MICROBIOLOGICAL AETIOLOGY OF PNEUMONIA IN CHILDREN UNDER FIVE YEARS AT KENYATTA NATIONAL HOSPITAL, KENYA

A DISSERTATION PRESENTED IN PART FULFILMENT FOR THE DEGREE OF MASTER IN MEDICINE (PAEDIATRICS) OF THE UNIVERSITY OF NAIROBI

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This book is dedicated to

KIKI and CIIRU

for their patience and understanding during

MAMA's busy moments

ETHICAL CONSIDERATION

A copy of the study proposal was submitted to the Ethical and Research Committee Kenyatta National Hospital, Nairobi. Permission to carry out the study was granted.

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ABBREVIATIONS

Adeno	Adenovirus
ARI	Acute respiratory infections
BOSTID	Board on Science and Technology for International Development
Coag. neg. Staph	Coagulase negative Staphylococci
E. coli	Escherichia coli
Flu A.	Influenza type A
H. influenzae	Haemophilus influenzae
HSV	Herpes simplex virus
IF	Immunofluorescence
KEMRI	Kenya Medical Research Institute
Kleb.	Klebsiella
Kwash.	Kwashiokor
LA(s)	Lung aspirate(s)
mcg	Microgram
MDCK	Mardin-Darby Canine Kidney
M-Kwash	Marasmic-Kwashiokor
Mo	Months
NPA(s)	Nasopharyngeal aspirate (s)
P_1P_3	Parainfluenza types 1 and 3
RCM	Robertsons cooked meat media
RSV	Respiratory syncytial virus
Sp.	Species
SPS	Sodiumpolyanethol sulfonate
VRC	Virus Research Centre
VTM	Virus transport media
WF_1CO_2	Whole human fetal fibroblosts in 5% humidified carbon dioxide.

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SUMMARY

Percutaneous needle aspiration of the lung was performed on 45 children admitted to Kenyatta National Hospital, over a 3 month period, to determine aetiology of pneumonia.

Positive bacterial cultures were obtained from 49% of the lung aspirates. The commonest bacteria was coagulase negative staphylococcus, 52% followed by Escherichia coli, 16%, Klebsiella, 12%. Haemophilus influenzae contributed to 8% of the isolates, the pneumococcus was not isolated.

Positive viral isolates were obtained in 47% of the lung aspirates. Respiratory syncytial virus, RSV was the most commonly encountered virus. No measles virus was found.

A combined bacterial and viral aetiology was found in 22% of the 23 lung aspirates that underwent complete bacterial and viral studies. Bacteria alone were found in 39% and viruses in 17%.

The coagulase negative staphylococcus was resistant to the penicillins. Gram negative organisms were fairly sensitive to the aminoglycosides.

Minor complications occured in 5(11.9%) of the children, all of whom recovered spnontaneously.

INTRODUCTION

Acute respiratory infections (ARI) are a leading cause for utilization of health services worldwide. They account for 20-40% of the children attending outpatient clinics and 12-35% of admissions of children into hospitals (1).

The most important cause of death is acute infection of the lower respiratory tract in the form of bronchiolitis or pneumonia. Pneumonia alone accounts for about 75% of all deaths reported for acute respiratory infections (2,3). Infants and to a lesser extent children aged below 5 years have the highest mortality due to ARI Mortality may even be as high as 27% of all deaths recorded (2).

In a review of the admissions into the acute care Paediatric Ward of Kenyatta National Hospital, Kenya, ARI were the commonest cause of admissions, accounting for 41% of all the admissions. Broncho-pneumonia formed the majority, 74%. A review of the deaths showed ARI was the leading primary cause of death, accounting for 22% of the total (4,5).

A wide variety of organisms may cause acute respiratory infections. Overall viral agents predominate (1, 6). They are estimated to be responsible for over 90% of the cases of community acquired disease of the upper respiratory tract, and a lesser, but considerable proportion of cases of the lower respiratory tract (1,6). The viral infection may be complicated by bacterial super-infection or cause a severe disease, ending in death (7). Viral infections act as suppressors of the normal respiratory antimicrobial defence thus predisposing to pathogenic bacterial invasion of the lower respiratory tract (1,6).

The most frequent viral agents of lower respiratory tract disease in infants and young children are the respiratory syncytial viruses (RSV), and the adenoviruses, the parainfluenza types, influenza A and B viruses (1,6).

Recent work suggests that bacterial pathogens play a greater role as a cause of lower respiratory tract disease in developing than in developed countries (1,6). The most frequent agents in young children are Streptococcus pneumoniae and Haemophilus influenzae. Staphylococcus aureus predominates in the first six months of life and is probably a complication of measles in older children (1,6). The prevalence of bacterial infections may be favoured by impaired immunity in malnourished children, insufficient immunization coverage, and lack of early health care (1,6).

Aetiological diagnosis of pneumonia in infants and young children is difficult. Clinical features found useful in detecting severe forms of acute lower respiratory infections such as cyanosis, flaring alae - nasi, chest retractions, and coarse crepitations, may not be useful in distinguishing the various syndromes, even to the experienced and well trained clinician (2,8,9). They are not useful in identifying the pathogen responsible. Whereas chest X-rays are useful in detecting pneumonic changes, radiologically normal chest findings have been found in upto 25% of children with the clinical diagnosis of pneumonia. Pneumonic changes were found in a significant proportion with other respiratory tract syndromes (10). Except mycobacteria tuberculosis, Klebsiella and staphylococcal pneumonias, most other bacterial pneumonias have no specific identifying characteristics on chest X-rays (11-12).

Features to distinguish bacterial from viral pneumococcal radiologically have been suggested and attempted (13) but it is believed to be difficult, if not impossible to distinguish the two (11,12). Bacterial cultures of throat swabs and nasophyryngeal secretions are misleading because of the high carriage rate of respiratory bacterial pathogens (1,6,14) Transtracheal aspiration has been rarely applied due to the technical difficulties (15) and potential complications (16). The sensitivity of blood cultures is quite low, only 30% bacterial isolation in pneumococcal pneumonias and 10% of all pneumonias (6). Rapid immunological techniques such as latex agglutination, counter-immuno-electrophoresis or coagglutination have not yet been found very useful in determining bacterial aetiology due to relatively low specificity and sensitivity (6,17).

Lung puncture aspirates have until now offered the best way of determining the aetiology of pneumonia in children (1,6,17-25). Mimica (19) demonstrated the specificity of the procedure, by showing the lung aspirate to be sterile from children with no clinical or radiological evidence of pneumonia. In the studies where it was not possible to determine how many patients had been treated with antimicrobials before lung aspiration, bacterial cultures were positive in 16-33% (20,21). In untreated children bacterial isolation rates of upto 60% have been shown (17,18,19,22,23). Few studies have attempted viral isolation (18,19,20,24), most showing very low viral isolation rates, ranging from 0% to 5% (19,18). In developed countries a low bacterial isolation rate, 11%, has been demonstrated from lung aspirates (24), in keeping with the view that most lung infections in developed countries are caused by non-bacterial agents.

