AETIOLOGY OF PNEUMONIA IN CHILDREN AGED 2 – 59MONTHS WITH SEVERE AND VERY SEVERE PNEUMONIA IN THE HIV AND THE POST HIB VACCINATION ERA AT KENYATTA NATIONAL HOSPITAL.

BY

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DECLARATION

I certify that this is my original work and has not been presented for any academic program in any other academic institution.
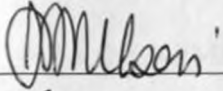
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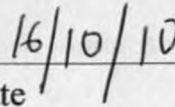This dissertation has been submitted to the University of Nairobi with our approval as University Supervisors.
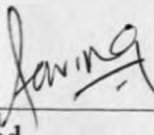
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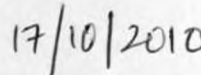
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I would like to express my sincere appreciation to:-

LIST OF ABBREVIATIONS

AIDS   Acquired Immune Deficiency Syndrome

ALRI   Acute Lower Respiratory tract Infection

ARI   Acute Respiratory tract Infection

BAL   Bronchoalveolar Lavage

CRDR   Centre for Respiratory Disease Research

HIV   Human Immunodeficiency Virus

Hib   Haemophilus influenza type b

IS   Induced Sputum

IF   Immunofluorescence

KEMRI   Kenya Medical Research Institute

KNH   Kenyatta National Hospital

NPA   Nasopharyngeal Aspirate

PCP   Pneumocystis pneumonia

PEU   Pediatric Emergency Unit

PJP   Pneumocystis Jirovecii Pneumonia

SHO   Senior House Officer

SPSS   Statistical Package for Social Sciences

UON   University of Nairobi

WHO   World Health Organization

TMX-SMX   Trimethropin / Sulphamethoxazole combination

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ABSTRACT

BACKGROUND

Pneumonia is the leading cause of death in children under five. Knowledge of the current pathogens causing paediatric pneumonia is scarce. We hope to demonstrate the bacterial pathogens currently contributing to the burden of pneumonia in children less than 59 months.

OBJECTIVES

The goal of this study was to determine the patterns of bacterial pathogens isolated from blood cultures in children aged 2 to 59 months admitted with severe and very severe pneumonia. Our secondary objectives were to determine the correlates of culture positive bacterial pneumonia and the prevalence of Pneumocystis *jirovecii* pneumonia in those children also infected with HIV.

DESIGN

Descriptive cross-sectional study

SETTING

Kenyatta National Hospital situated in Nairobi, Kenya and is one of the 2 national tertiary hospitals and is also the teaching hospital of the University of Nairobi.

STUDY DURATION

June 2009 to September 2009

POPULATION

Children aged 2-59 months who presented at Paediatric Emergency Unit with WHO criteria of severe and very severe pneumonia.

METHOD

Blood for culture was collected from 335 patients who met the inclusion criteria. HIV antibody tests were done on 306 patients. Confirmatory PCR was done seroreactive patients aged less than 18 months. NPA was done on 29 seropositive children aged between 2 - 24 months to screen for PCP.

RESULTS

Bacteremia was found in eleven patients giving a prevalence of 3.3%. The significant pathogens isolated were Streptococcus pneumonia 5(45.5%), Salmonella typhimurium 3(27.3%), Escherichia coli 2(18.2%) and Pseudomonas 1 (9%). H.influenza was not isolated. Pneumocystis jirovecii was isolated in 4 out of 29 HIV infected children, giving a prevalence of 13.8%.

CONCLUSION

The bacterial yield obtained was low (3.3%) making conclusions difficult. H.influenza was not isolated. PCP prevalence among HIV infected children was 13.8% and therefore remains an important cause of morbidity.

RECOMMENDATIONS

Most patients had prior antibiotic exposure hence we may have under-estimated bacteremia. Similar studies at lower level facilities where patients first receive care will provide more accurate information.

Streptococcus pneumonia was the predominant pathogen isolated highlighting need for the pneumococcal vaccine to be introduced as part of the routine childhood vaccinations.

The children found with PCP had not received co-trimoxazole prophylaxis. Emphasis should be placed on antenatal testing of all pregnant women and initiation of early co-trimoxazole prophylaxis in all HIV exposed and infected children.

INTRODUCTION AND LITERATURE REVIEW

Pneumonia is defined as inflammation of the parenchyma of the lungs and is a substantial cause of morbidity and mortality in childhood especially in children under 5 years of age worldwide. In developing countries, World Health Organisation(WHO) provides a clinical definition of pneumonia as an acute episode of cough or difficulty in breathing associated with an increased respiratory rate[1].

The annual incidence of pneumonia in children younger than 5 years of age is 34–40 cases per 1000 in Europe and North America. In the developing world, the incidence is several folds higher[2]. The mortality due to pneumonia remains very high worldwide with more than 2million children dying annually[3]. Pneumonia was ranked the leading cause of deaths in children under five accounting for approximately 20% of childhood deaths[4]. In a review of admissions and mortality records of patients in paediatric wards at Kenyatta National Hospital (KNH), a teaching hospital in Nairobi, Kenya, approximately 250 patients are seen per month with severe and very severe pneumonia. This constitutes approximately 30% of all paediatric admissions in one month with a case mortality rate of approximately 10%. These deaths are potentially preventable if appropriate clinical and laboratory tools are made available to facilitate early detection of pneumonia, identification of the pathogens involved and initiation of appropriate therapy.

Immunization has a lot of potential to decrease the morbidity and mortality caused by pneumonia in developing countries. Several vaccines have been developed against organisms that cause pneumonia this include the polysaccharide protein conjugate

vaccines that protect against H. influenza type b (Hib) and Streptococcus pneumoniae. The two vaccines were licensed for use in the developed countries in 1990 and 2000 respectively. This resulted in a dramatic reduction of diseases caused by the targeted serotypes. Routine use of the Hib vaccine in developing countries has also confirmed its effectiveness.[5, 6] A study in South Africa that recruited children less than 2yrs who had received the H. influenza type b vaccine, showed that there was 54% reduction of pneumonia in HIV infected and 90% reduction in HIV uninfected children. Overall pneumonia reduction in all children was 78 %.[7]

In Kenya, the Hib vaccine was introduced in 2001 and is currently part of the routine vaccination given to every child according to the Ministry of Health guidelines. According to the World Health Organisation, a child is considered fully vaccinated if he or she has received a BCG vaccination against tuberculosis; three doses of DPT vaccine to prevent diphtheria, pertussis, tetanus (or three doses of pentavalent which includes 2 additional vaccines Hib and hepatitis b vaccine) and one dose of measles vaccine. According to the Kenya Demographic Health Survey 2008, 86 percent of children country wide had received the recommended three doses of DPT/pentavalent.[8] The pneumococcal conjugate vaccines is not yet available for routine vaccination in Kenya hence streptococcus pneumonia remains a big challenge, More recent studies on bacteraemia are now showing that 'atypical pathogens' are becoming more important.[9]

AETIOLOGY

Pathogens that commonly cause childhood pneumonia include respiratory viruses (most frequent isolates being respiratory syncytial virus, adenovirus, parainfluenza virus, influenza A and B viruses) and bacteria (most frequent isolates being Streptococcus pneumonia, Staphylococcus aureas, Haemophilus influenza type b and non typhoidal salmonella). Most serious episodes of pneumonia are caused by bacterial infections [1]. Less common causes include Mycobacterium tuberculosis, chlamydia, fungi and Pneumocystis jirovecii.

PRE- HIB VACCINATION ERA

Haemophilus influenza type b was one of the most important causes of bacterial pneumonia in young children but, with the routine use of effective vaccines, it has now become less common (See table 1).

Forgie et al. [10, 11] conducted two hospital-based studies. The first study recruited children less than one year of age, bacterial infections were most common and predominantly involved Streptococcus pneumoniae (20%) or Haemophilus influenza (11%). The second study which recruited children aged between 1yr and 9yrs, a bacterial infection was identified in 57 (77%), a viral infection was seen in 25 (34%) and 18 (24%) had mixed viral-bacterial infections. The bacterial pathogens most frequently identified were Streptococcus pneumoniae and Haemophilus influenza found in 61 and 15% of patients, respectively.

A study done by Wafula et al in KNH, looked at aetiology of acute respiratory infection using blood culture and nasopharyngeal aspiration for bacterial isolation, and the most common isolates were Haemophilus influenza ,Streptococcus pneumoniae and Staphylococci.[12]

A study by Kariuki et al in 1987, at the Kenyatta National Hospital used samples from lung aspiration to determine the aetiology of pneumonia. The results showed that the most commonly bacteria isolated were coagulase negative staphylococcus 52%, followed by Escherichia coli 16%, Klebsiella 12% and Haemophilus influenza 8% [13].

