vitro growth of the specific bacteria.

This procedure was carried out using a microtitre method and growth inhibition at the lowest concentration of the specific antibiotic was taken to be the MIC.

The critical MIC concentrations, also known as breakpoint concentrations, denoting susceptibility and intermediate and full resistance, are given in Table 3.2.

TABLE 3.1 *Reference zones of inhibition indicating degrees of resistance*

	Zones of inhibition in mm denoting		
ANTIBIOTIC	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
OXACILLIN	≥21	13-19	≤13
CHLORAMPHENICOL	≥19	15-19	≤14
TETRACYCLINE	≥19	14-19	≤15
ERYTHROMYCIN	≥20	16-20	≤16
CLINDAMYCIN	≥21	19-21	≤19
RIFAMPICIN	≥20		≤20
COTRIMOXAZOLE	≥19	16-18	≤15

Etest methodology

All penicillin-resistant isolates as determined by the oxacillin disc diffusion test, as well as cotrimoxazole-resistant isolates, were further tested using an Etest (Ab Biodisk, Solna, Sweden), as described by Jorgensen *et al*[18].

Inoculum preparation and inoculation density were as for disc susceptibility testing. Etest strips were applied to the surface of a completely dry Mueller Hinton laked blood agar or sheep blood agar plates. Plates were incubated at 35°C for 24 hours.

Using aseptic techniques discs containing the following antibiotics were placed firmly on the agar surface of the respective blood agar plates:

5% sheep blood agar	*Mueller Hinton laked blood agar*
Oxacillin (1 ug)	Cotrimoxazole (25ug made up of trimethoprim, 1.25 ug and sulfamethoxazole 23.75ug)
Chloramphenicol (30ug)	
Tetracycline (30ug)	
Erythromycin (15ug)	
Clindamycin (2ug)	
Rifampicin (5ug)	

Plates were incubated overnight at 35°C.

The presence of a definitive zone of inhibition for each antibiotic (see following table) was regarded as susceptible.

S pneumoniae ATCC # 49619 was used for quality control purposes both for the optochin as well as the susceptibility testing.

These control procedures were put up concurrently, on a weekly basis. The zone sizes (expressed in mm) which were used as interpretation guidelines are given in Table 3.1.

3.2.1 Nasopharyngeal swabs

After 24 hours incubation, the blood agar plates with gentamicin were examined for typical colonies of *S pneumoniae*, producing alpha-haemolysis and exhibiting a draughtsman shape (colonies that are mucoid or flattened with a depressed centre.)

Five colonies showing the abovementioned features were plated out onto horse blood agar for confluent growth and an optochin disc was placed in the centre.

The blood agar plate was incubated for 24 hours at 37°C. A zone of inhibition of at least 14 mm around the optochin disc was regarded as typical of *S pneumoniae*.

3.2.2 Antimicrobial susceptibility testing

The antimicrobial susceptibility test employed the Kirby-Bauer technique and was performed according to the National Committee for Laboratory Standards (NCCLS) guidelines[7]. A suspension of the overnight growth was prepared in peptone water, equivalent to a 0.5 McFarland standard. A swab from which the excess fluid has been expressed was used to inoculate as uniformly as possible one Mueller Hinton laked blood agar and one 5% sheep blood agar.

The paediatric ward was visited on a daily basis by myself or a laboratory technologist. Daily temperature checks were performed on the incubator and the refrigerator containing the media. We ensured that all instructions given at the outset of the project were being followed. Replacing and/or replenishing stocks of media was carried out by ourselves.

3.2 In the laboratory

Pneumococci were identified in the routine microbiology laboratory by the following procedures:

1. Gram-stain: gram-positive, lanceolate-shaped, capsulated diplococci that may form short chains.

2. Haemolysis: the organism produces α - haemolysis on blood agar. The haemo-lysis is greenish in colour and is also known as a viridans-type haemolysis

3. Culture: *S.pneumoniae* grows best on chocolate (heated blood) agar, incubated in a carbon dioxide enriched atmosphere. *S.pneumoniae* is catalase negative.

4. Identification: Pneumococci are soluble in bile salts, and are susceptible to ethyl hydrocupreine hydrochloride (optochin). In this study only optochin susceptibility was used to identify the strains. Using a 6mm disc, a zone of inhibition of at least 14mm in diameter is indicative of susceptibility.

For children with acute respiratory infection it is essential that the clinical specimens be collected before antimicrobial therapy is begun, and all specimens should be processed in the bacteriology laboratory as soon as possible. Instructions to effect these mandatory steps were given and generally adhered to.

CHAPTER 3 PROCEDURES FOLLOWED IN THE STUDY

3.1 In paediatric casualty ward

On admission to the casualty ward and upon confirmation of the inclusion criteria listed in chapter 2, the following was done:

1. A calcium alginate nasopharyngeal swab (Calgiswab: Inolex Corporation) was taken from each child using the pernasal route

2. A blood culture (Oxoid Signal Blood Culture System) was taken on each child at the same time.

3. On completion of the nasopharyngeal swab and the blood culture the child was immediately started on antibiotic therapy (procaine-penicillin).

4. Trained nursing staff were responsible for

   - plating the nasophatyngeal swab directly onto a blood agar plate containing horse blood and 5μg/ml of gentamicin. These plates were incubated for 24 hours . ' in the incubator in the paediatric casualty section of the hospital.

   - putting the blood culture bottles in the incubator in the ward immediately after they had been collected.

5. The blood cultures were incubated at 35°C for 24 hours in the incubator in the paediatric Casualty ward.

2.6 Laboratory investigations

a) Upon admission the following specimens were taken on each child prior to the administering of antibiotics:

- A calcium-alginate swab (Calgiswab; Inolex Corporation) was used to collect a nasopharyngeal specimen.
- 5-10 ml of blood was collected and placed in a blood culture bottle (Oxoid Signal Blood Culture System) immediately
- 5 ml of uncoagulated blood was collected for a white blood cell count.

b) On discharge the parent or guardian of the child was requested to bring the child back 6 weeks later if the nasopharyngeal swab proved to be positive.