Work done on the aetiology of ARI in Kenya (8,26) utilized blood cultures and nasopharyngeal aspirates for bacterial isolation, and virus identification by immunofluorescence on the nasopharyngeal aspirates. The results showed Streptococcus pneumoniae, Haemophilus influenzae and staphylococci to be the commonest bacterial isolates, while enteroviruses, respiratory syncytial virus, rhinoviruses and measles virus were the commonest viruses, suggesting a possible aetiological role. The more specific method of lung aspiration for lower respiratory tract infections was not utilized.

Appropriate management of ARI requires identification of specific causative agents and knowledge of their antimicrobial sensitivity. Since this information is lacking for patients seen at Kenyatta National Hospital, the author undertook the study.

AIMS

1. To determine the viral and bacterial agents responsible for pneumonia in children admitted to Kenyatta National Hospital, Kenya.

2. To determine the antimicrobial sensitivity pattern of the bacteria isolated.

MATERIALS AND METHODS

The study took place at the Paediatric wards of Kenyatta National Hospital, Kenya, for 12 weeks, the period extending from 1st August, 1986 to 31st October, 1986.

Selection of Study Patients

The study population was selected from children aged 5 years and less admitted to the Paediatric Wards with clinical evidence of pneumonia. Antero-Posterior and lateral views of chest X-rays were taken to help determine the site of the pneumonic process. Only patients with pneumonia at a site suitable for lung aspiration, as described by Shann and Biddulph (27) and as illustrated in Appendix I, were included in the study. A history of any medicine use during the acute illness was taken to identify patients who had had prior antimicobials. Patients who died of pneumonia were also included in the study. Any patient with a history suggestive of pulmonary tuberculosis was excluded from the study.

Nutritional status of the patients was determined using the Welcome classification of malnutrition (28).

A data collection sheet (Appendix IV) was completed for all study cases. An informed consent was obtained from the parent or adult guardian.

Specimen collection

Blood cultures

From each study patient, 2 mls of blood was collected by venepuncture from the antecubital fossa after sterile skin preparation with 1% iodine in 70% alcohol solution. This sample was aseptically inoculated into two blood culture media. Trypticase soy broth with liquoid (SPS) for aerobes and Trypticase soy broth with 10% thioglycollate for anaerobes, 1 ml each as recommended on the label on the bottles, for bacterial isolaton.

Nasopharyngeal Aspirates (NPA)

Nasopharyngeal aspirate collection was done with the child seated on the mother's lap, with the head immobilized between mother's left hand and chest. A sterile plastic nasogastric feeding tube, size 8, with a blunt end and 2 wide openings near the tip was connected to a mucus trap ("Nunc Intermed", Denmark) and a suction pump giving a negative pressure of not less than 100 mmHg. With the pump on and tube pinched the tube was gently worked down one nostril to an estimated distance half-way between earlobe and tip of the nose. While here the tube was released, its tip moved up and down, rotated, then finally withdrawn, the NPA being sucked up into the mucus trap. If the NPA was not enough, the process was repeated through the other nostril. The NPA was divided into 3 portions. The first portion was inoculated into Robertson's cooked meat media (RCM), for bacterial isolation. Direct smears were prepared with the third portion for immunoflourescence staining.

Lung Aspirates (LA)

Lung aspirates were obtained by percutaneous needle aspiration of the lung using a 23G x 1" needle attached to a disposable 10ml. Luer-lok tip syringe with good suction pressure. The procedure as described by Shann and Biddulph (27), and as illustrated in Appendix 1, was followed. The anterior chest wall was avoided to minimize anxiety in the patient. Patients who died of pneumonia had lung aspirations performed on them within $^1/_2$ hour after death. All lung aspirations were done once only, after sterile skin preparation with 1% iodine in 70% alcohol solution. The lung aspirate obtained diluted in $^1/_2$ml of sterile saline was divided into 3 portions and handled in exactly the same manner as the nasopharyngeal aspirates.

Handling of bacteriology specimens

Blood cultures, nasopharyngeal and lung aspirates were all subcultured onto blood agar, chocolate agar and MacConkey media after 18-24 hours incubation at 37°C. All bacterial isolates were identified using the methods of Cowan and Steel (29). Antimicrobial sensitivity testing was done on bacterial isolates whenever possible. The disc diffusion method using Oxoid multodisks containing eight different agents was used. The bacteria were plated on diagnostic sensitivity test (DST) agar incubated overnight at 37°C with the multodisks properly in place. Disc contents are shown in Appendix II.

Handling of virology specimens

Nasopharyngeal and lung aspirates in VTM were transported on wet ice to the Virus Research Centre (VRC) laboratories at Kenya Medical Research Institute (KEMRI). VTM was prepared from Hank's Balanced Salt Solution (HBSS) with 1% albumin and antibiotics to support labile viruses such as repiratory syncytial virus. In the laboratory, the specimens were kept at $4^{o}C$ for inoculation into viral tissure cultures as soon as possible. Whenever immediate transportation to KEMRI was not possible, specimens were kept at $4^{o}C$ in the ward for a period not exceeding 24 hours.

0.1mls of each specimen was inoculated into 1 tube each of 3 cell lines: Vero, MDCK and WF_1CO_2 (Appendix III). Whenever possible, all cultures were examined daily under the microscope for the appearance of cytopathic effects. MDCK cells were followed for 21 days, Vero cells for 28 days and WF_1CO_2cells for 42 days.

Direct smears of the aspirates were prepared on microscope slides. The slides had five or six $1cm^2$squares engraved on them using a diamond pencil. The smears were prepared within the squares, air-dried then transported in covered slide racks to the same laboratories as the specimens in VTM. The smears were immediately fixed in acetone at $4^{o}C$ for 10 minutes then stored at $-70^{o}C$ until a significant number of slides were collected for immunofluorescence staining. Standard procedures for identification of all the viruses were followed (30).

RESULTS

A total of 45 cases were recruited into the study. Of these 26 were males and 19 females giving a male to female ratio of 1.36:1. The age range was 2 months to 60 months, with a mean age of 18 months. The majority, 71%, of the children were less than 24 months of age, as shown in Table 1.

Table 1: Distribution of study cases by age and sex

AGE GROUP	SEX		TOTAL	% of TOTAL
	M	F		
0 - 11 mo	11	8	19	42.2
12 - 23 mo	9	4	13	28.9
24 - 35 mo	5	2	7	15.6
36 - 47 mo	0	3	3	6.7
48 mo	1	2	3	6.7
TOTAL	26	19	45	100%
% of TOTAL	57.8%	42.2%	100%	

As shown in Table II, more than half of the study population were well nourished, a third underweight while 11% had frank malnutrition.

Table II: Nutritional Status of study cases

Nutritional status	Frequency	Percentage
Normal	25	55.6%
Underweight	15	33.3%
Kwashiorkor / M-Kwash / Marasmic	5	11.1%
TOTAL	45	100%

Of the 45 patients 56% had received no treatment while 44% had had some form of treatment before admission into the study. This was either antimicrobials or non antimicrobial therapy.