POST- HIB VACCINE ERA

Aetiological studies, done in developing countries suggest that *Streptococcus pneumoniae* may now be the most common bacterial cause of pneumonia worldwide[11, 14]. In blood culture investigations of bacteraemia in children found to have clinical signs of pneumonia, non typhoidal salmonella have also been isolated with increasing frequency. A study done in Malawi showed that non typhoidal salmonella were the second most common blood culture isolate after S. pneumoniae among children with radiologically confirmed severe pneumonia[15].

A more recent study done to establish the burden, incidence, and types of community acquired bacteraemia among children admitted to a rural hospital in Kenya, found that 866 of 14,787 (5.9%) children who were 60 or more days of age had bacteraemia.

Streptococcus pneumoniae, non typhoidal Salmonella species, Haemophilus influenza, and *E. coli* accounted for more than 70% of isolates[9].

THE ROLE OF HIV

HIV/AIDS is a major cause of infant and childhood morbidity and mortality in Africa. In children under five years of age, it accounts for 7.7% of mortality worldwide[8]. Pneumonia is the leading cause of hospitalization in HIV infected children. With the increasing prevalence of the HIV/AIDS the management of pneumonia has become even more challenging. The causative organisms are similar in both HIV infected and uninfected children, however in severe immunosuppression increased prevalence of opportunistic infection complicates the management of these children. Pneumocystis jirovecii, a fungus causing pneumocystis pneumonia was until recently the most prevalent opportunistic infection in patients infected with the human immunodeficiency virus (HIV) [1, 16]. The name *P. jirovecii* is now used to distinguish the organism found in humans from physiological variants of pneumocystis found in other animals and was first proposed in 1976, in honor of Otto Jirovec, who described pneumocystis pneumonia in humans. PCP (originally pneumocystis carinii pneumonia), which was widely used has been retained for convenience and stands for pneumocystis pneumonia [17].

PCP is a common first indicator of AIDS in HIV infected children less than 1 year old [18]. Data from studies conducted in areas where the population prevalence of HIV in adults is high report frequent isolation of Pneumocystis jirovecii, with median age of presentation at 2-3 months [19]. Necropsy studies from some African countries have consistently shown

that *Pneumocystis jirovecii* is common in children with HIV infection dying of pneumonia [20, 21]. The advent of HAART and use of co- trimoxazole as prophylaxis on everyone found to be infected with the HIV virus has reduced the incidence of PCP. However the burden of disease attributable to PCP has remained poorly described mainly due to scarcity of resources to diagnose and manage it. Although PCP has also been reported in HIV negative children, especially those with malnutrition and chronic respiratory disorders,[22] it is most commonly associated with HIV infection [19].

The prevalence of Pneumocystis carinii pneumonia was studied by Bakeera-Kitaka et al. [20] in children aged 2-60 months presenting with severe pneumonia at Mulago Hospital, Kampala. They found 20(16.5%) out of the 121 children recruited, irrespective of their HIV status to have PCP. Prevalence was found to be higher in the HIV infected group (41.6%) when compared to the HIV negative group (2.6%) and 12 (60%) were below 6 months of age. Case fatality of those with PCP was 40%.

In Malawi Graham et al[19], studied prevalence of PCP in children aged 2 to 60months with acute severe pneumonia. PCP was identified in 16 (10.6%) out of the 150 children with radiologically confirmed severe pneumonia. All were HIV-positive and younger than 6 months. The case fatality rate of children with PCP was 62%.

In a South African study, Zar et al.[21] investigated the incidence and associated features of PCP in HIV-infected children. Of the 151 HIV-infected children 15 (9.9%) had PCP and it was the AIDS-defining infection in 13 of 64 (20%). Case fatality rate was 47%.

A necropsy study in Zambia by Chintu et al[23] investigated the aetiology of lung disease in African children dying from respiratory illnesses. Autopsy restricted to the chest was undertaken in 264 children aged 1month to 16 years. HIV prevalence was found to be 68% and pyogenic pneumonia was the leading cause of death irrespective of the HIV status. P. jirovecii was the most frequently isolated organism in the HIV-infected children aged 0 to 5months.

In Kenya Kose et al [24] determined the prevalence of PCP in HIV exposed children aged 2 to 24 months admitted with severe pneumonia at the Kenyatta National Hospital, 18 (14%) out of the 130 cases studied had PCP. Majority (78%) of the children of the children with PCP died within 48hrs of admission. Table 1 below is a summary of patterns of bacteria isolated form blood culture and prevalence of PCP in studies done in developing countries

Table 1: Summary of aetiology studies showing bacterial pathogens isolated from blood culture and immunofluoresent assay technique in the pre and post Hib vaccine and HIV Era.

Author, study design	Setting	Sample size	Inclusion criteria	Tests	Results
Ekalaksananan,[25] 2001 case series	Thailand Teaching hospital	74	Under 5 years of age with cyanosis or chest indrawing	Blood culture nasopharyngeal aspirate, enzyme immunoassay	S.pneumonia (8.9%) H. influenzae (8.9%) Mixed infection 8/74 (10.8%) C. trachomatis 5/76 children
Forgie,[26] 1992 Cohort study	Gambia, rural community	500	Children < 5 years resident in 7 villages in a rural area of The Gambia	NPAs for enzyme immunoassay; Blood culture;	Bacterial infection: 32 (14%) common isolates — S. pneumonia, H. influenza.
Forgie,[10] 1991a Case series	Gambia, paediatric referral hospital	91	Infants < 1 year of age hospitalized with pneumonia	Blood culture; lung aspirates;	Bacterial infections: 27 (30%) cases, Streptococcus pneumoniae (20%); Haemophilus influenza (11%).
Forgie,[11] 1991b Case series	Gambia, paediatric referral hospital	74	Children 1 to 9 years hospitalized with severe pneumonia	Blood culture; lung aspirate; nasopharyngeal aspirate	Bacterial infection: 57 (77%); S. pneumoniae and H. influenza found in 61 and 15% of patients, respectively
Berkley,[9] 2005	Kilifi District hospital		Children > 60days admitted	Blood culture	Bacterial isolate: S. pneumonie,non typhoidal salmonella, H. influenza, E. coli
Graham,[19] 2000 Case series	Malawi, hospital	150	Children 2 months - 5 years hospitalized with severe pneumonia)	Blood cultures Immunofloresence on NPAs used to test for PCP PCR to detect HIV CXR.	Bacterial pathogen: 21/150 cultures showed growth (14%); S. pneumoniae (8) and non-typhoidal salmonellae (7). 16 cases of PCP among 150 children: all cases were HIV positive.
Bakeera-Kitaka,[20] 2004 Case series	Uganda, Teaching Referral Hospital	121	Children 2-60 months with severe pneumonia	Sputum fluorescence microscopy	PCP prevalence: 16.5% (20 out of 121) 18/43-HIV infected 2/78-not HIV infected
Zar,[21] 2000 Case series	South Africa, teaching hospital	151	HIV infected children hospitalized with pneumonia	Immunoflourescence and silver stain	PCP prevalence: 15/150 (9.9%; 95% CI 5.5 to 15.5)
Bii,[24] 2006	Kenya, Teaching Referral hospital	130	HIV exposed children with severe pneumonia	Sputum induction Immunoflorencence .	PCP prevalence: 8/60 (13%) Bacterial isolates: Klebsiella(43%), E. coli (18%),Staph aureas (13%) were most frequent isolates.

LABORATORY DIAGNOSIS

The scarcity of data on the aetiology of community acquired pneumonia is mainly as a consequence of lack of simple reliable methods for establishing diagnosis.[27] The currently available tools for the diagnosis of acute lower respiratory tract infections in children have low sensitivity and are grossly underutilized. Most of the children presenting to a health facility with symptoms and signs suggestive of LRTIs are frequently offered antibiotic treatment without any prior laboratory investigations.

The gold standard method for diagnosis of pneumonia would be to take a sample directly from the infected area of the lung. Lung puncture has been shown to have better sensitivity than other tests but is a very invasive procedure therefore not routinely performed and is reserved only for very ill children with specific radiological findings. A few studies on lung punctures done in Africa yielded high bacteria growth[13, 28].

Culture of sputum in children is not usually performed because sputum is difficult to obtain in young children and cultures performed on specimen obtained from upper respiratory tract or sputum generally do not reflect the cause of lower respiratory infection.

Throat swabs are not suitable due to the high carriage rate of bacterial respiratory pathogens in throats of healthy children.

In 1996 Ramsey et al[29] found that antigenuria was present in 4% of asymptomatic children and 16% of children with acute otitis media as well as 24% of children with acute LRTI. This specificity is too poor for it to be a helpful test in diagnosis.