At this stage a final nasopharyngeal swab using a Calgiswab was to be taken.

4.3.3 Antibiotic resistance patterns of nasopharyngeal isolates

Of the resistant strains, 43 (51%) were mono-resistant, 33 (39%) were resistant to two drugs and 9 (11%) isolates to 3 antimicrobial agents. The various antimicrobial resistance patterns and their corresponding serotypes are given in Table 4.9 and Table 4.10. The incidence rates of resistance to penicillin, cotrimoxazole, tetracycline, chloramphenicol, erythromycine and rifampicin were 52%, 28%, 18%, 9.4%, 1.2% and 1.2%, respectively.

4.3.4 Serotypes of *S pneumoniae*

The serotypes of the pneumococcal isolates from the nasopharynx and blood of children with pneumonia are given in Table 4.11.

Of the 23 blood culture isolates, 22 were available for serotyping and antibiotic susceptibility testing. The comparative data of these isolates and strains isolated from nasopharyngeal swabs are given in Table 4.9 and Table 4.11.

Only one isolate from the blood culture group was shown to be different from the corresponding nasopharyngeal isolate. This finding suggests strongly that nasopharyngeal swab results are likely in a large percentage of cases to reflect the pneumococcus causing invasive pulmonary pathology in cases of pneumococcal pneumonia. In cases of non-pneumococcal pneumonia some nasopharyngeal swabs may of course still yield positive pneumococcal cultures.

With regard to the antibiotic susceptibility results of the 22 available blood culture isolates 17 (72%) were resistant to penicillin while five (23%) were in addition also resistant to cotrimoxazole. The corresponding serotypes were 19F (19 isolates) and 19A, 14 and 21 (all one isolate each) while one blood culture isolate was contaminated and could not be typed or tested for drug susceptibility.

TABLE 4.8 Antibiotic resistance in nasopharyngeal isolates of S pneumoniae from Maputo children

Antibiotic	Disc diffusion results			MIC-confirmed resistance			Etest results (only strains resistant on screening were tested)		
	R[1] (%)	I R[2] (%)	S[3]	R (%)	I R(%)	Total (%)[4]	R(%)	I R (%)	Total tested
Oxacillin/penicillin	57 (45)	16 (13)	55	1 (0.8)	66 (52)	67(52)	17 (13)	54 (42)	73
Tetracycline	18 (14)	7 (5)	103	20 (16)	3 (2.3)	23(18)		Not done	
Cotrimoxazole	24 (19)	9 (7)	95	33 (26)	3 (2)	36(28)	21 (16)	0	53
Chloramphenicol	4 (3.1)	8 (6)	116		Not done			Not done	
Erythromycin/Clindamycin	2 (1.6)	0	126		Not done			Not done	
Rifampicin	2 (1.6)	-	126		Not done			Not done	

1 = Fully resistant
2 = Intermediately resistant
3 = Susceptible
4 = Total number of strains showing resistance

4.3.2 Antibiotic susceptibility testing

Once confirmed as *S pneumoniae*, antimicrobial susceptibility testing was performed on all isolates.

On screening for resistance on disc diffusion testing, all penicillin- and cotrimoxazole-resistant isolates were retested by Etest in Maputo while MIC determinations involving these two antibiotics, as well as tetracycline, were performed on all the isolates at the SAIMR in Johannesburg. The antibiotic susceptibility results of the nasopharyngeal isolates are shown in Table 4.8.

Based on MIC confirmation of resistance findings, the incidence of penicillin resistance was 52% overall with only 0.8% of the carriers harbouring fully resistant strains while all but one isolate was intermediately resistant to this antibiotic. These penicillin resistance data also reflect those of ampicillin and amoxycillin which exhibit MIC's similar to those of penicillin in the case of *S pneumoniae* isolates.

Resistance rates to cotrimoxazole, tetracycline and chloramphenicol were 28%, 18% and 9% respectively, while those to the other antimicrobial agents, including erythromycin, clindamycin and rifampicin were low (less than 3%).

The Etest results compared very well with those of the MIC determinations while the disc diffusion tests performed in Maputo tended to overestimate resistance.

4.3.2.1 Age-related carriage of antibiotic-resistant pneumococci

From Table 4.6 it can be seen that there was a much higher prevalence of antibiotic resistant pneumococci in children less than three years of age (75 out of 82; 91%) compared with the older children (10 out of 46; 22%). The difference was highly significant ($P<0.001$; Chi-squared, Yates corrected).

TABLE 4.7 Type of house in relation to carriage of Streptococcus pneumoniae

Carrier status	Cement house (n)	Straw hut(n)	Unknown	Total(n)
Non carriers	108	148	16	272
Carriers	47	54	27	128
R-Carriers[1)]	22 (47%)	45 (83%)	18 (67%)	85 (66%)

1) Carriers of antibiotic-resistant pneumococci with percentage in group in brackets

Of the 47 pneumococcal strains from carriers who lived in cement houses, 22 (47%) were resistant as opposed to 45 resistant strains out of 54 carriers (83%) who lived in straw huts ($P=<0.001$; X^2 13.4, Yates corrected). These findings suggest that the antibiotic resistance was more likely to occur in children from poor households who live in straw huts compared with the presumably higher socio-economic group living in cement houses.

4.3 Characterization of pneumococcal isolates

4.3.1 Isolation from nasopharynx and blood

As previously mentioned, nasopharyngeal specimens were initially cultured for the presence of *S pneumoniae* only.

All isolates were then confirmed by various laboratory methods.

Of the 128 cases who had *S pneumoniae* isolated from nasopharyngeal swabs, 23 (18.0%) in addition had the organism recovered from blood cultures. No blood culture-positive children had a negative nasopharyngeal culture. The overall positive blood culture rate in the 400 children with pneumonia was 5.8%.