Table III: Distribution of cases in respect of prior treatment

Group	Frequency	Percentage
No prior treatment	25	55.6%
Prior treatment	20	44.4%
TOTAL	45	100%

BACTERIOLOGY RESULTS

Forty three out of 45 aspirates were successfully processed for bacteriological isolation. 21 of these specimens (48.8%) yielded one or more bacteria . As shown in Table IV bacterial isolation rate did not differ between the group that had received prior treatment and that which had not received prior treatment.

Table IV: Bacterial isolation from 43 Lung Aspirates

	LA-Bacterial result			
GROUP	+VE	-VE	TOTAL	%TOTAL
NO PRIOR TREATMENT	12	12	24	50.0%
PRIOR TREATMENT	9	10	19	47.3%
TOTAL	21	22	43	48.8%

+VE = Positive

-VE = Negative

Out of the 21 positive aspirates 25 bacteria were isolated as shown in Table V. The commonest organism was the coagulase negative staphylococcus 13(52%) followed by the gram negatives, E. coli and Klebsiella sp., 16% and 12% respectively. H. influenza contributed to 8% of total isolates while Streptococcus pneumoniae was not isolated.

Table V: Bacteria isolated from Lung Aspirates

LA BACTERIA	NO PRIOR TREATMENT	PRIOR TREATMENT	TOTAL	% TOTAL
H. influenza	2	-	2	8%
E. coli	2	2	4	16%
Klebsiella sp.	-	3	3	12%
Coag. neg. staph.	9	4	13	52%
OTHERS:				
Eiknella	-	1	1	12%
Lactobacillus	1	-	1	
Diptheroids	1	-	1	
TOTAL	15	10	25	100%

As shown on Table VI, all bacteria were fairly distributed across the age groups except E. coli which was not found beyond 24 months of age, but the numbers are too small for any valid conclusions to be made.

Table VI: Distribution of bacterial isolates by age groups

BACTERIA	AGE GROUP (MONTHS) 0 - 11	12 - 23	⩾24	TOTAL
H. influenza	1	-	1	2
E. coli	1	3	-	4
Klebsiella sp.	1	1	1	3
Coag. neg. staph.	4	3	6	13
Others:	1	1	1	3
Total	8	8	9	25
% Total	32%	32%	36%	100%

The severely malnourished children and the organisms isolated from them were too few for any valid conclusions to be made but it appears that infection with well-recognised pathogenic bacteria like H. influenzae, coagulase negative Staphylococcus, E. coli and Klebsiella species was less common in children with severe malnutrition.

Table VII: Distribution of bacterial isolates by nutritional status

BACTERIA	NUTRITIONAL STATUS			TOTAL
	NORMAL	UNDER WT	KWASHIORKOR M-KWASH MARASMIC	
H. influenza	2	-	-	2
Coag. neg. staph.	10	3	-	13
E. coli	1	2	1	4
Klebsiella sp.	-	3	-	3
Others: (Eiknella, Diptheroid bacilli Lactobacillus)	-	2	1	3
TOTAL	13	10	2	25
% TOTAL	52%	40%	8%	100%

Forty pairs of blood were submitted for bacterial cultures. Only one pair yielded organisms, Klebsiella, giving a bacterial isolation rate of 2.5%.

25 Nasophryngeal aspirates were submitted for bacterial isolation. 21 of these yielded 26 bacterial isolates as shown in Table VIII, giving an upper respiratory tract bacterial carriage rate of 84%. It was mainly of Gram negative organisms: Klebsiella and E. Coli, 34.6% and 30.8% respectively. There was no pneumococcal nasopharyngeal carriage.

Table VIII: Bacterial isolates from the nasopnaryngeal aspirates (NPA)

Bacteria	No prior treatment	Prior treatment	Total isolates	% Total
H. influenza	1	-	1	3.8%
Klebsiella sp	4	5	9	34.6%
E. coli	8	-	8	30.8%
Coag. neg. staph.	4	-	4	15.4%
Others:				
Salmonella sp.	1	-	1	
Haemolytic strep.	1	-	1	
Proteus sp.	-	1	1	15.4%
Branhamella catarrhalis	-	1	1	
Total	19	7	26	100%
% of Total	73.1%	26.9%	100%	

Only 7 cases yielded the same bacteria from the lung aspirate and nasopharyngeal aspirate. Coagulase negative Staphylococcus was paired 4 times, E. coli 2 times and Klebsiella once. Each time the coagulase negative Staphylococcus was isolated from the nasopharynx, it was also isolated from the lung aspirate, signifying a pathogenic role if found in the upper respiratory tract.

VIROLOGY RESULTS

45 lung aspirates (LA) and 24 Nasopharyngeal aspirates (NPA) were submitted for viral studies. Only 33.3% of the specimens were processed through all the 3 cell lines used, i.e. 21 days in MDCK, 28 days in vero cells and 42 days in WF_1CO_2. 46 (66.6%) of the specimens did not go through WF_1CO_2 cells because of repeated contamination of this cell line grown in the 24-well plastic tissue culture plates (Linbro).

Table IX: Distribution of specimens submitted and processed for viruses

Specimen	Number submitted	Number (%) Processed
LA	45	15 (33.3%)
NPA	24	8 (33.3%)
TOTAL	69	23 (33.3%)

Two methods used for viral isolation were, Immunofluorescence and tissue cultures. 38 direct smears underwent immunofluorescence studies, and this comprised 23 LAs and 15 NPAs, as shown in Table Xa. A viral isolation rate of 52.6% was demonstrated by this method. 23 specimens were cultured through the 3 cell lines, comprising of 15 LAs and 8 NPAs. A viral isolation rate of 56.5% was demonstrated by this method as shown in Table Xb.

Table Xa: Virus isolation by Immunofluorescence on direct smears

	IF Result		
Specimen	+ve(%)	-ve	Total
LA	9 (39.1%)	14	23
NPA	11 (73.3%)	4	15
TOTAL	20 (52.6)	18	38

Table Xb: Virus isolation by Tissue Culture Method

	Tissue Culture Results		
Specimen	+ve(%)	-ve	Total
LA	7 (46.7%)	8	15
NPA	6 (75)	2	8
TOTAL	13 (56.5)	10	23

Nasopharyngeal aspirates seem to give a better virus yield (73.3% and 75%) compared to lung aspirates (39.1% and 46.7%) regardless of the method used for isolation.

Viruses isolated are shown on Table XI. A total of 24 viruses were identified. The most commonly encountered virus was RSV, 62.5% of total isolates. All isolates from the lungs were single. Multiple isolates comprising RSV and HSV were found once from the nasopharynx. Positive viral isolations from both NPA and LA were found in 6 patients, while identical NPA and LA results were obtained in 5 patients, 4 being RSV and the other influenza A. Only 5 patients with viral isolations on nasopharyngeal aspirates were negative on lung aspirates. The viruses involved were Parainfluenza type 1, in 3 patients and RSV in 2 patients.