Blood culture is an important laboratory investigation for the aetiological diagnosis of bacterial pneumonia but it is only useful when pneumonia is associated with bacteraemia (i.e., bacteraemic pneumonia). The yield from blood culture in patients with pneumonia has ranged from 10% to 30% [27]. Although the isolation by blood cultures are generally low from samples obtained from children with pneumonia, studies have identified several clinical and laboratory predictors of a positive blood culture. A study by Berkley J et al. [30] found that small volumes of inoculation and the use of antibiotics may compromise the sensitivity of blood cultures. The sensitivity of blood cultures was found to fall by almost one third when cultured samples of 1 ml were compared with those of 3 ml. Recent antibiotic use was noted to reduce blood-culture yields by 62 to 73 percent in patients with severe or fatal disease. [30] In a Gambian study children aged below 5 years, who presented at a rural dispensary with pneumonia, high temperature, rapid respiratory rate, dehydration, nasal flaring, grunting, dullness to percussion, bronchial breathing and diminished breath sounds were all found to be predictors for a positive blood culture.[31] Although multiple blood cultures give a marginal increase in bacterial yield compared with single cultures, they are rarely used in developing countries because of the additional costs, the general lack of facilities and the overwhelming burden of diseases requiring laboratory investigations.

The sensitivity of testing nasopharyngeal aspirate samples to isolate of Pneumocystis jirovecii is not known several methods have been used to make a diagnosis of PCP. Sputum induction with nebulised hypertonic saline another technique for PCP diagnosis has high risks of cross infection especially in our setting where we have a high prevalence of tuberculosis. Bronchoalveolar lavage is not available for our study due to expense and lack of expertise to perform the procedure in children. Samples obtained by open lung or transbronchial biopsy provide greater diagnostic sensitivity than respiratory secretions but are difficult procedures to perform and carry high risk of complication if done by an inexperienced person. Percutaneous lung aspiration would provide a higher yield of *P jirovecii* however, this technique is rarely used because of the danger of air leaks in PCP. A study done in Malawi used Immunofluoresent assay on nasopharyngeal aspirate samples and identified 16 cases (10.6%) of PCP out of 150 children with radiologically confirmed severe pneumonia ,this prevalence was within the range found by other studies that use sputum induction.[23]

JUSTIFICATION

Pneumonia is still a major cause of mortality and morbidity in developing countries and is currently the leading cause of mortality in Kenyatta National Hospital.

Current WHO treatment recommendations for low and middle income countries (LMICs) are largely based on the most likely aetiological organisms identified in studies conducted before or around the period when the guidelines were developed over 15 years ago [32, 33].

No current data is available to show the common bacterial pathogen causing severe pneumonia post Hib vaccine.

The maturing HIV epidemic has probably resulted in a change in the spectrum of pathogens causing pneumonia particularly in African children. The impact of these changes on treatment recommendations is not yet clear and therefore there is necessity for more research in order to reduce the current disease burden.

UTILITY

This study will therefore help to identify the current bacterial pathogens contributing to the burden of severe and very severe pneumonia and thereby help in making decisions about antibiotic policy.

STUDY OBJECTIVES

Primary objectives

- To determine the patterns of bacterial pathogens isolated from blood cultures in the HIV and post Hib vaccine era in children aged 2 to 59 months admitted with severe and very severe pneumonia at KNH.

Secondary objective

- To determine the correlates of culture positive bacterial pneumonia.
- To determine the prevalence of Pneumocystis *jirovecii* pneumonia in HIV infected children aged 2-24months admitted with severe and very severe pneumonia at KNH.

METHODOLOGY

STUDY DESIGN

Descriptive cross sectional study.

STUDY SITE

The study was carried out in the KNH Paediatric Emergency Unit (PEU) and in the paediatric general wards. KNH is situated in Nairobi, Kenya and is the teaching hospital of UON; it is also one of the two national tertiary referral hospitals. The hospital caters for patients referred from health facilities all over the country and serves as a primary health facility for those who live in Nairobi and its environs. PEU is the clinical unit where all sick children first encounter the clinicians before admission to the wards or treated as outpatients and discharged home.

SAMPLE SIZE

245

Calculated using the Fishers Formula for prevalence studies:

$$n = \frac{Z^2\,p\,(1-p)}{d^2}$$

Where: n= sample size

z= table value for the standard normal deviate which corresponds to a significance level of 5 %(=1.96)

p= estimated prevalence of bacterial isolation in blood culture in children with severe and very severe pneumonia (10 – 30%).

d= degree of accuracy set +/- 5%

- If p=10%,   n=3.84×0.1×0.9/0.0025 =**138**

- **If p=20%,   n= 3.84 ×0.2×0.8/0.0025 =245**

- If p=30%,   n= 3.84×0.3×0.7/0.0025=**322**

- If p=40%,   n=3.84 ×0.4×0.6/0.0025=**368**

NPA Sample size

This sample was taken from a subset of the children enrolled into the study who were found to be HIV exposed aged between 2 to 24months; these criteria were based on results of several studies done that show that PCP is rarely isolated among children who are HIV negative and older than 24months of age.

Assumptions made:

1. Prevalence of HIV infection is 10.2%[34].

2. The number of patient that we expected to recruit in 3months = 600

   n= 10% 0F 600 = 60

Using the Fishers formula

$$n= \frac{Z^2 \ p \ (1-p)}{d^2}$$

$60 = 3.84 \times 0.14 \times 0.86 / d^2$

$d^2 = 0.007056$ $d = 0.084 \ (8\%)$

p =14 %(derived from Kose's study)

z= table value for the standard normal deviate which corresponds to a significance level of 5 %(=1.96)

With a sample size set at 60 patients, and prevalence set at 14% we will report our results with degree of precision of +/ - 8%

STUDY POPULATION

We recruited all children aged 2-59 months who presented with WHO criteria of severe and very severe pneumonia and satisfied the inclusion criteria.

SUBJECT SELECTION

1. INCLUSION CRITERIA

- Children aged 2- 59months.
- Children who had the clinical signs and symptoms of severe and very severe pneumonia using standard WHO criteria. (See Appendix 1).
- Informed consent given by parent or adult caregiver.

2. EXCLUSION CRITERIA

- Children whose parents or guardians withdrew consent for participation in the study.
- Known chronic renal, cardiac or primary neurological abnormality predisposing child to pneumonia.
- Clinical diagnosis of severe malnutrition (marasmus/ kwashiorkor).
- Admission from outpatient clinic specifically for treatment of TB.

- Referral from another inpatient facility because of pneumonia treatment failure.

SAMPLING METHODS

This study was part of a larger study that aimed at comprehensively studying all children aged 2-59months with severe and very severe pneumonia. A team of 8 investigators worked in shifts at the Pediatric Emergency Unit on a 24 hour basis during weekdays and weekends and attended to every consecutive patient who came into the unit and met the inclusion criteria

STUDY PROCEDURES

Clinical pathway of identified cases

All children aged 2- 59months who presented to KNH PEU with cough and/or difficulty in breathing were screened at the triage for any signs of severe or very severe pneumonia using the standard WHO criteria.

All children who satisfied the inclusion criteria and did not have any of the exclusion criteria were invited to participate in the study. The study plan was explained to the caretaker of the recruited child and a written consent to participate in the study was sought. (See appendix 3- consent form).

Where consent was obtained a comprehensive history was taken and a thorough physical examination was conducted and documented by the principal investigator. The interview

was done using a standard structured questionnaire (See study questionnaire- Appendix 2). All the emergency cases that were seen received treatment as a first priority with study data collected only after stabilization of the child.

The flow chart below summarizes the participant's progress.

Screening

| 487 Children 2 – 59 months of age with cough or difficulty in breathing + Lower chest wall indrawing or danger sign |

| 28 Children with chronic cardiopulmonary conditions, meningitis, cerebral palsy, severe malnutrition | → Exit

Bronchodilator

| 73 Wheeze responsive to bronchodilator | → Discharge

| 96 Wheeze non-responsive to bronchodilator |

| 386 Children assessed and admitted for follow up to day 5 |

| 51 Blood culture results not available | → Exit

n= 335

| **Blood culture available** |

| 306 HIV Rapid test |

n =29

| **Nasopharyngeal aspirate done** |

| PCR (<18 months) |

Laboratory investigations

All the children enrolled into the study had 2 to 3mls of peripheral blood drawn for blood cultures, HIV test and all other relevant blood tests.