Demographic data

The prevalence of pneumococcal carriers in different age groups and the average number of children under the age of 5 years in the respective households, are given in Table 4.6.

TABLE 4.6 Relationship between carriage and household size

Age Group (months)	Resistant *S pneumoniae*	Carriers (%) in group	No of children in age group	Average No. of children in household
≤ 18	59 (94%)	63 (28%)	224	3.9
19 - 36	16 (84%)	19 (25%)	77	3.8
≥ 37	10 (22%)	46 (47%)	99	4.3
TOTAL < 60	**85 (66%)**	**128 (32%)**	**400**	**4.0**

The children over three years of age came from slightly larger families and their pneumococcal carriage rate was appreciably higher than in the younger age groups. On the other hand, the younger children had a much higher carriage rate of antibiotic-resistant strains (See Section 4.4.2.1)

Type of habitation

The type of habitation of all children was divided into 3 categories ie. cement, straw hut and unknown. 39% of children resided in a cement abode and 51% in a straw hut. Information on 43 (11%) of the children was unavailable. (Table 4.7).

PREENCHER NOS QUADRADINHOS

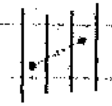
- [ ] Mãe e/ou pai ausente
- [ ] Gémeos
- [ ] peso ao nascer inferior a 2,5 kg
- [ ] Irmãos com malnutrição
- [ ] Migração recente da família
- [ ] ....
- [ ] ....
- [ ] ....

ESCREVER NA COLUNA DO MÊS APROPRIADO

Desmame
Diarreia
Sarampo
Pneumonia
Kwashiorkor

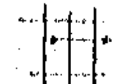
Nova gravidez da mãe
Tosse convulsa
Anemia
Outras doenças

BOM SINAL



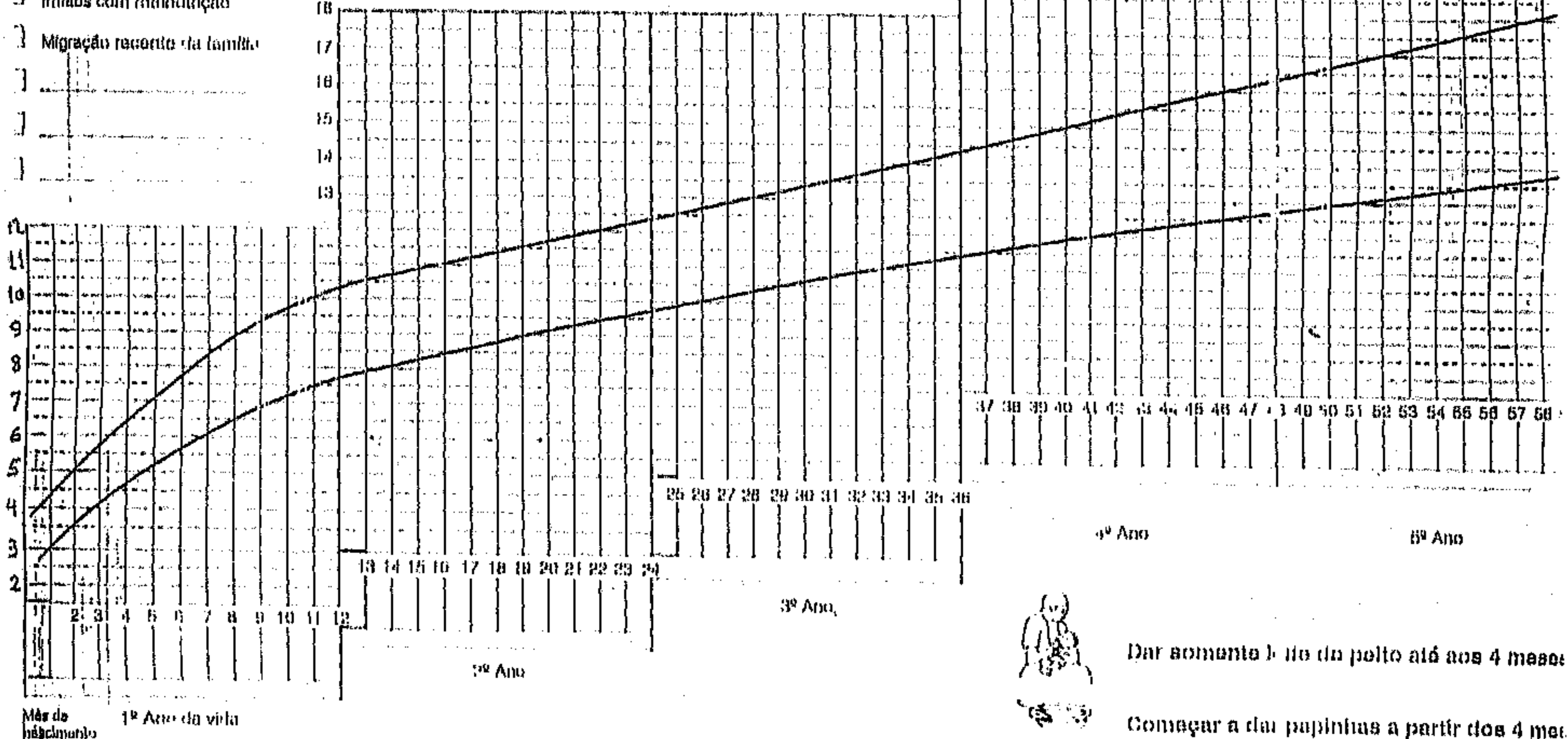
Bom, quer dizer que a criança está a crescer bem

SINAL DE ALARME



Perigo, procure saber porque não cresce

MUITO PERIGO



Dar somente leite do peito até aos 4 meses

Começar a dar papinhas a partir dos 4 meses

Continuar a dar de mamar até aos 2 anos

On analysis of the weight by age and based on percentile charts, malnutrition in children falling under the third percentile level occurred mainly in children between 13 - 60 months of age. The better nutritional status of infants less than one year of age probably relates to breast feeding which is practiced extensively in this age group. The culture-positive children (carriers) weighed slightly less than the culture-negative children and the differences in weight between the two groups was again most noticeable in those 13 - 60 months of age.