Table XI: Viruses isolated

	SPECIMEN			
VIRUS	LA	NPA	TOTAL	% TOTAL
Respiratory syncytialvirus (RSV)	8	7	15	62.5%
Parainfluenza type 1 (P_1)	1	3	3	12.5%
Herpes simplex virus (HSV)	1	1	2	8.3%
Influenza type A (Flu A)	1	1	2	8.3%
Adenovirus	1	-	1	4.2%
Parainfluenza type 3 (P_3)	1	-	1	4.2%
TOTAL	12	12	24	100.0%
% TOTAL	50%	50%	100%	

No measles virus was isolated. A total of 9 patients has suffered measles diaganosed on clinical features. Three developed the rash 24 hours after recruitment into the study. Six presented after appearance of the rash at periods ranging from 4 to 10 days, only 4 had NPA direct smears processed, none of which had measles virus detected. However, 2 isolates of Para-influenza type 1 and one RSV were isolated from these 4 direct smears.

Virus isolation by age groups is shown on Table XII. A fairly uniform distribution of the viruses across the age groups was noted.

Table XII: Virus isolation by age groups

	AGE GROUPS (MONTHS)			
VIRUS	0-11 MO	12-23 MO	⩾24 MO	TOTAL
RSV	2	3	3	8
Others:				
(P_3, Adeno, Flu A, HSV)	2	-	2	4
Total	4	3	5	12

Only 23 lung aspirates underwent complete bacterial and viral studies. An aetiological factor was identified in 18 (78.3%) of the cases. Bacteria alone were found in 39.1% of the cases. Bacteria and viruses combined were found in 21.7% of the cases and viruses alone in 17.4%. No organisms were found in 21.7% of the cases, as shown on Table XIII

Table XIII: Distribution of aetiological factors from 23 LAs

Aetiology	Frequency		Percentage	
Bacteria only	9		39.1%	
Bacteria + Virus	5	18	21.7%	78.2%
Virus onlyu	4		17.4%	
No Isolate	5		21.7%	
TOTAL	23		100.0%	

Bacteria are the most important cause of pneumonia in our set up. Viruses also contributed significantly whether alone or in combination with bacteria.

RADIOLOGY RESULTS

Chest X-rays of 42 patients were available for reporting. As shown on Table XIV there were more bronchopneumonic changes, 66.7% than lobar peneumonic changes, 28.6%. A combined broncho and lobar pneumonic changes was seen in 4.7% of the X-rays.

Table XIV: Distribution of pneumonic changes in 42 chest X-rays

Feature	Frequency	Percentage
Lobar pneumonia	12	28.6%
Bronchopneumonia	28	66.7%
Broncho- + Lobar pneumonia	2	4.7%
TOTAL	42	100.0%

Forty chest Xrays were available for comparison with the corresponding lung aspirate results. Organism isolation rate is as shown on Table XV.

Table XV: Lung aspirate result compared to radiological feature

	LA Result		
Radiological Feature	+ve(%)	-ve	Total
Broncho-	15 (55.5%)	12	27
Lobar-	6 (54.5%)	5	11
Broncho + Lobar	-	2	2
Total	21 (52.5%)	19	40

Only 52.5% of the radiological pneumonias yielded a positive result. There was no difference in the organism yield between lobar (54.5%) and bronchopneumonic changes (55.5%).

Table XVI: Organism isolated compared to radiological feature

	Organisms isolated from LA				
Radiological Feature	Bacteria Only	Viruses Only	Bacteria + Viruses	No Organ-ism	Total
Broncho-	11	1	3	12	27
Lobar-	3	2	1	5	11
Broncho + Lobar	-	-	-	2	2
Total	14	3	4	19	40

Radiological features are not specific to organisms causing them, both bacteria and viruses being found in either broncho or lobar pneumonias. Four pleural effusions were seen. Two yielded no organism, one yielded coagulase negative staphylococcus and the other Eiknella with RSV.

ANTIMICROBIAL SENSITIVITY

Multodisk sensitivity tests were done on afew of the coagulase negative staph., Klebsiella sp., E. coli, Lactobacillus and Eiknella. H. influenza were not tested. Sensitivity testing was done on five out of 13 coagulase negative staphylococci from LA specimens. 100% showed resistance to Penicillin and Methicillin. None were resistant to Minocycline and Lincomycin.

Table XVII: Antimicrobial sensitivity of coagulase negative Staphylococci

Antimicrobial	Study numbers 05	07	08	09	20	% Resistant
Penicillin G	R	R	R	R	R	100%
Methicillin	R	R	R	R	R	100%
Ampicillin	R	R	R	S	R	80%
Erythromycin	R	R	R	S	R	80%
Cotrimoxazole	R	R	R	S	R	80%
Chloramphenicol	R	S	R	S	S	40%
Minocycline	S	S	S	S	S	0%
Linocmycin	S	S	S	S	S	0%

R = Resistant

S = Sensitive

Antimicrobial sensitivity was done on the four Klebsiella species isolated from the lungs. All of these showed resistance to Ampicillin and Sulfonamide. Minimal resistance is shown to Aminoglycosides and Chloramphenicol, as shown on Table XVIII.

*The Klebsiella isolated in combination with RSV showed resistance to all antibiotics tested. The patient had been on Benzyl penicillin and Gentamicin for 5 days.

Table XVIII: Antimicrobial sensitivity of Klebsiella sp.

Antimicrobial	Study numbers 15	20	31	3*	% Resistant
Ampicillin	R	R	R	R	100%
Sulfonamide	R	R	R	R	100%
Tetracycline	R	R	S	R	75%
Cotrimoxazole	R	S	S	R	50%
Streptomycin	R	S	S	R	50%
Gentamicin	S	S	S	R	25%
Kanamycin	S	S	S	R	25%
Chloramphenicol	S	S	S	R	25%

Three of the four E. coli isolated from the lung aspirates were tested for antimicrobial sensitivity. As is shown in Table XIX the E. coli isolated appreared to be relatively sensitive to all of the antimicrobials tested.

Table XIX: Antimicrobial sensitivity of Escherichia coli

Antimicrobial	Study numbers 15	28	32	% Resistant
Ampicillin	R	S	S	33.3%
Sulfonamide	R	S	S	33.3%
Chloramphenicol	S	S	S	-
Gentamicin	S	S	S	-
Kanamycin	S	S	S	-
Streptomycin	S	S	S	-
Cotrimoxazole	S	S	S	-
Tetracycline	S	S	S	-

COMPLICATIONS

Forty-two lung apirations were done on live children, and in these, complications occured in 5 (11.9%). The two complications observed were laminar pneumothorax, detected only on chest Xray, in 7.1% of the cases, and transient haemoptysis in 4.8% as shown below in Table XX.