The definitive laboratory diagnosis of pneumonia is dependent on the culture or detection of casual microbial agent recovered from sterile sites, using aseptic and standard procedures. The quality of specimens received for processing in the laboratory determines the success in isolation of the pathogens. The blood samples taken for cultures are at risk of contamination by normal commensals. To prevent this, strict aseptic procedures were adhered to throughout the process of collection, transport and investigation of the clinical specimen. (See blood sampling SOP Appendix 6)

Once delivered to the laboratory, specimen of blood was incubated at 37^0 C sampling from culture bottles and subsequent plating on sheep blood agar, chocolate agar and Macconkeys agar was done regularly at 24, 48 and 72 hours. In case of a growth, the isolates was processed using standard bacteriologic techniques consisting of colony, morphology, gram stain, catalase, oxidase and other biochemical identification tests. Specimen was declared sterile when no growth was seen after 72hrs of incubation. (See blood culture SOP Appendix 8). The parallel testing method was employed for HIV testing; this was done using rapid kits from Determine (Abbot Laboratories) and Immunocomb (Orgenics), both kits test for antibodies against HIV1 and HIV 2 in whole blood. The patient was considered seropositive when both tests were positive or negative

when both were negative. No discordant test was found. All HIV exposed children less than 18 months of aged had PCR blood test done on blotting paper at UON Virology laboratory to confirm infection. (See SOP for rapid HIV testing - Appendix 7)

In addition all HIV exposed children aged between 2months to 24months underwent nasopharyngeal aspiration. (See NPA SOP – Appendix 4) The specimen obtained was sealed and sent to the mycology laboratory at the Centre for Respiratory Diseases Research (CRDR) at Kenya Medical Research Institute (KEMRI). The NPA was then analyzed for presence of P. *Jirovecii* with an indirect immunofluoresent assay technique using DETECT immunoflourescence Kit made by Shield Diagnostics of Dundee of UK. (See PCP SOP Appendix 5)

TREATMENT AND FOLLOW UP

The patients were treated according to standard Government of Kenya guidelines for severe or very severe pneumonia after ensuring that they had no known allergies or contra-indications to recommended antibiotics. Supportive care with oxygen, fluids and feeds was provided. Management after admission was guided by a clinical care pathway but with definitive decisions made by the ward-based consultant team responsible for the patient.

All the HIV seropositive children were treated with antibiotics appropriate for very severe pneumonia and with high dose oral cotrimoxazole as empiric treatment for Pneumocystis pneumonia according to WHO guidelines. Those who had any clinical or

immunological criteria for initiation of HAART were initiated on medication after adherence and nutritional counseling was done.

Data

(a) Data storage

All the clinical data was collected at admission and recorded onto pre-formatted data collection sheets. The confidentiality of the participants was preserved. The data was entered into a purpose designed database with the participants identified by only a unique study code – participant names and hospital inpatient numbers was not included. At the point of data-entry range and validity checks were incorporated to prevent data entry errors

(b) Data management/analysis

Data was entered into pre coded questioners (Appendix 2) and entered using the Epi data programme. The data was examined for outlying or inconsistent values and the distribution of continuous data was examined to check for normality before analysis. Categorical data was tabulated and assessed using chi-square test. Descriptive statistics including rates and percentages were determined, proportions were calculated with appropriate confidence limits and means, with standard deviations, or medians, with inter-quartile ranges, derived as appropriate to provide descriptive summaries of the data. Univariate and multivariate correlates of positive blood cultures and positive NPAs were determined using the chi- square test and fisher's exact test. Results were presented using frequency tables, bar graphs and pie charts. Analysis was conducted with EPI INFO and STATA v9.2 (Stata Corporation, Texas, and USA).

ETHICAL CONSIDERATIONS

The study was designed to comply with international ethical guidelines and those of KNH and KEMRI Ethical Research Committee.

1. The nature of the study was explained to the parent or guardian before recruitment.

2. Consent was obtained in writing from the parent or guardian and after adequate explanation (see appendix 3) for enrolment in this study. Patients were free to withdraw from the study without penalty.

3. The data was entered with the participants identified by only a unique study code – participant names and hospital inpatient numbers was not included.

4. Results during the study were used wherever possible to guide patient care. Antibiotic therapy was adjusted, as needed, based on culture and sensitivity results from blood cultures and after reviewing HIV testing results.

RESULTS

During the study a total of 487 patients presented with cough and/ or difficulty breathing, plus lower chest wall indrawing and were screened at the PEU. We excluded 152 children including 28 (5.7%) children who had history of known chronic conditions and severe malnutrition, 73 (15%) wheezers who responded to bronchodilator therapy, and 51(10%) children for whom blood cultures were not available. Thus, 335 children were included in the study.

Characteristics of the study population

Out of the 335 children recruited into the study, 163 were males and 172 female giving a male to female ratio of 1: 1.06. The median age was 9 months (age range was 2 to 59 months). Most of study participants (66%) were infants.

The pneumonia severity classification was derived using data collected on admission and based on the WHO guidelines. 148 (44.2%) children were classified as severe pneumonia while 187 (55.8%) as very severe pneumonia.

Majority of the patients 210(62.6%) had history of previous antibiotic use before admission (confirmed from prescriptions, referral notes or medication bottles), coamoxiclav and macrolides in most cases; 56 (16.9%) had not taken any antibiotics and 69 (20.5%) did not know what medication the patient had received.

On the fifth day post admission 175 patients (52.2%) had been discharged, 121 (36.1%) were still undergoing treatment as inpatients, 13(3.9%) had absconded from the wards, and 26 (7.8%) were deceased. (See table 2 below)

Table 2: Characteristics of the study population

Variables	Frequency n= 335	Percentage
Demographic indicators		
Sex		
Male	163	48.7
Female	172	51.3
Age groups		
2 – 11months	212	66
12 – 23months	66	20.6
24 - 59months	43	13.4
n = 321		
History and signs		
Diagnosis		
Severe pneumonia	148	44.2
Very severe pneumonia	187	55.8
HIV status		
Positive	33	10.7
Negative	276	89.3
n =309		
Previous antibiotic use		
No	56	16.9
Yes	210	62.6
Unknown	69	20.5
Outcome		
1. Discharged	175	52.2
2. Deceased	26	7.8
3. Absconded	13	3.9
4. still admitted	121	36.1

Figure 1: Prevalence of bacteremia among children with severe and very severe pneumonia
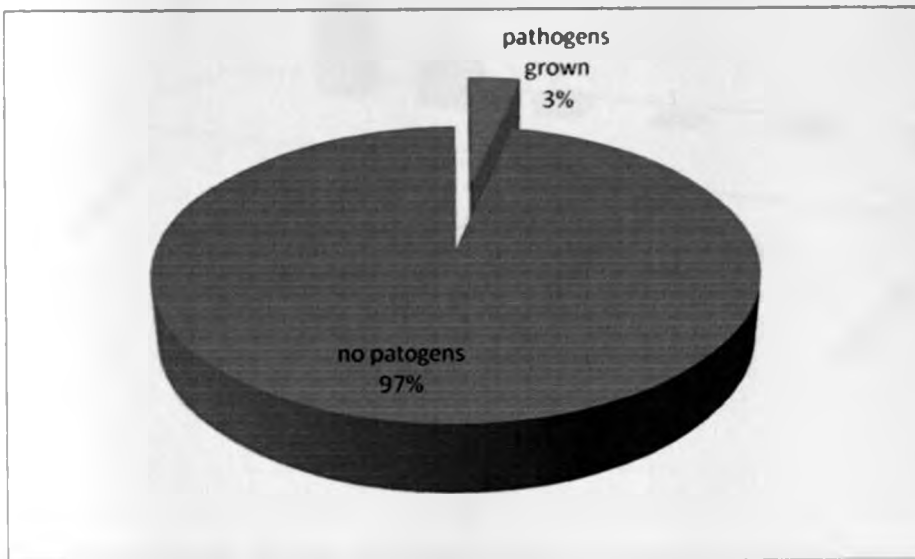
Out of the 335 children recruited 11 had blood cultures that grew significant organisms giving a bacterial isolation rate of 3.3%.

Figure 2: Patterns of organisms isolated from blood culture
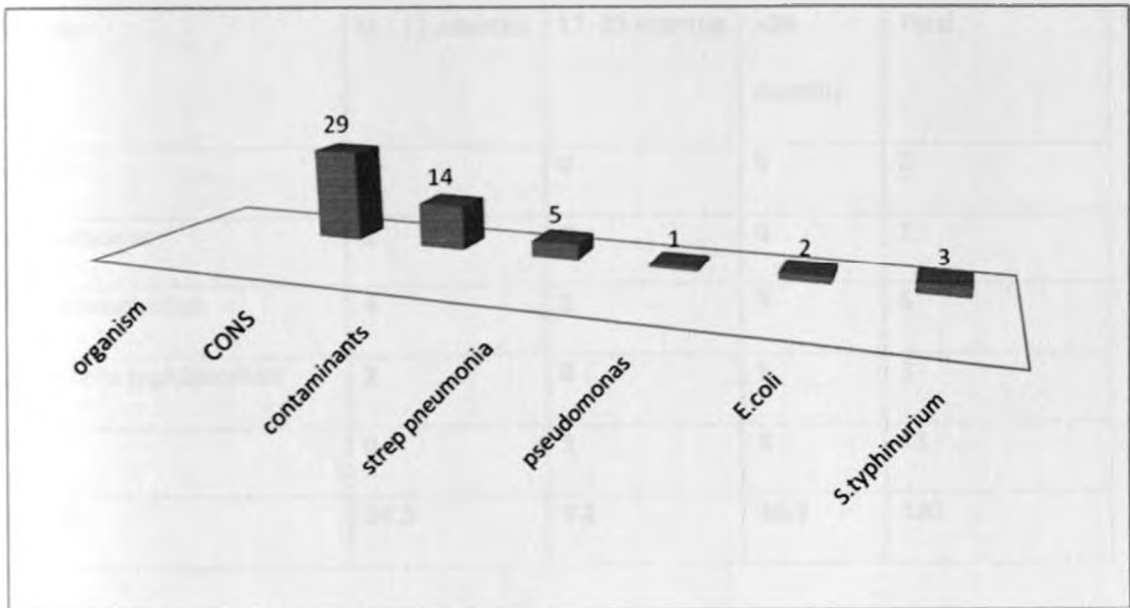
The most common isolate was Streptococcus pneumonia 5(9.3%), followed Salmonella typhinurium, Escherichia coli and Pseudomonas, 3(5.5%), 2(3.7%) and 1 (1.9%) respectively. Haemophilus influenza was not isolated in any of the blood samples. Other organisms grown were 29 (53.7%) Coagulase negative staphylococcus, 6(11.1%) Micrococcus, 7 (13%) mixed organisms, 1(1.9%) Diptheroids. The latter organisms were considered to be contaminants and included amongst the patients with no pathogen grown in blood culture for analysis.