A health card which is used for all children in Mozambique is illustrated on page 33. The percentile (P_{50} - P_{50}) is used as an indicator of the nutritional status of the child. When the child's weight was $\leq P_{50}$ and this figure was less than 60% of the P_{50} figure (see chart), the child was considered to have marasmus[19, 20].

These criteria are more useful for the assessment of the individual because it is generally accepted that the decreases for the age may be evidence of previous or chronic malnutrition and decreased weight for height may be evidence of recent or relatively acute onset of malnutrition[19, 20].

In Table 4.5 height and weight data relating to the nutritional status of children are recorded according to age groups. The last column indicates the ideal weight, which is based on a chart reflecting the relationship between age, height, brachial circumference and weight.

TABLE 4.5 Age-related height and weight measurements

Age group (months)	No. of children culture positive	Culture positive group Mean and range		All children Mean and range		Ideal weight (kg)
		Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	
1 - 3	3	34.9 (48-50)	6.1 (5-7.2)	34.9 (48-50)	6.1 (5-7.2)	5.0
4 - 6	9	60.2 (55-69)	7.3 (4.5-9.8)	61.1 (55-85)	7.6 (4.5-9.8)	8.2
7 - 9	18	81.6 (54-93)	8.8 (3.9-12.8)	79.9 (54-98)	9.3 (3.9-12.8)	7.5
10 - 12	21	87.0 (65-102)	10.2 (7.3-14)	86.3 (65-110)	10.0 (7.2-15.8)	10.0
13 - 24	31	80.0 (60-110)	9.7 (5.5-14)	81.3 (60-110)	10.8 (5.5-14)	12.0
25 - 48	38	94.7 (82-106)	10.8 (9.5-15.5)	92.6 (76-106)	11.2 (7.5-15.5)	14.0
49 - 60	8	93.4 (95-114)	12.2 (12.9-15.5)	85.6 (90-114)	14.8 (12-17)	15.8

TABLE 4.4 Chest X-ray findings

X-ray findings [1]	No. of cases with positive x-ray findings	
	Culture - Positive	Culture - Negative
Left upper lobe	36 (31%)	78 (30%)
Left lower lobe	50 (41%)	48 (19%)
Right upper lobe	16 (13%)	56 (23%)
Right lower/middle lobe	10 (8%)	30 (12%)
Bilateral pulmonary	48 (39%)	18 (7%)
Normal	10 (8%)	48 (19%)
Not done/Not available	6 (5%)	24 (10%)
Number x-rayed	122 (95%)	248 (91%)

(1) More than 1 lobe were involved in some patients

Of the 128 carriers, X-ray examination of the chest was performed on 122 and 48 showed bilateral pulmonary involvement (39.3%) compared with only 18 out of 248 non-carriers that were X-rayed (7.3%). The difference was statistically significant ($P<0.001$; Chi-squared, Yates corrected). There were, on the other hand, only 10 normal *X*-ray findings in the carrier group (8.2%) as opposed to 48 (19.4%) in the non-carrier group. Lower lobe involvement occurred in 60 (49%) of carriers as opposed to 78 (31%) in the non-carrier group. It is interesting that 112 out of 122 pneumonia cases with a positive nasopharyngeal swab from whom x-ray findings were available showed an abnormal x-ray finding giving a predictive value of 92% for a positive nasopharyngeal swab. The specificity, sensitivity and negative predictive indices of nasopharyngeal cultures were 83%, 36% and 19%, respectively.

4.2 Clinical findings

There were 179 females and 221 males included in the study. Nasopharyngeal carriers comprised of 64 females and 64 males.

The temperatures upon admission ranged between 37,5°C and 40.2°C with a mean of 38,9°C.

The clinical data and X-ray findings are given in Tables 4.3 and 4.4.

The average heart and respiration rates for healthy children are about 120 beats per minute and 20 respirations per minute, respectively, but both heart and respiration rates vary with age in children, being higher in infants compared with older children. As all of the admitted children presented with tachycardia the mean heart rate was 131 beats per minute and the mean respiratory rate was 48 respirations per minute.

As expected, most of the children showed a leucocytosis with a mean leucocyte count of $15,79x10^9$/l and a range of $3,60 - 28,20x10^9$/l for the group as a whole.

TABLE 4.3 Clinical data of children admitted to study

Manifestation	Mean evaluated	Range	Number of cases
Temperature	38.9°C	37.5 - 40.2°C	400
Respiratory rate/minute	48	36 - 51	400
Heart rate/minute	131	120 - 160	400
Leucocyte count/litre	$15.79x10^9$	$3,6 - 28,20x10^9$	400

From Table 4.2 it is clear that most cases came from Polana Canico (51 children) and Matola (45 children).

Polana Canico (zone S on map) is an extremely poor area (Canico means straw) where no clinics with treatment facilities are available. There are however small health centes which refer very ill patients directly to the Hospital Cental de Maputo. Children from this suburb have a high pneumococcal carriage rate of 36 out of 51 (70.6%) as well as a high rate of resistance 31 out of 36 (86.1%). It should be noted that by far the largest number of antibiotic-resistant isolates came from this poor area of Maputo, 31 from this single suburb as opposed to 55 from the other 25 suburbs, giving a mean of only 2.2 per suburb for the latter group.