Table XX: Complications of lung aspiration

Complication	Frequency	Percentage	
Transient haemotysis	2	4.8%	
Laminar pneumothorax	3	7.1%	11.9%
No complication	37	88.1%	
TOTAL	42	100.0%	

Mortality:

Four children died during the course of the study, giving a mortality rate of 9.5%. One child died at two days and another two weeks after lung aspiration. Post-procedure chest X-rays did not show pneumothorax in these patients. Two others died at 6 hours and 11 hours after lung apsiration. Both had severe dehydration following diarrhoea. Post procedure chest X-rays could not be obtained. There were no clinical features to suggest pneumothorax or haemothorax. Post-mortems were not done on any of them. The four children were malnourished (3 underweight, 1 Marasmic).

DISCUSSION

Kenyatta National Hospital is a referral hospital which also serves as a primary health care centre for the city of Nairobi and its environs. The study population consisted of moderate to severe cases of pneumonia since they were ill enough to be treated as inpatients.

The short period of study, the limited number of specimens that could be handled by the laboratories per day, and occassional lack of X-ray films, limited the number of cases that were recruited into the study or completely studied.

The majority (71%) of the study cases were less than 24 months of age, in keeping with the observation that severe forms of ARI are common in the younger children and particularly those below one year (2,6,19,20,21).

Antimicrobial serum levels could not be determined. A history of medicine use was therefore obtained, to identify patients who had had prior antimicrobial therapy. Although 56% of them denied prior treatment, bacterial isolation rate in this group, (50%) did not differ from the group that admitted prior treatment (47%). Therefore, history alone was not enough to distinguish the two groups. This is not surprising, Kenyatta Hospital being a referral hospital, is likely to receive patients who have had prior treatment, antimicrobials included.

Bacterial isolation rates have varied form 13-79%, being higher in the preantibiotic era and in patients who had not received antimicrobials (17,19,20,22,24). Where prior antimicrobial therapy could not be determined, bacterial isolation rates of 33% and 16% have been shown in Calcutta and Colombia, respectively (20,21). In this study it was 49%, within the wide range reported.

Only organisms of known pathogenicity were considered to be of aetiological significance in the Calcutta study (20). Isolations of "non pathogenic" bacteria were not found in the Colombia study (21). In this study, any organism isolated from the lungs was considered to be aetiologically significant since the lung has been shown to be sterile in noraml individuals (19,31,32). Mimica (19) showed cultures of needle aspirates from the lungs of 13 normal children to be all sterile. "Non-pathogenic" organisms had been commonly found in malnourished children, while infection with well recognized pathogens was less common in children with severe malnutrition in the study by Mimica. The number of malnourished children and the organisms isolated from them in this study were too small for any conclusions to be made on them.

Streptococcus pneumoniae and Haemophilus influenzae have been shown to be the commonest cause of pneumonia in childhood, comprising about 80% of the positive findings. This information was from untreated children (17,18,24). The types of organisms isolated in this study most likely represent organisms associated with moderate to severe disease, but of a limited variety due to lack of seasonal variation which has been shown to affect the types of organisms isolated in this environment (8,26). The commonest bacteria isolated was the coagulase negative Staphylococcus, 52% followed by Escherichia coli, 16% and Klebsiella species, 12%. Haemophilus influenzae contributed 8% of the total isolates; pneumococcus was not found. Pneumococcus is highly susceptible to antimicrobials especially penicillin and its derivatives, being markedly and rapidly inhibited by concentrations as low as 0.02-0.05 mg/ml of penicillin (34). In this study although prior antimicrobial use could not be determined, the type of sensitivity pattern shown by the organisms, especially coagulase negative staphylococcus' resistance to penicillin and its derivaties, and significantly (80%) to the others suggests prior exposure of the organisms to these antimicrobials. This

could have contributed to the failure to isolate the pneumococcus and a low isolation rate for H. influenzae. Bedside inoculation of culture media, which was not feasible in this study, has been shown to enhance yield of fastidious organisms (17,18,22,24,30). Transport media such as RCM used in this study support little or no growth of organisms (33), and unless the inoculum contains a significant number of organisms, they are unlikely to be isolated. An enrichment medium or nutrient broth to promote growth of organisms has been found usefull (15,17,19,20,21,23,30). An example of a satisfactory general enrichment media is Thioglycollate medium without indicator (34) which was not available at the time of this study.

Gram stain of aspirates obtained was overlooked in this study; but this has been shown to improve bacteria identification (17,21).

Pneumococci and H. influenzae have complex nutritional requirements (34). Some studies have employed special methods to favour their isolation (15,18,20,23). These included use of Rabbit or Horse blood as culture media, Gentamicin in blood for pneumococcus, Bacitracin chocolate agar and intraperitoneal inoculation of mice (15) for H. influenzae. Organisms resembling pneumococcus have also been identified using the Quellang reaction with type specific sera (15). None of these were employed in this study. Although growth of organisms is apparent within 24 hours, longer incubation, and possibly further subcultures at 48 hours and 72 hours for aspirates and blood subcultures on days 1,3 and 7 are recommended for a better yield (34). In this study, specimens were considered negative if no growth was obtained after a single subculture done 18-24 hours after incubation at 37°C, due to limited facilities, All these could have contributed to the lower yield of organisms.

In developing countries, few workers have attempted virus isolation (18,19,20,24) with results ranging from zero to 5% viral isolation rates (18,19). Laboratory facilities have been the major drawback in most of these studies. For the same reason results obtained in this study were based on only one third of the specimens submitted. However, viral isolation rates of 39.1% by immunofluorescence and 46.7% by tissue culture methods on lung aspirates were obtained. RSV was the most commonly encountered virus, contributing to 63% of the total viruses isolated, a finding similar to Wafula's (8) and to developed countries (1,6,7). Similarly, other viruses included Parainfluenzae, influenza types and adenovirus. In this study, Parainfluenza type 1 was only found in the upper respiratory tract. Although Wafula (8) found RSV commonly in association with pneumonia, the major viruses included enteroviruses and measles which were not found in this and other studies (18,19,20,24). Measles diagnosed on clinical grounds has subsequently been shown to have other viruses in this and other studies (20,24).

Out of 23 cases where specimens underwent both bacterial and viral studies, 22% combined aetiology was found. This suggests significant contribution to aetiology of pneumonia by both bacteria and viruses, bacteria being the most important. Bacteria alone were found in 39% of the 23 cases studied for both bacteria and viruses. Failure to identify an organism in 22% of the cases suggests some other factors such as chlamydiae, mycobacteria tuberculosis, fungi etc which were not looked for. The contribution to pneumonia aetiology by both bacteria and viruses, whether a primary or secondary role, is not quite clear. Viruses have been shown to act as suppressors of the normal respiratory antibacterial defence by impairing the mucociliary clearance and altering the functions of neutrophils, alveolar macrophages and T-lymphocytes (1,6).

Although RSV has been associated with certain specific syndromes of ARI in infancy, as a whole viral isolation did not seem to vary with age.