Table 3: Distribution of bacterial isolates by age groups

Bacteria	0 – 11 months	12 -23 months	>24 months	Total
E. coli	2	0	0	2
Pseudomonas	1	0	0	1
Strep pneumoniae	1	1	3	5
Salmonella typhimurium	2	0	1	3
Total	6	1	4	11
Total %	54.5	9.1	36.4	100

As shown in the table 3 above the gram negative organisms E. coli and S. typhimurium were mostly isolated among children aged below 12 months. Streptococcus pneumonia predominated among children aged more than 24 months; however the numbers were too small for any valid conclusions to be made.

Table 4: Characteristics of the study population with cons as isolate compared to true negatives

Variables	CONS n= 29	TRUE NEGATIVES n= 295	P value (χ^2)
Sex			
Female	16(55.2%)	151(51.2%)	0.68
Age groups			
2 – 11months	22(78.6%)	184(65%)	0.15
12 – 23months	2(7.1%)	63(22.3%)	
24 - 59months	4(14.3%)	36(12.7%)	
	n =28	n =283	
Diagnosis			
Severe pneumonia	10(34.5%)	130(44.1%)	0.32
Very severe pneumonia	19(65.5%)	165(55.9%)	
HIV status			
Positive	4(14.3%)	238(89.1%)	0.58
Negative	24(85.7%)	29(10.9%)	
	n= 28	n =267	
Previous antibiotic use			
No	4(13.8%)	51(17.3%)	
Yes	20(69.0%)	186(63.1%)	0.81
Unknown	5(17.2%)	58(19.7%)	
Mortality			
1- discharged	12(41.4%)	157(53.2%)	
2- deceased	1(3.4%)	24(8.1%)	0.37
3- absconded	0(0%)	13(4.4%)	
4- still admitted	16(55.2%)	101(34.2%)	

Coagulase negative staphylococci were isolated in 29/335 (8.7%) samples. We explored the possibility these bacteria represented true pathogens. In table 4 we compare demographic and clinical characteristics of patients in whom coagulase negative staphylococci were isolated and those with cultures reported as negative. Significant differences in the characteristics compared would suggest that the two groups represent unique populations. Our analyses showed no statistically significant differences between the groups, suggesting that the two groups are homogenous.

Figure 3: Population vaccinated against h. Influenza vs. Unvaccinated in children aged between 4 months and 59 months with severe and very severe pneumonia
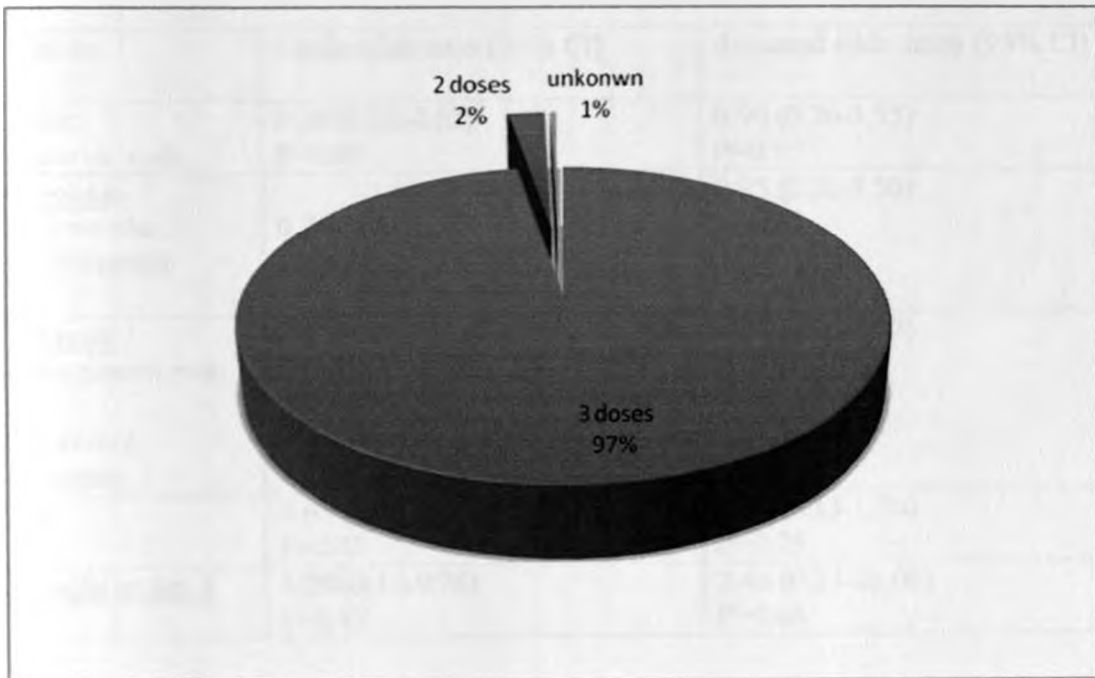
Information about vaccination with the pentavalent vaccine was taken from 265 of the patients recruited. We analyzed children above the age of four months for number of doses received because at this age we expected all infants to have received all the 3 doses as per the KEPI and Ministry Of Health schedule. 258(97.4%) had received all the three doses, 6 (2.3 %) two doses, 1(0.3%) did not know the vaccine status of the patient. None of the patient above four months of age had received less than 2doses of the vaccine.

Table 5: Univariate analysis of the correlates of positive blood culture in children admitted with severe and very severe pneumonia

Variables	Crude odds ratio (95% CI)	Adjusted odds ratios (95% CI)
Gender Female vs. male	0.78 (0.23-2.62) P=0.69	0.96 (0.26-3.55) P=0.95
Age groups 2 – 11months 12 – 59months	0.76 (0.21-2.78) P=0.68	0.95 (0.26-3.56) P=0.95
Diagnosis Severe pneumonia vs. Very severe pneumonia	0.29(0.07-1.11) P = 0.05	0.28 (0.06-1.27) P=0.10
Fever	0.60 (0.18-2.03) P=0.41	0.46 (0.13-1.70) P=0.25
Mortality at day 5	1.20 (0.15-9.76) P=0.87	2.46 (0.23-26.06) P=0.46

In our univariable analyses for clinical predictors of positive blood cultures as shown in table 5, the diagnosis of very severe pneumonia was modestly associated with positive blood cultures when compared against severe pneumonia OR 3.50 (0.90-13.58) P = 0.05. No other significant associations were apparent. Our multivariable analyses did not show any clinical signs to be predictive of positive blood cultures. However the ability to detect statistical associations was compromised by the low power, a consequence of the small proportion of positive blood cultures.