Motala, Zone C on the map, on the other hand is a suburb on the outskirts of Maputo and has a well established primary care clinic where patients can be treated and seriously ill patients be referred to hospital. There are also two nearby hospials and patients will only be referred to the Hospital Central de Maputo when these two hospitals are full. Children from this suburb had a very low carriage rate of 7 out of 45 (15.6%) which could be explained by the possibility that preliminary treatment may have been instituted at the clinic before referral of patients to the central hospital. Such treatment may have influenced the isolation of nasopharyngeal pneumococci.

The addresses of about a quarter of the children were not recorded and most of these came from areas outside central Maputo (99 out of 400).

TABLE 4.2

Carriage status and carriers of antibiotic-resistant pneumococci according to suburb:

Zone	Suburb	Non-carriers	Carriers	Resistant Isolates
A	BAIRRO FERROVIARIO	2	5	3
B	MAXAQUENE	10	5	2
C	MATOLA	38	7	5
D	MALANGA	15	2	2
E	MKADGUINE	8	1	1
F	BAIRRO CENTRAL	5	7	5
G	ALTO MAE	26	4	3
H	MAFALALA	3	1	1
I	MATUTUINE	2	1	1
J	HULENE	4	3	1
K	CHAMANCULO	12	3	1
L	MICHAFUTINE	5	2	0
M	PATRICE LUMUMBA	5	5	3
N	LAULANE	4	1	1
O	INFULENE	2	5	2
P	COSTA DO SOL	7	2	1
Q	25 DE JUNHO	4	1	0
R	AERPORTO	5	1	1
S	POLANA CANICO	15	36	31
T	NHAGOIA	2	1	1
U	MAVALANE	5	6	3
V	DJAVELA	1	1	1
W	POLANA CIMENTO(1)	4	1	1
X	BENFICA	2	2	1
Y	JARDIM	4	1	0
Z	MALHANGALENE	3	4	2
Unknown or outside of Maputo		79	20	12
	Total	**272**	**128**	**85**

For future surveillance of pneumococcal resistance patterns in Mozambique, improvement of the disc diffusion technique is required as in the present study this method tended to over estimate the frequency and levels of resistance. Improved quality assurance, including external quality assessment participation may help to acheve this. The Etest gave good results but tended to overestimate levels of penicillin resistantce. The cost of this test is, however, prohibitive in the Mozambique setting.

5.3.4 Antibiotic policy for Mozambique/Maputo, based on the findings of the present study

According to World Health Organization recommendations, penicillin should be available in all countries. It is an essential drug chosen because of its effectiveness in the case of many infections in developing countries and the fact that it is relatively inexpensive. Even though high levels of penicillin resistance have been demonstrated, this antibiotic can still be used effectively in the treatment of pneumonia[22]. The efficacy of penicillin treatment of pneumonia in a population where the incidence of penicillin-resistant pneumococci is extremely high has again been demonstrated in the present study.

In Mozambique penicillin, chloramphenicol, and to a lesser extent, cotrimoxazole and tetracyclines are the most frequently used antibiotics and they may select for resistance due to overuse and possible abuse. In pulmonary lobar pneumonia for example, penicillin is one of the most effective antibiotics, due to its easy absorption into the blood stream and high bactericidal levels achieved in the lungs. This is however not the case in meningitis and cases of otitis media as this antibiotic has greater difficulty crossing the blood-brain barrier and into middle-ear tissues[22].

Chloramphenicol is frequently administered to patients in Mozambique. It is a broad spectrum antibiotic and is predominantly bacteriostatic. Severe but uncommon side effects include aplastic anaemia and pancytopenia. For this reason it is probably unwise to use it in the treatment of pneumonia, despite its low cost. However, in poor developing countries there is often no option but to use this antibiotic as none other may be available or affordable.

reasons for this finding may have been that because only cases admitted to hospital were investigated, it is possible that unrecorded antibiotic administration may have occurred before the nasopharyngeal swabs were taken.

It is noteworthy that the nasopharyngeal carrier rate of 71% in children from Polana Canico, the poorest suburb in Maputo where patients are directly referred to the hospital, is in the higher range of those recorded in the literature[4,8,33].

5.3.2 Prevalence of antibiotic resistance in nasopharyngeal pneumococci in Maputo children

The antibiotic resistance incidence of 66% in *S pneumoniae* in Maputo children during the study period was extremely high. The prevalence rate for penicillin resistance of 52% is slightly higher than that in neighbouring South Africa[5,1,25], while those of cotrimoxazole (28%) and tetracycline (18%) are also higher than at the Chris Hani Baragwanath Hospital in Soweto, Johannes-burg[4,25]. The higher incidence in the infants and children under 3 years of age is in accordance with other studies in the literature[4,21,35]. However, the excessively high rate of resistance to penicillin and other antibiotics in this age group requires explanation. Two possible factors may have played a role. One is that fairly rapid spread of a clone within serotype 19F could have occurred in the population studied and that presently unknown risk factors e.g. crowded conditions, ant. [illegible] usage and/or frequent upper respiratory infections played a role. The other scenario is that the evolution of drug resistance had taken place over a prolonged period and resistance accumulated gradually to its present high levels.

5.3.3 Significance of antibiotic resistance findings

This study was very important as it provided a baseline of relevant information on antibiotic resistance in *S pneumoniae* which, up to now, was unavailable. This information could form the basis for the rational treatment of pneumonia, meningitis and otitis media in Maputo.

study died, giving a case fatality rate of 0.5%. Both had positive blood cultures and died soon after admission. The low mortality in this group of children is not surprising as bacterial pneumonia which is usually caused by *S pneumoniae* usually respond well to antibiotic treatment provided appropriate treatment is given relatively early in the disease[8,22]. It is probably fair to suspect that the case fatality rate is likely to be much higher in the more remote rural regions of Mozambique[27].

The fact that patients responded to penicillin treatment of their pneumonia despite the fact that more than 50% of the causative pneumococcal strains were "resistant" to this antibiotic is not surprising. As indicated earlier penicillin resistance, although highly relevant in the menigitis setting, has no bearing on pneumonia where penicillin levels in the diseased lungs are much higher than the MIC's or MBC's of the pneumococcal strains causing the pneumonia[22].