For successful isolation of any virus, collection of specimens in the acute phase of the infection is recommended, when virus is present in high concentrations (35). 49% of the children in this study had been ill for more than 3 days. The uncontainated primary cell lines such as Primary Monkey kidney (PMK) or Human Embryonic Kidney (HEK) cells are recommended for isolation of respiratory viruses. Alternatives suggested being Hep-2, MDCK, LLC-MK_2 and MRC-5, (35) most of which were not available at the time of this study. Vero and WF_1CO_2 had been adopted for this purpose by the KEMRI (VRC) laboratories (Appendix III). Hep-2 cell line, the human heteroploid cell line derived from carcinoma of the larynx, is recommended for isolation of RSV and adenovirus. MDCK cell line, a diploid cell from the Madin-Darby Canine Kidney, is recommended for influenza. LLC-MK_2 cell line, a heteroploid cell line from the rhesus monkey kidney, is recommended for isolation of parainfluenza viruses. MRC-5 cell line, a diploid fibroblast cell line from human embryonic lung, is recommended for isolation of Cytomegalovirus, Rhinoviruses and Herpes viruses. Enteroviruses are best isolated using primary cell lines. Measles virus can also be isolated using primary cell lines (35).

Organisms isolated from the upper respiratory tract are of doubtful aetiological role in severe forms of ARI. In this study only 16% of the cases had the same bacteria isolated from lungs and nasopharynx simultaneously. Similarly poor concordance between upper and lower respiratory tract isolates has been found by other workers (17,19,22,23,30), Bacteria from the upper respiratory tract have been

used to indicate carriage rate. Streptococcus pneumoniae has been chosen for this purpose (1,6). An isolation of upto 100% in healthy children has been reported in developing countries and less than 50% in the developed countries (1,6). Wafula (8) found a carriage rate of 14.2%, 80% was reported in Papua a New Guinea (18) and upto 85% in Senegal (6). A zero percent rate was found in this study, possibly for the reasons discussed earlier.

Blood cultures were not found useful in determining pneumonia aetiology in this and most other studies (6,17,20,23). An isolation rate of 2.5% was found. Wafula (8) found a rate of 23.2% overall for all ARI. No pathogens were isolated in the Calcutta study (20).

Radiological features were not found useful in determining the aetiology of pneumonia in this and other studies (17,15,22,24). Wafula and Muruka (10) found normal chest X-rays in 25% of the children with clinical diagnosis of pneumonia, whereas pneumonic changes were found significantly in children with the diagnoses of brochiolitis, upper respiratory tract infection, croup, bronchospasm and mixed infections. The discrepancy between clinical diagnosis and radiological findings partly explained by the known lag of radiological changes behind clinical features may have resulted in many children with early pneumonia being excluded from this study in which pneumonia had to be diagnosed clinically before chest X-rays could be obtained. The unspecificity and inadequacy of clinical features relied on in diagnosis of pneumonia noted in many studies (8,10,19,36) may also have contributed to exclusion of a number of true early pneumonias and therefore delay in collection of specimens in the present study. More bronchopneumonic changes than lobar were noted as elsewhere (10,24). There was no difference in organism isolation rate between the two changes. The diffuse interstitial process

considered compatible with viral pneumonia was not reported. It was not possible to differentiate viral from bacterial pneumonia by looking at the features and distribution on a chest X-ray, neither was it possible to tell which one was going to yield a positive result on lung aspiration (11,17,19,22,24).

Complications following percutaneous needle aspiration of the lung have been fairly minor. These include transient haemoptysis, pneumothorax and local subcutaneous emphysema. In this study, the complication rate was 11.9%, within the range reported in most other studies of 2.4% to 16% (17,24). Pneumothorax was only diagnosed on chest X-rays. Most of the cases of pneumothorax have been minor and self-limiting, a few have required chest-tube drainage (18,19,21). In this study all recovered spontaneously. A high rate of complications has been observed in immunocompromised children, 32% (37) and complex pneumonias of underlying life threatening disease, 43.6% (30). Contraindications to this procedure should therefore include immunocompromised children, evidence of air-trapping in form of emphysema, bullae and pneumothorax, bleeding and clotting disorders, atherosclerosis, pulmonary hypertension, severe uncorrected hypoxia, and where mechanical positive pressure ventilation is likely to be required. Although lung aspiration is a fairly safe and reliable diagnostic procedure in the correctly selected patient, it should be reserved for cases of pneumonia of questionable aetiology unresponding to the standard management.

For efficient case management within primary health care, and as a component to the strategy to control of ARI, antimicrobial use should include those to which the organisms concerned are sensitive. Often this is challenged by increasing resistance of the organisms to the commonly

used antimicrobials. In recent years, relative penicillin resistance by the pneumococcus has been reported (37-40) with higher levels and more frequent prevalence in the developing countries. Studies at Kenyatta National Hospital indicate some resistance to penicillin by pneumococcus (41). Increasing ampicillin resistance by Haemophilus influenzae has also been reported (42). It therefore means that close surveillance on therapeutic efficacy and microbial drug sensitivity is necessary. Not much can be commented on the antimicrobial sensitivity obtained in this study, because of the small numbers involved, but some resistance is suggested by the rather unusual coagulase negative staphylococcus isolated in this study.

Resistance observed in other studies is relative in most cases and can be overcome with injectable penicillins, if necessary at higher concentrations. In many developing countries including Kenya, penicillin still remains the first line of treatment for acute pulmonary infections (1, 6) unless other more extensive studies suggest otherwise.

CONCLUSION

1. Bacteria are the commonest case of pneumonia in our set up. Viruses also contribute significantly, singly or in combination with bacteria.
2. The rather atypical pattern of bacteria demonstrated probably reflects the pattern associated with moderate to severe disease in a tertiary care centre or probably, a situation peculiar to our environment.
3. Upper respiratory tract secretions, blood cultures and chest X-rays are not very useful in determining the aetiology of pneumonia in our set up.
4. Antimicrobial sensitivity done on a few of the organisms suggests some resistance to the commonly used antimicrobials.
5. Lung aspiration is a fairly safe diagnostic procedure, but it should be reserved for pneumonias of questionable aetiology unresponding to the standard management.

RECOMMENDATIONS

1. A further study combining urban and rural populations is suggested.
2. A longer period of study to allow for seasonal variation, which is known to affect both incidence of ARI and types or organisms concerned.
3. Determination of serum antimicrobial levels to identify patients who have had prior antimicrobials.
4. Use of more selective methods for isolation of bacteria such as pneumococcus and H. influenzae
5. Continued surveillance of antimicrobial sensitivity of organisms responsible for ARI since there is evidence to suggest resistance to some of the commonly used antimicrobials.
6. Penicillin remains the first line of treatment for acute pulmonary infections.

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REFERENCES

1. A programme for controlling acute respiratory infections in children: Memorandum from a WHO meeting, Bull. WHO 62:47, 1984.