Figure 4: The prevalence of HIV infected patients with severe and very severe pneumonia
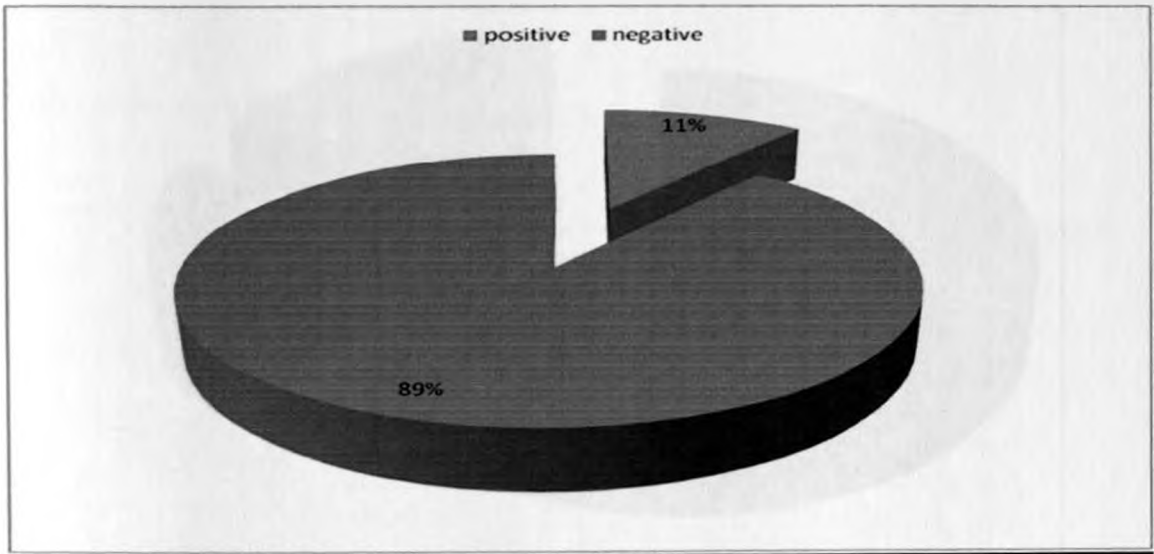
A total of 306 of patients recruited with severe and very severe pneumonia accessed HIV testing using rapid antibody tests; all patients who were less than 18 months with positive antibody test underwent a PCR test to confirm infection. In total, 26 children did not access HIV testing because their caretakers declined testing or they did not have a scheduled blood drawn for the test during their admission or ward care, 3 did not have blood culture results and therefore exited the study. The antibody results were positive in 33 (10.7%) of the children while 276 (89.3%) had negative results this is depicted by Fig 4 above. Majority, 26(76%) of the children with positive antibody test were less than 18months and underwent a PCR test to confirm diagnosis of these, 20(77%) were infected and 6 (23%) were negative.

Figure 5: Prevalence of Pneumocystis jirovecii pneumonia among HIV infected children aged 2 to 24 months with severe and very severe pneumonia
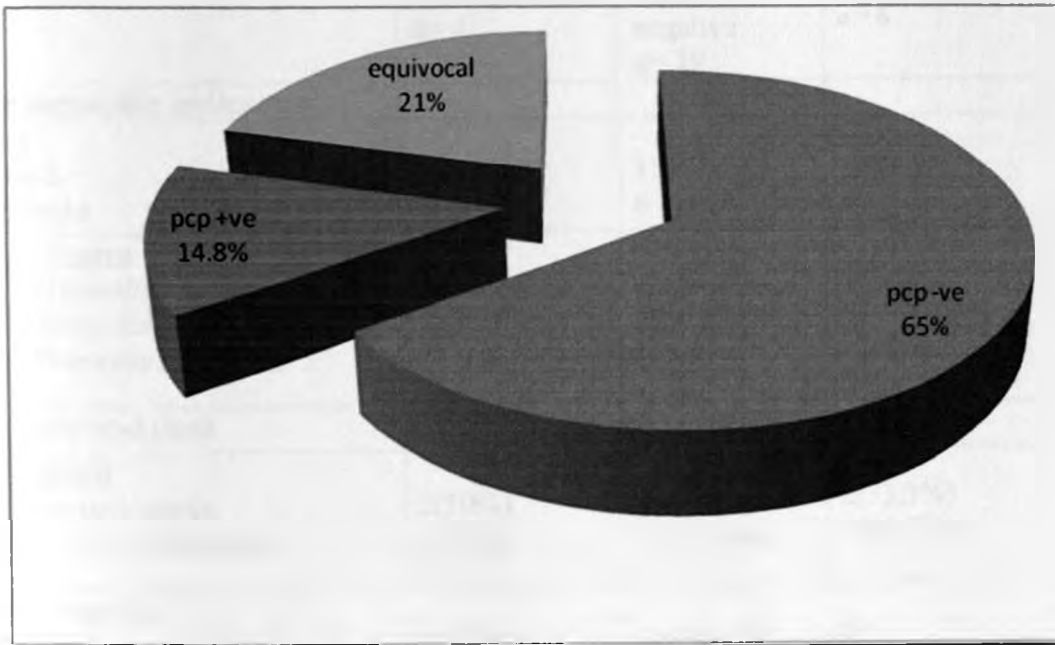
Of the 33 HIV seropositive children 29 had nasopharyngeal aspiration done to check for pjp. 4 tested positive for Pneumocystis jirovecii, giving a prevalence of 13.8% in HIV infected children with severe and very severe pneumonia, 12% in all HIV exposed children and 1.2% in unselected population of children presenting with severe and very severe pneumonia (we assumed that pjp is absent in all children who are HIV negative children and those above 24 months of age). The children found infected with PCP were all HIV infected and aged < 6 months. None of the confirmed PCP cases had received co-trimoxazole prophylaxis. 6(21%) samples scored equivocal (<5 oocysts) and were all from HIV negative children. (HIV exposed). No death had occurred among these children by day 5 of admission.

Table 6: Comparison of PJP positive patients, PJP negative and patients with equivocal results

Variables	NPA positive n= 4	NPA negative n= 19	Equivocal results n = 6
Demographic indicators			
Sex			
Male	3(75%)	13(68.4%)	3(50%)
Female	1(25%)	6 (31.6%)	3(50%)
Age groups			
2 – 11months	4(100%)	16(84.2%)	4(80%)
12 -23months	0	2(10.5%)	0
24 59months	0	1(5.3%)	1(20%) n=5
History and signs			
Diagnosis			
Severe pneumonia	2(50%)	9(47.4%)	2(33.3%)
Very severe pneumonia	2(50%)	10(52.6%)	4(66.7%)
PCR results			
Positive	4 (100%)	16(94.1%)	0
Negative	0 n=4	1(5.9%) n= 17	5(100%) n=5
Spo2			
Severe hypoxia	0	1(5.6%)	0
Mild to moderate hypoxia	1(50%)	14(77.8%)	4(80%)
No hypoxia	1(50%)	3(16.6%)	1(20%)
Temperature			
Fever (>37.7C)	1(25%)	16(84.2%)	5(100%)
No fever(< 37.7C)	3 (75%)	3 (15.8%)	0
Co-trimoxazole prophylaxis			
Yes	0	1(7.1%)	0
No	3 (100%)	12(85.7%)	4(100%)
Unknown	0 n=3	1(7.1%) n=14	0 n=4
Outcome			
1- discharged	1(25%)	4(21.1%)	1(16.7%)
2- deceased	0	2(10.5%)	0
3- absconded	0	0	1(16.7%)
4- still inpatient	3(75%)	13(68.4%)	4(66.6%)

Table 7: Multivariate analysis of the correlates of positive nasopharyngeal aspirate for Pneumocystis pneumonia in seropositive children admitted with severe and very severe pneumonia

	NPA – VE	Equivocal
VARIABLES	2	3
gender	3.614	10.97
	(0.113 - 115.3)	(0.412 - 292.3)
agegrp2	0.438	0.472
	(0.036 - 5.379)	(0.044 - 5.055)
diagnosis1	0.962	0.367
	(0.019 - 48.29)	(0.0069 - 19.36)
PCR	0.812	1.442
	(0.003 - 227.2)	(0.0069 - 300.5)
hypoxiad1	0.207	0.156
	(0.004 - 9.959)	(0.0031 - 7.715)
fever	1.969	0.703
	(0.068 - 56.84)	(0.027 - 17.81)
mortality	0	0
	(0 -)	(0 -)

The table above is a multivariate analysis using NPA positive for PJP as the baseline.

Reported are odds ratios and their 95% confidence intervals. None of relationships are

significant at the 95 % level.

DISCUSSION

This study highlights the prevalence of bacteremia and the changing pattern of the causative organisms in children with severe and very severe pneumonia.

The mean age of the study population was 13 months, with a median age of 9 months. Majority, (66%) of the study population was below one year .This is in keeping with the observation from similar studies previously done that found severe forms of ARI been more frequent in the younger children[34, 35].

We derived the pneumonia severity classification using data collected on admission and based on the WHO guidelines. The number of children found with severe pneumonia and very severe pneumonia was 148 (44.2%) and 187 (55.8%) respectively. Children found with very severe pneumonia in our study was much higher than other studies done in KNH and other regions [9,36]. This could be due to the fact that majority (47%) of our study population was referred from other health facilities and because the study had comprehensive coverage thereby capturing even patients who came in at night.

In our study only organisms of known pathogenicity were considered to be of aetiological significance. The most frequently isolated pathogen was Streptococcus pneumonia 5(45.5%), followed by Salmonella typhinurium 3(27.3%), Escherichia coli 2(18.2%) and Pseudomonas 1 (9%). Haemophilus influenza was not isolated in any of the blood samples. In children who were less than 12 months old, Salmonella typhinurium and E. coli were the predominant isolates and in those above 12 months of age Streptococcus pneumonia was the most common. In contrast to our findings, several studies done in the

pre Hib period revealed H. influenza as a common pathogen in blood culture isolate Forgie et al, Gambia cultured H.influenza in 11% and S pneumonia in 20% of isolates.[10] Kariuki et al in KNH, studied aetiology of pneumonia before and H.influenza was one of the common isolates 8%.[13] Wafula et al , KNH also looked at the aetiology of pneumonia usning blood cultures and NPAs in the pre Hib period and they found that H influenza, S. pneumonia and staphylococcus were the most common isolates.[12] Studies done in the post Hib era have shown almost similar result to our study, several implicating Streptococcus pneumonia as the most common isolate and H influenza being less commonly isolated from blood culture found in children with severe and very severe pneumonia, with Non typhoidal salmonella being increasingly isolated [9, 14, 15].