Whether any of the children in the present study suffered from underlying HIV disease is uncertain but pneumococcal pneumonia and bacteraemia are more common in HIV-positive adult patients, even when their CD_4 cell counts are still relatively high[30]. Pneumococcal isiates from such patients tend to be more commonly resistant to antibiotics than HIV-negative control patients.

5.3 Significance of antibiotic resistance findings

5.3.1 Carrier rates

Although the actual rates of pneumococcal carriage for the study of the prevalence of resistance patterns and serotypes present in communities are not of primary importance, a sufficient number of isolates which is also representative is required for meaningful results[2].

The pneumococcal carrier rate results from the present study, centralized at the Hospital Central de Maputo, apear to be lower than in other developing countries[1,23,25]. One of the

Mozambique, the incidence of antibiotic resistance in pneumococci and other bacteria is unknown.

5.2 Clinical aspects of the Maputo study

5.2.1 Hospital facilties in Maputo

Apart from the Hospital Central de Maputo, there are three other fairly large hospitals in Maputo that admit children from surrounding areas. Our hospital, being a reference hospital, accepts mainly referred cases.

5.2.2 The status of the hospitalized children

The population of children entered into the study came from a very poor socio-economic background. The poorest suburb of Maputo, Polana Canico, also provided the largest number of children with pneumonia admitted to the hospital. These children had a high carrier rate as well as a high rate of antibiotic resistance among the isolates.

With regard to the nutritional status of the children, those below one year of age tended to be well nourished, probably because they were largely breastfed. The older children in the study were generally below the ideal weight for their age and reflect the state of poverty and deprivation prevailing in the wake of the recent civil war in the country where malaria, diarrhoea and respiratory disease are rampant[26,27,28,29].

5.2.3 Clinical features of pneumonia cases

The children with pneumonia showed the classical signs and symptoms as well as radiographic features of this disease. It is interesting that the children with positive nasopharyngeal swabs had a greater propensity for the development of bilateral pneumonia. The patients responded well to penicillin or chloramphenicol and only two out of the 400 children in the

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Pneumococcal resistance worldwide

S pneumoniae remains the most common bacterial cause of pneumonia[7,8,24]. Worldwide, approximately 15 million children under 5 years of age die annually from preventable diseases. The majority of these deaths occur in developing countries[7]. Among these, respiratory infections predominate and the pneumococcus is responsible for about 80% of severe pneumonias in children in Africa[24].

As mentioned in Chapter 1, antibiotic resistant pneumococci were first described to be fully and multiply resistant to penicillin in 1977 and 1978, respectively[1,2]. Since then a dramatic increase in the incidence of pneumococci resistant to penicillin and other antimicrobials has been noticed[4,23]. A marked increase in resistance is currently seen in some European countries such as Spain, France, Hungary, Slovakia as well as South Africa and the United States of America[4,22,23,25].

For many years penicillin was the standard treatment for pneumococcal infections ie. the drug of choice. Strains with MIC's between 0.12ug/ml and 1.0µg/ml have historically been designated as relatively resistant to penicillin and those with MIC's greater than 1.0µg/ml as resistant. However, these critical concentrations, also called breakpoint concentrations, are only relevant in cases of meningitis and possibly otitis media in the case of penicillin and not to pulmonary infections[22]. A note of caution is appropriate in connection with the use of chloramphenicol for the empiric treatment of acute bacterial meningitis in developing countries where there is a high incidence of penicillin-resistant pneumococci. Patients with pneumococcal meningitis caused by penicillin-resistant strains do not respond well to chloramphenicol treatment[3,23].

Surveillance and ongoing research programmes are costly and difficult to establish in a country with severe financial constraints and in some underdeveloped countries such as

4.3.7 Treatment data

The most common treatment regimen involved intra-muscular procaine penicillin. However, chloramphenicol and occasionally cotrimoxazole were sometimes used depending on the availability of penicillin.

Previous taking of antibiotics

Of the 400 children included in the study, only 3 children were known to have received an antibiotic before admission to hospital. At admission, 1 child was currently taking nalidixic acid and 2 were on amoxycillin. It was not possible to obtain meaningful information from the parents or guardians of patients regarding prior antibiotic exposure.

4.3.8 Case fatality rate

Two children entered into the study died shortly after being admitted to the Paediatric Unit. Both cases died as a direct result of their pneumonia and had positive blood cultures. The case fatality rate of 0.5% was therefore quite low.

4.3.6 Six-week follow-up results

We were unable to get a 6-week follow-up swab from 73 of the 128 children, leaving 55 children who could be swabbed after discharge. Although various attempts were made to locate all the children, this was not always possible.

Thirty (55%) of the children tested at 6 weeks, still had *S pneumoniae* present in their nasopharyngeal swabs and of these 20 isolates were resistant to one or more antibiotics.

The carriage rate of resistant strains after six weeks (20 out of 30; 67%) was identical to resistance rate on admission to hospital (86 out of 128; 67%).

The serotypes of resistant strains and their resistance patterns are given in Table 4.12.

*TABLE 4.12 **Serotypes and resistance patterns six weeks after hospitalization***

Serotype	No. of strains	Resistance to			
		Pen[1]	Pen Cot	Pen Tet	Pen,Tet, Cot
19F	15	9	3	1	2
23F	3	1	1	1	
14	1	1			
6B	1		1		

1) Pen = penicillin; Cot = cotrimoxazole; Tet = tetracycline

4) In one patient the blood culture isolate was a penicillin- and tetracycline-resistant type 21 while the corresponding nasopharyngeal isolate was penicillin-resistant and a type 23F.