2. Clinical Management of acute respiratory infections in children: a WHO memorandum.
Bull WHO 59: 707, 1981

3. Bulla, A. Hitze, L.
Acute respiratory infections: A review.
Bull. WHO 50:481, 1978

4. Mortality figures for the Republic of Kenya, 1980, Statistics from the Ministry of Health, Republic of Kenya.

5. Mortality figures for Paediatric Observation Ward, Kenyatta National Hospital, 1981

6. Pio, A., Loewski, J., tenDam, H.G.
The magnitude of the problem of acute respiratory infections.
International Workshop on Acute Respiratory infections in children, Syudney, 27-31 August 1984.

7. Downham, M.P., Gardner, P.S., McQuillin, J., Ferris, J.A.J. Role of Respiratory viruses in childhood mortality
Br Med J. 1: 235, 1975

8. Wafula, E.M., Tukei, P.M., Bell, T.M., Nzanze, H., Ndinya-Achola, J.O., Hazlett, D.T.G., Ademba, G.R., Pamba A.
Aetiology of acute respiratory infections in children aged below 5 years in Kenyatta National Hospital.
East Afr Med J 62: 757, 1985

9. Wafula, E.M., Tukei, P.M., Bell, T.M., Nzanze, H., Ndinya-Achola, J.O., Hazlett, D.T.G., Ademba, G.R., Pamba A.
How should Primary Health Workers diagnose and treat acute respiratory infections?
East Afr Med J 61: 736, 1984

10 Wafula, E.M., Muruka FJ., Chest X-rays in children with a acute respiratory infections or bronchospasm at Kenyatta National Hospital
East Afr Med J 61: 900, 1984

11. Caffey, J.
Pediatric X-ray Diagnosis (7th Edn)
Vol.I, Yearbook Medical Publishers, Inc. Chicago, 1978 pg. 394

12. Behrman, R.E., Vaughan, V.C.III.
Nelson Textbook of Pediatrics (12 Edn)
WB. Saunders Campany, Philadelphia, 1983, pg 1046

13. Swischuk L.E., Hayden C.K.,
Viral vs. Bacterial pulmonary infections in children (Is roent-genographic differentiation possible?).
Pediatr Radiol 16: 278, 1986

14. Loda
Occurence of D. Pneumonia in upper respiratory tract of children.
J Pediatr 87: 1087, 1975

15. Davidson, N., Tempest, B., Palmer, D.L. Bacteriological diagnosis of acute pneumonia
JAMA 235: 158, 1976

16. Spencer, C.D. Beaty, H.N.
Complications of transtracheal aspiration
N. Engl J Med 286: 304, 1972

17. Silverman, M., Stratton, D., Diallo, A., Egler, LJ. Diagnosis of acute bacterial pneumonia in Nigerian children.
Arch Dis Child 52: 925, 1977.

18. Shann, F., Germer, S., Hazlett, D.T.G., Grattan, M., Linnemann, V., Payne, R.
Aetiology of pneumonia in children in Goroka Hospital
Papua New Guinea
Lancet 2: 537 1984

19. Mimica, I., Denoso, E., Howard, J.E., Ledermann, W. Lung puncture in the aetiological diagnosis of pneumonia.
Am J Dis Child 122: 278, 1971

20. Hughes, J.R., Sinha, D.P., Cooper, N.R.,
Shah, K.V., Bose KS,
Lung tap in childhood
Pediatrics 44:477, 1969

21. Garcia, O.D., Hugo, T.S., Alvaro, U.P., Nancy A.D. Lung puncture aspiration as a bacteriologic diagnostic procedure is acute penumonias in infants and children.
Clin. Pediatr 10: 346, 1971

22. Kalra, S.K., Sasidharan, T., Vatwani, V., Sarkar, P. Lung punctrue: a diagnostic aid in childhood pneumonia.
Indian Pediatr 18: 727, 1981

23. Diakparomre, M.A., Obi J.O.
Aetiological diagnosis of pneumonia in childhood by lung puncture
Nigerian J Pediatr. 8:61, 1981

24. Escobar, J.A., Dover, A.S., Duenas, a., Leal, E., Medina P. Etiology of respiratory tract infections in children in Coli, Colombia.
Pediatrics 57: 123, 1976

25. Rapkin, R.H.
Bacteriologic and clinical findings in acute pneumonia in childhood
Clin Pediatr 14: 130, 1975

26. Wafula, E.M., Ndinya-Achola, J.O., Tukei, P.M., Hazlett, D.T.G., Bell, T.M. ADEMBA, G.R.
Preliminary results of investigations into the aetiology and epidemiology of acute respiratory infections in children in a rural area of Kenya.
7th Annual Medical Scientific Conference of Kenya Medical Research Institute (KEMRI) and Kenya Trypanosomiasis Research Institute (KETRI). 3rd - 8th February, 1986.

27. Shann, F., Biddulph, J.
Lung aspiration, in Paediatrics for Doctors in Papua New Guinea, Rotary Club Waverley, Port Moresby, Papua New Guinea, 1983, p.185.

28. Editorial:
Classification of Infantile Malnutrition
Lancet 2: 302, 1970

29. Cowan, S.T.
Cowan and Steels' Manual for identification of medical bacteria (2nd Edn.). Cambridge University Press, 1977.

30. Palmer, D.L., Davidson, M., Lusk, R.
Needle aspiration of the lung in complex pneumonias
Chest 78: 16, 1980

31. Finland, M.
Diagnostic lung puncture
Pediatrics 44: 471, 1969

32. Laurenzi, G.A., Porter, T.T., Kase, E.H.
Bacteriologic flora of the lower respiratory tract.
N. Engl. J. Med 265: 1273, 1961

33. Ndinya-Achola, J.O.
Dept. of Medical Mircrobiology, School of Health Sciences,
University of Nairobi, Kenya.
Personal Communication

34. Lennette, E.H., Balows, A: Hausler, W.J., Sahdomy, H.J.
Manual of clinical microbiology, (4th Edn.)
American Society for Microbiology, Washington DC 1985.

35. BOSTID Research Grants Program on Etiology and Epidemiology of acute respiratory infections in children.
Manual of laboratory procedures for diagnosis of respiratory virus infections.