Eleven out of the three hundred and thirty five children had blood cultures that were definitely positive, giving a bacterial isolation rate of 3.3% among children with severe and very severe pneumonia. Our study may have underestimated the actual prevalence of bacterial pneumonia in the community. This figure was lower than that reported by Maina et al, 5.6%.[36] Several factors may have contributed to the lower yield: first it is well recognized that blood cultures are not very sensitive for detecting bacteremia; several studies done in developing countries have shown that the yield from blood culture in children diagnosed with pneumonia has ranged form 10 to 30%[9, 29]. Secondly, the technique, number, and timing of blood cultures, antibiotics administered before obtaining the specimen, quantity of blood with-drawn are also important determinants of the accuracy of blood culture results[9]. Small volume of inoculation and use of antibiotics may have compromised the sensitivity of blood cultures in the study. We aimed at taking

2 to 3ml of blood sample but in several occasions this was not achieved. Majority (62%) of the study patients had history of prior antibiotic; we were not able to determine the antimicrobial serum levels therefore exposure was established through prescriptions, referral notes and medication bottles. Berkley et al found that sensitivity of blood cultures fell by almost one third when culture sample of 1ml were compared with those of 3ml and recent antibiotic use reduced blood culture yields by 62 to 73% [9].

Special measures must be taken to avoid contamination of the blood culture media by normal skin flora during the process of collection. Contamination of blood cultures can occur even when the most exacting techniques for collection and processing are used. We adhered to strict aseptic procedures throughout the process of collection, transport and investigation of the clinical specimen to minimize contamination. Despite this we cultured a very high rate of contaminants: 43 out of 335 samples (12.8%) when compared to what has been earlier reported[12, 13]. Organisms grown were 29 (8.7%) Coagulase negative staphylococcus, 6(1.8%) Micrococcus, 7 (2.1%) mixed organisms, 1(0.3%) Diptheroids. Coagulase-negative staphylococci (CONS) are most frequent constituents of the normal flora of the human skin.[37] Staphylococcus epidermidis constitutes more than half of resident staphylococci.[37] Other studies have shown that despite the frequency of isolation of CONS, only 4 to 12 percent of patients with blood cultures positive for these organisms are estimated to have significant bloodstream infections.[38] During analysis we explored the possibility that these bacteria represented true pathogens. Our analyses showed no statistically significant differences in demographic and clinical characteristics when patients in whom CONS were isolated compared to those with cultures reported as

negative, suggesting that the two groups are homogenous. However the relatively small number of children with positive cultures for coagulase negative staphylococci may have rendered the test underpowered to demonstrate any differences between the groups.

Significant progress has been made in the control of bacterial pneumonia with the development of bacterial polysaccharide - protein conjugate vaccines for the two most common pathogens in childhood pneumonia, H. influenza type b and S. pneumonia. These vaccines have led to a drastic decline in pneumonia caused by these pathogens in the countries where they are used routinely [5, 7]. In a study by Cogwill et al, in Kilifi, Kenya, impact of Hib vaccine was studied and they found that its introduction into routine childhood immunization reduced Hib disease by 12% of its baseline level in children aged below 5 year [6]. We analyzed children above the age of 4 months to check for status of pentavalent vaccination, an assumption was made that by this age most children should have received all doses at 6, 10 and 14 weeks as required by WHO and Ministry of Health guidelines. We found that 97% of the children aged between 4 to 59 months who were enrolled in the study had received the 3 doses as required. This may have been a major reason for the absence of H. influenza in any of our blood culture samples. However other reasons that may have contributed to the failure to culture the H. influenza are; firstly because of their small size, pleomorphism and poor uptake of stain by some isolates, they are sometimes difficult to visualize. Identification of micro-organisms on smear requires at least 10^5 bacteria/ml; failure to visualize them does not therefore preclude their presence. Secondly culture of H. influenza requires prompt transport and processing of specimen because it is a fastidious organism, despite the fact

that we had standard operating procedures on sample collection and transport we still encountered challenges especially in delivering and storage of samples collected at night. Thirdly H. influenza require a carbon dioxide-enriched atmosphere, hemin (factor X), and nicotinamide adenine dinucleotide (NAD, factor V) for in vitro growth; therefore, isolation from clinical specimens on solid medium requires the use of chocolate agar or other X and V factor supplemented media [6]. Despite ascertaining that these conditions were met by the laboratory to enhance growth of the organism, it is well know that the organisms are very fastidious and therefore culture rates remain low. Finally, most strains of H. influenza are susceptible to beta-lactam agents (e.g. penicillins such as amoxicillin, or second or third-generation cephalosporins) fluoroquinolones, macrolides and amino glycosides. Majority of the study population (62%) had been exposed to one or more of the mentioned antibiotics within a week prior to enrollment.

In our study caretakers were provided with information on HIV disease and then the HIV rapid test was offered. Out of the 335 children enrolled into the study 306 (91.3%) accessed HIV testing with all children below 18 months found to have a positive antibody test having a PCR virologic test to confirm infection. The study revealed a HIV prevalence of 10.7%. Previous studies done in KNH have found prevalence ranging from 12.7% by Warurua et al in 1992 among the general paediatric admissions,[39] to 15.8% by Inwani et al among the infants mothers presenting for delivery in maternity unit.[40] Other studies looking at HIV prevalence in children with pneumonia in Africa found prevalence ranging from 35.5% to 60% [23, 24, 27]. All the seropositive children aged less than 24 months underwent nasopharyngeal aspiration to test for presence of PCP.

Despite PCP still being a major cause of morbidity and mortality in HIV infected children with severe and very severe pneumonia in the developing countries, very few studies have been done to show the magnitude of the disease. Our study is unique from the one previously done in Kenya in that it examines prevalence of PCP in HIV infected children using nasopharyngeal aspiration in severe and very severe pneumonia. Kose et al, looked at the prevalence of PCP in all HIV exposed children age less than 24 months irrespective of infection status with severe pneumonia using sputum induction, they found a prevalence of 14%. The most sensitive method of diagnosing PCP is DNA by PCR; this method was not available for this study. We used Immunofluoresent technique assay which has been shown to give rapid diagnosis. Previous studies have used sputum induction with nebulised saline for diagnosis, we were not assured of the safety of this procedure in severely hypoxic patients and we also had no access to protective gear for the investigator to perform the procedure. Bronchoalveolar lavage and transbronchial biopsy which have been shown in previous studies as successful methods to diagnose PCP were not available for this study. We found the prevalence of PCP similar to that reported by Kose et al at 13.8%. All the children infected with PCP were below 6 months of age and were HIV infected. Similar results have been reported in several African studies, in Malawi Graham et al reported a prevalence of 10.6% using NPA and immunofluoresent assay [23]. Zar et al in South Africa reported a prevalence of 9.9%, this study used both sputum induction and NPA methods [25]. Bakeera- kitaka et al, in Uganda, using sputum and immunofluoresent assay, revealed a prevalence of 41.6% in HIV infected children; all the infected children were less than 6months of age [24].

Outcome for PCP in our study was good; none of the infected children had died by day 5 post admission. This is different from results obtained in similar studies previously done which found PCP to be associated with poor outcome. Many factors may have contributed to this difference in outcome; although the number of children found to be infected was too small to give our study any power to detect statistical associations, we took measures to give quality care to all children admitted, the availability of rapid HIV tests in the PEU made it possible to know the children HIV status on admission, all the children found to be HIV seropositive with severe and very severe pneumonia were immediately initiated on high dose oral co-trimoxazole to be continued for 21 days. Pulse oximetry was made readily available in the Paediatric Emergency Unit during the study period and this helped in identifying children with hypoxia who were then instantaneously initiated on oxygen. In addition these children were treated with the first line treatment for severe or very severe pneumonia according to the Ministry of Health guidelines.

STUDY LIMITATIONS

The time in which the study had to be completed was limited because was done as part of fulfillment of the MMed paediatrics program. A longer duration of data collection would have potentially yield a larger sample size that would have allowed greater precision in reporting of estimates for proportions or odds ratios. In addition, it would have helped in reducing the possible influence of seasonal variation on the reported estimates thereby improving generalisability.

Cost was a limitation as most of the material, equipment and laboratory resources required to carry out the study was very expensive thereby limiting the number of patients recruited.