TABLE 4.11 Serotypes of antibiotic-resistant nasopharyngeal and blood culture isolates

Serotype	Number of positive N P Swabs[1)]	Blood cultures[2)] No (Resistance patterns)	Identical Anti-biogram Swab/B/C	Identical Serotype Swab/BC
19F	42 (47.7%)	19 (9P, 4Pcot,6S[3)])	19	19
14	12 (14.0%)	1 (P)	1	1
23F	6 (7.0%)	0	No[4)]	No[4)]
6B	4 (4.7%)			
6A	3 (3.5%)			
1B	4 (4.7%)			
13	2 (2.3%)			
28	2 (2.3%)			
19A	2 (2.3%)	1 (P)	1	1
9N	2 (2.3%)			
7	2 (2.3%)			
4	2 (2.2%)			
21	0 (0%)	1 (P)	No[4)]	No[4)]
29	1 (1.2%)			
5	1 (1.2%)			
10	1 (1.2%)			
24	1 (1.2%)			
Contaminated		1		
Total	**85**	**23**	**21**	**21**

1) Number of nasopharyngeal swabs
2) Number of blood cultures and resistance patterns in brackets
3) S Susceptible

TABLE 4.10 Resistance patterns of six common paediatric serotypes compared with others

Serotypes	Resistant strains			
	Pen R[1](%)	Single[2](%)	≥2R[3](%)	Total R[4]
19F	30 (71)	31(50)	21(50)	42
14	10 (83)	5(42)	7(58)	12
23F	4 (67)	4(67)	2(33)	6
6B	4 (100)	2(50)	2(50)	4
6A	3 (100)	1(33)	2(67)	3
19A	2 (100)	1(50)	1(50)	2
All 6 types	53 (77)	34(49)	35(51)	69
Other	14 (88)	9(56)	7(44)	16
Total	**67(79)**	**43(51)**	**42(49)**	**85**

1) All strains resistant to penicillin, singly or multiply resistant
2) Resistant to one antibiotic only
3) Strains resistant to more than one antibiotic
4) Total number of resistant strains.

TABLE 4.9 Resistance patterns and serotypes of nasopharyngeal isolates based on breakpoint concentrations given in Table 3.1and Table 3.2

Serotype	*RESISTANCE TO*										
	Pen[1)]	*Tet*[1)]	*Cot*[1)]	*Chl*	*Rif*[1)]	*Pen/Cot*	*Pen/Tet*	*Pen/Cot/Tet*	*Cot/Tet*	*Eryth/ Cli*[1)]	*Total (Nasopha-ryngeal isolates)*
19F	13(9)[2]	2	1	4	1	15 (4)		2	1	2	42[(3)]
14	3 (1)[3)]	1			1	1	1	5			12 (1)
23F	1		3			2	1				6
6A	1					1		1			3
6B	2					1		1			4
19A	1 (1)						1				2 (1)
1B	1	1					2				4
13	1					1					2
28	1	1									2
9N	1						1				2
7							1				1
4						1 (1)					1 (1)
21	0 (1)[4)]										0 (1)[4)]
29	1										1
5	1										1
10	1										1
24							1				1
Total	**28(12)**	**5**	**4**	**4**	**2**	**22 (5)**	**8**	**9**	**1**	**2**	**85 (17)**

1) Pen, Tet, Cot, Chl, Ery, Cli, Rif denote penicillin, tetracycline, cotrimoxazole, chloramphenicol, erythromycin, clindamycin and rifampicin, respectively
2) The numbers of blood cultures showing particular resistance patterns are given in brackets.
3) One of the two nasopharyngeal isolates showed high level resistance (MIC, 2 µg/ml)
4) This strain was isolated from the blood culture but not from the nasopharynx

4.3.5 Antibiotic resistance patterns in relation to serotypes

The antimicrobial resistance patterns of the different *S pneumoniae* serotypes are given in Table 4.9 (all isoaltes) and in Table 4.10 (patterns of the six most common serotypes isolated from children worldwide).

Serotype 19F was by far the most common type isolated (42; 49.4%) followed by type 14 (12; 14.0%), type 23F (6; 7%) 6B (4; 4.7%), 1B (4; 4.7%) and 6A (3; 3.5%).

The very high prevalence of pneumococcal resistance in the Maputo children suggests that clones of *S pneumoniae* may well be prevalent in Mozambique and DNA fingerprinting may well yield rewarding findings, especially with regard to serotype 19F, because this strain is so common in the community studied, as well as serotype 23F, a clone of which has been shown to have spread to many countries, including South Africa[11]. Especially striking is the large number (31) of antibiotic-resistant pneumococcal isolates found in children coming from the suburb Polana Canico.

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Monitoring and surveillance of pneumococcal resistance and if possible, resistance in *Haemophilus influenzae* from the nasopharynx of children attending paediatric units at Hospital Central de Maputo as well as surrounding hospitals, should be implemented. Ideally such surveillance should be ongoing.

Data generated in this way should be reviewed frequently to establish any obvious trends and patterns, but at least every 3 years update surveys should be performed.

As serotype 19 F was very common in my study, it would be interesting to see any changes that may occur with regards to resistance and serotypes in the next few years. The use of antibiotics should also be closely monitored to prevent abuse and a consequent increase in resistance patterns. Epidemiological surveillance should be extended nationally as a follow-up to the study of pneumonia.

The introduction of the conjugated polysaccharide vaccine into the community, especially in children under 2 years of age, who have a deficient immune system with regard to mounting as humoral antibody response to polysaccharide antigens, could play an important role in combatting pneumococcal disease in Mozambique. Whether this promising but expensive vaccine will become available in Mozambique, and be affordable, only time will tell.

nasopharyngeal swabbing procedure. Various attempts were made to locate the outstanding cases, but most of these proved fruitless.
Only 2% of the total number of cultures submitted to Johannesburg were either non-viable or contaminated upon receipt in the laboratory at the SAIMR.

5.6 Conclusions and recommendations

In Mozambique due to the socio-economic conditions as a consequence of the war and ongoing drought, there is much suffering amongst children from acute and chronic malnutrition which is highly prevalent in our country[26,27,28,29].