36. Leventhal, J.M.
Clinical Predictors of pneumonia as a guide to ordering chest roentgenograms
Clin Pediatr 21:730, 1982

37. Chaudhary, S., Hughes, W.T., Feldman, S.
Percutaneous transthoracic needle aspiration of the lung.
Am J. Dis Child 131: 902, 1977

38. Sash, A.H., Malionee, J.P., Tarpay, Thornsbery, C.T. Relative resistance to Penicillin in the pneumococcus.
JAMA 243: 1824, 1980

39. Gratten, M., Naraqi, S., Hansmen, D.
High prevalence of Penicillin - insensitive pneumococci in Port Moresby, Papua New Guinea
Lancet 2: 192, 1980

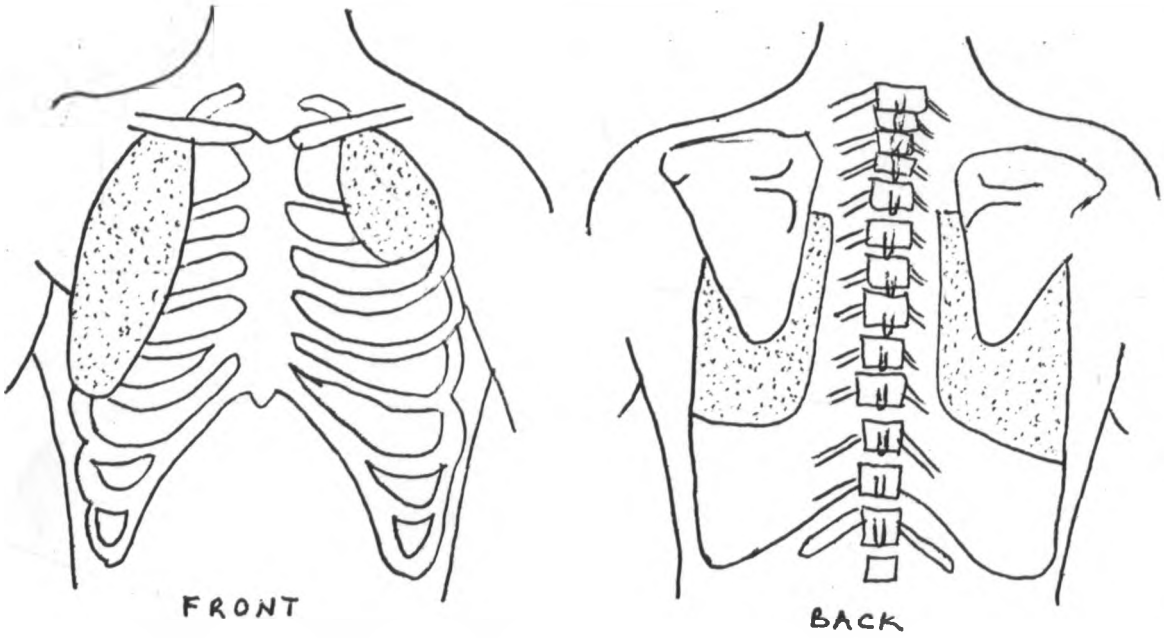
40. Jacobs M.R., Koornhof, H.J., Robins-Browns, R.M., Stevenson, C.M.
Emergence of multiply resistant pneumococci.
N. Engl. J. Med, 299: 735, 1978

41. Wamola, I.A. Mirza, N.B., Nsazumuhire, H.
Penicillin resitant pneumococcal meningitis in Kenyatta National Hospital, Kenya
East Afr Med J 58: 12, 1981.

42. Syrioupoulou, V., Schefele, D., Smith, A.L., Perry, P.M.
Increasing incidence of ampicillin resistance in H. influenzae
J Pediatr 92:889, 1978

APPENDIX I - LUNG ASPIRATION

1. Take a chest X-ray, Antero-posterior and Lateral views. Aspirate only it there are chest X-ray changes or clear clinical localising signs of pneumonia.
2. Aspirate the appropriate zone from either anterior or posterior chest wall (not from laterally), depending on the lateral X-ray and whether there are more crepitations anteriorly or posteriorly.
3. Aspirate ONLY the shaded areas of the diagram:



4. An assistant holds the child sitting up with hands over the head.
5. Clean the skin with alcohol-iodine solution.
6. With a 23 gauge needle on a 10ml syringe, draw up 1ml sterile saline.
7. A second assistant grasps the childs chest firmly in expiration just before you aspirate.

8. Push the needle quickly into the chest to the depth required, just above a rib.

9. Inject $^{1}/_{2}$ml of saline into the lungs. With needle at same position exert maximal suction, then slowly withdraw the needle over 2-3 secs, while exerting strong suction.

10. Observe child closely for next hour for signs of pneumothorax (increasing tachypnoea, cyanosis). Repeat chest X-ray within 24 hours.

APPENDIX II ANTIMICROBIAL SENSITIVITY DISCS

Multodisks from Oxoid (Oxoid Ltd., Basingstoke Hants, U.K.) were used for antimicrobial sensitivity testing.

Composition of disc used on Garm positive organisms

Penicillin G	(1 i.u)	Cotrimoxazole	(25mcg)
Methicillin	(5mcg)	Chloramphenicol	(30mcg)
Ampicillin	(10mcg)	Minocycline	(30mcg)
Erythromycin	(15mcg)	Lincomycin	(2mcg)

Composition of disc used on Gram negative organisms

Ampicillin	(25mcg)	Kanamycin	(30mcg)
Chloramphenicol	(30mcg)	Streptomycin	(10mcg)
Sulfonamide	(200mcg)	Cotrimoxazole	(25mcg)
Gentamicin	(10mcg)	Tetracycline	(25mcg)

APPENDIX III - VIROLOGY TISSUE CULTURE CELL LINES

The following 3 tissue culture cell lines for identification of viruses were used in this study.

1. Vero cell line

   A continuous line from the African green monkey kidney cells.

2. MDCK cell line

   A diploid cell from the Madin-Darby Canine Kidney.

3. WF_1CO_2 cell line

   A diploid cell from whole-human fetal fibroblasts, contained in multiwell plates in a humidified atmosphere of 5% carbon dioxide.

APPENDIX IV - DATA SHEET

A Date ..... Name .....

Study No. ..... Study Group A/B/C.....Sex M/F

B Age .... Weight .....kgs Edema (Yes/No)

Nutritional Status:

1. Normal
2. Underweight
3. Kwash/M-Kwash/Marasmus

C Duration of illness ..... days 1-3 4-7 8+

D Duration of treatment ..... days 0 1-3 4-7

E Drugs given

	Yes	No
Penicillin V.	_____	_____
Ampicillin	_____	_____
Procain Pen	_____	_____
Benzyl Pen	_____	_____
Chloramphenicol	_____	_____
Cotrimoxazole	_____	_____
Gentamicin	_____	_____
Other	_____	_____
Don't know	_____	_____

F Radiology Findings

Before LA:

After LA:

G Bacteria Isolated:

	Blood	NPA	LA
Strep. pneumoniae	____	____	____
H. influenzae	____	____	____
Staphylococci	____	____	____
Klebsiella sp.	____	____	____
E. Coli	____	____	____
Other (specify)	____	____	____

H Virus Isolated:

	NPA	LA
RSV	____	____
Parainfluenza (1/2/3)	____	____
Adenovirus	____	____
Measles	____	____
Other (Specify)	____	____

I ANTIMICROBIAL SENSITIVITY OF LA ORGANISM

ORGANISM	ANTIMICROBIAL
(i) _____	S._____
(ii)_____	R._____
	S._____
	R._____

J. COMPLICATIONS

1) Haemoptysis

Yes/No

Onset

Duration

Management

2) Pneumothorax Yes/No

Onset

Duration

Management

3) Others (Specify) _____

K. OUTCOME: DISCHARGED/DIED/OTHER (Specify) _____