As is well known, there is a vicious cycle between infectious diseases and malnutrition[15,19,20]. Many a time a malnourished child with pneumonia does not get to the hospital or health care centre for various reasons eg lack of transport or lack of knowledge on the mother's part. The undernourished child is also far more exposed to infections especially of the respiratory tract due to concomitant overcrowded conditions[26,27,28,29].

Penicillin resistance in our study was high at 52%. However, penicillin is still one of the cheapest drugs available and it can still be prescribed for the treatment of pneumonia. However the ideal antibiotic would be ceftriaxone (3rd generation cephalosporin) for the empiric treatment of acute bacterial meningitis. Due to severe financial constraints the country will not be able to afford this atibiotic at present. However, my recommendation to the Ministry of Health would still be the implementation of a policy that would allow the adininistration of ceftriaxone to critically ill patients with acute menigitis, irrespective of whether the patient could pay for treatment or not. Such a policy will have to be strictly controlled. If this is possible, consideration could also be given for ceftriaxone to be reserved for cases of typhoid fever, gonorrhoea and chancroid.

may have the same propensity to spread and add to the already high incidence in regions with a high endemic prevalence of resistant strains.
In pneumococcal disease, fortunately, there is the prospect of the use of improved pneumococcal vaccines in the near future, which should cover against high risk patients and specifically young children who would benefit from the conjugate vaccine[9,30]. Another high risk group that could be targeted are HIV-positive patients who are prone to pneumococcal pneumonia and bacteraemia even when their CD_4 counts are still high[31].
Older children and adults respond well to the polysaccharide vaccine[8]. However, the group which would benefit most from the use of a conjugate vaccine would be children especially those under two years of age who do not produce adequate antibody levels to unconjugated polysaccharide antigens.

All of the serotypes identified in our study are already present in the polysaccharide vaccine, but it is important that the serotypes that are common in Mozambican children should be represented in the conjugate polysaccharide vaccine. To ensure this, periodic surveys or ongoing surveillance of serotypes prevalent in children in Maputo would be required.

5.5 Problems encountered during the study and lessons for developing countries

Due to the high illiteracy levels in the country, it was often difficult to establish whether the child had been on any medication prior to hospitalization. It was therefore possible that some children were on treatment when the initial nasopharyngeal swab was taken.

There was a possibilty that some of the sisters in the Casualty Department may not have followed strictly the instructions on the proper procedures for correct specimen collection. Most of the problems in this study occurred in the initial stages and were corrected as a result of continuous communication between the SAIMR and ourselves.

There were specific problems with regard to the collecting of the 6-week post-discharge swabs from patients. Only 73(18.3%) of the 400 cases presented for the follow-up

Blood culture *S pneumoniae* isolates

The blood culture isolates belonged to serotype 19F (nineteen isolates) and to types 14, 19A and 21 (one isolate each).

One additional blood culture isolate was not typed as it was lost due to contamination. The serotypes found in this study are also common in children of most other countries and the conjugate pneumococcal vaccines planned for the future would cover against disease caused by the Maputo strains[4,9,23,25,31]. The large number of 19F isolates which may also constitute a cluster in the Polana Canico suburb suggest spread of clones within this serotype in Maputo and DNA fingerprinting of 19F isolates could establish whether this is the case.

Improvements in socio-economic status with better housing, living standards and nutrition are likely to decrease the risk of acquiring pneumonia in future. The present conditions of poverty including overcrowding and malnutrition, however, aggravate the situation by also increasing the mortality rate of patients with established infections. Malnutrition is especially common in children from 3-5 years of age. Breastfeeding is strongly encouraged but the common trend is that by the time the child is between 2 and 3 years of age there is another sibling in the household leading to the inevitable disadvantage to older children. There is a high attack rate of pneumococcal infection in this low socio-economic population group. The mortality rate in such populations remains high especially in rural areas where access to antibiotics and appropriate treatment are often problematic. Many children are critically ill, particu-larly those with certain negative prognostic features. In the present study the case fatality rate was very low, probably because the children were hospitalized early and treated with penicillin and chloramphenicol which both constitute appropriate therapy in this population group.

Serotype 23F has spread widely in Europe and from there to other continents and countries, including the United States and South Africa[21]. It is possible that other serotypes such as 19F

Studies carried out at the Chris Hani Memorial Hospital showed that patients with meningitis caused by penicillin-resistant pneumococci treated with chloramphenicol did not respond well and therefore should not be used in pneumococcal menigitis in regions where the incidence of penicillin-resistant pneumococci is high[3]. This is certainly the case in Maputo.
Presently the third generation cephalosporins, cefotaxime or ceftriaxone constitute the agents of choice for the initial treatment of acute bacterial meningitis in infants, excluding the neonatal period, and older children and adults. Those two agents are easy to use and can be administered intramuscularly or intravenously. However due to their high cost it is not possible to include them in the treatment regimens listed in the essential drug lists (EDLs) of developing countries. Their use should be approved nationally, by governments and administered selectively for the treatment of meningitis in hospitalised children.

With regard to the treatment of pneumonia in Maputo, penicillin, ampicillin or amoxycillin would be appropriate, despite the high incidence of penicillin-resistant pneumococci[22]. However, treatment with cotrimoxazole may be problematic as a large percentage of pneumococcal isolates in this study proved to be resistant. The same probably also applies to the treatment of otitis media with this therapeutic combination[22]. The use of tetracyclines is contra-indicated in young children.

5.4 Pneumococcal serotypes in Maputo

This study demonstrates the common serotypes found in 128 patients.

Nasopharyngeal *S pneumoniae* isolates

19F was the most common serotype (47.7%), followed by serotype 14 (14%), then type 23F (7%), type 6B and 1B both with 5%, while serotypes 6A, 13, 28, 19A, 9N, 7, 21, 29, 5, 10 and 24 also featured in the study.