Kenyatta National Hospital is a tertiary facility therefore most of the patients are referred from lower-level hospitals. This lead to enrolment of "sicker" patients than those attending other hospitals in Kenya thus limiting the applicability of the results to these other health facilities. Furthermore most of these patients have already been exposed to antibiotics, either self medicated or from another health facility, so the study probably under-estimated the actual prevalence of bacteremia and it probably also selected for the severe end of the spectrum. It may also explain the higher number of atypical organisms cultured as cause of pneumonia which may have occurred secondary to nosocomial infection. Similar studies at lower level facilities where patients first receive care will provide more accurate info on the prevalence of pathogens in community acquired pneumonia.

Some of the data collected relied on the memory of the caregivers, which may have been inaccurate. This was addressed in part by careful structuring of the questions directed at the caregivers to limit their liability to recall bias. However, despite these limitations the study has provided invaluable data that will help in improving our understanding of pneumonia in children at KNH.

CONCLUSION

1. The bacterial yield obtained from this study was low (3.3%) making conclusions difficult. Although Streptococcus pneumonia was the predominant bacterial isolate, it was found in only 5 out of the 385 children studied. H.influenza was not isolated.

2. The prevalence of PCP among HIV infected children with severe and very severe pneumonia was 13.8% and is therefore an important cause of morbidity.

RECOMMENDATIONS

1. Most of the children recruited into this study had received antibiotics and thus we may have under-estimated the prevalence of bacteremia and selected for the severe end of the spectrum. Similar studies at lower level facilities where patients first receive care will provide more accurate information on the prevalence of pathogens in community acquired pneumonia.

2. Streptococcus Pneumonia was the most common pathogen isolated in this study highlighting the need for the pneumococcal vaccine to be introduced as part of the routine childhood vaccinations.

3. All the HIV infected children found with PCP were not on co-trimoxazole prophylaxis as recommended by MOH. This highlights the need to place emphasis on antenatal testing of all pregnant women in order to make early diagnosis in the children and initiate early co-trimoxazole prophylaxis in all HIV exposed and infected children.

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C c

APPENDIX 1 - WHO GUIDELINES ON CLASSIFICATION OF PNEUMONIA

Signs & symptoms	Classification	Treatment
- Central cyanosis - Severe respiratory distress e.g. head nodding - Not able to drink	Very severe pneumonia	- Admit - Appropriate antibiotics - Manage airway - Oxygen - Treat high fever
- Chest in drawing	Severe pneumonia	- Admit - Appropriate antibiotics - Oxygen -Treat high fever.
Fast breathing -60b/min <2/12 -50b/min 2- 11/12 -40b/min 1- 5 yrs Definite crackles on Auscultation	Pneumonia	- Home care - Oral amoxyl - Advise when to return for follow up. (after two days)
- No signs of either severe or very severe pneumonia	No pneumonia Cough/cold	-Home care -Soothe throat with lozenges -Give safe cough remedy -Continue feeds.

APPENDIX 2 - DATA COLLECTION FORM

Please fill ALL sections in by interviewing the patient's caregiver

1.0 Registration					
1.1 Questionnaire Serial No.		1.2 Patient's Hospital No.		1.3 Date (dd/mm/yy)	[_|_] H [_|_] H [_|_]

2.0 Patient's Data

2.1 Preliminary assessment

2.1 History of cough	❏		[_0_] No	[_1_] Yes
2.2 If **yes** indicate duration in days	❏ Don't know	[_|_|_]		
2.3 History of difficulty in breathing	❏ Don't know	[_0_] No	[_1_] Yes	
2.4 If yes for 2.1 or 2.3, are the symptoms worse at night?	❏ Don't know	[_0_] No	[_1_] Yes	
2.5 Examine for central cyanosis (should be done before administration of **oxygen**)	[_0_] Absent	[_1_] Present		
2.6 Examine for visible severe wasting	[_0_] Absent	[_1_] Present		
2.7 Examine for oedema of kwashiorkor	[_0_] Absent	[_1_] Present		

3.0 Personal details

3.1 Gender	[_0_] Male	[_1_] Female		
3.2 Date of birth (dd/mm/yy) Enter at least year	❏ Don't know	[_|_] H [_|_] H [_|_]		
3.3 Time of admission (24 hr clock)	[_|_] H [_|_]			
3.4 Ward of admission	[_1_] 3A	[_2_] 3B	[_3_] 3C	[_4_] 3D
3.5 Current body weight in kg	[_____]			
3.6 Immunization status up to date as per KEPI schedule? Derive from caregiver history and child's card if available	BCG (0-1) (check for scar)	❏ Don't know	[_ _]	
Enter the number of doses received for each vaccine	OPV (0-4)	❏ Don't know	[_ _]	
	Pentavalent (0-3)	❏ Don't know	[_ _]	
	Measles (0-1)	❏ Don't know	[_ _]	

4.0 Treatments given for presenting illness prior to admission *(please request patient for any records including referral note, prescriptions, containers etc)*

4.1. Did the patient receive medication prior to admission?	❏ Don't know	[_0_] No	[_1_] Yes		
4.2. What kinds of medication did the patient receive?	❏ Don't know	[_1_] cough syrup	[_2_] Antibiotic	[_3_] Antipyretic	[_4_] Antimalarials
		[_5_] Herbal medicines	[_6_] Traditional treatments	[_7_] Anti-histamine	[_8_] Other (specify)

| 4.3 If antibiotics were given, which one(s) | ☐ Don't know | [_1_] Co-trimoxazole | [_2_] Amoxicillin | [_3_] Injectable penicillin |
| | | [_4_] Macrolide | [_5_] Cephalosporin | [_6_] Other (specify) |

5.0 TB/HIV History

5.1 Has the patient ever received prior treatment for TB?	☐ Don't know		[_0_] No	[_1_] Yes
5.2	If **yes**, specify duration of treatment in weeks		☐ Don't know	[_|_]
5.3 Has the child had diarrhoea lasting >14 days?	☐ Don't know		[_0_] No	[_1_] Yes
5.4 Has the patient been diagnosed as HIV positive in the past?	☐ Don't know		[_0_] No	[_1_] Yes
5.5	If yes for 5.4, is the patient currently on HAART?	☐ Don't know	[_0_] No	[_1_] Yes
5.6	Is the patient currently on cotrimoxazole prophylaxis?	☐ Don't know	[_0_] No	[_1_] Yes

6.0 Caregiver's data

6.1	Did mother attend ANC for the admitted child?	☐ Don't know	[_0_] No	[_1_] Yes
6.2	If yes for 6.1, how many times?		[_1_] Once [_3_] >2times	[_2_] Twice
6.3 Mother's HIV status at birth of patient.	☐ Don't know	[_0_] Negative	[_1_] Positive	[_2_] Will not disclose
6.4	If positive for 6.3, was PMTCT given?	☐ Don't know	[_0_] No	[_1_] Yes
6.5	If **yes**, Who received PMTCT?	[_1_] Mother only	[_2_] Baby only	[_3_] Both mother and baby
6.6	Has the patient ever breastfed from the mother?	☐ Don't know	[_0_] No	[_1_] Yes

Time after initial assessment Sign	0 hours (1ˢᵗ review) Note: Post bronchodilator therapy for wheezers Date: Time:			
7.1 Respiratory rate (breaths per minute)	[_ _ _]			
7.2 Temperature (°C)	[_ _ . _]			
7.3 Oxygen saturation (%)	[_ _ _ _]			
7.4 Central cyanosis (Examine after at least 3min on ambient air) [0] No [1] Yes	[_]			
7.5 Nasal flaring [0] No [1] Yes	[_]			
7.6 Grunting [0] No [1] Yes	[_]			
7.7 Head nodding [0] No [1] Yes	[_]			
7.8 Lower chest wall indrawing [0] No [1] Yes	[_]			
7.9 Wheeze audible/auscultatory [0] No [1] Yes	[_]			
7.10 Crepitations [0] No [1] Yes	[_]			
7.11 Level of consciousness (AVPU) [1] A [2] V [3] P [4] U	[_]			
7.12 Ability to drink [0] No [1] Yes	[_]			
7.13 Neck stiffness [0] No [1] Yes	[_]			
7.14 Severe pallor [0] No [1] Yes	[_]			
7.15 Sunken eyes [0] No [1] Yes	[_]			

8.0 Microscopy and culture results

8.1 Blood culture done? If no, proceed to 5.3.2	[0] No	[1] Yes		
8.2 Blood culture results: Growth obtained?	[0] No	[1] Yes		
8.3	*If **yes**, indicate organism isolated*	[1] *S. pneumoniae*	[2] *H. influenzae*	
		[3] *S. aureus*	[4] Other organism (specify)	

9.0 NPA done?	[_1_] Yes	[_2_] No		
10.1 Rapid test done?	[_0_] No	[_1_] Yes		
10.2	*If no*, indicate reason	[_0_] Consent not granted	[_1_] Kit unavailable	[_2_] Other reason (specify)
10.3 Determine®	[_0_] Negative	[_1_] Positive		
10.4 Bioline® assay.	[_0_] Negative	[_1_] Positive		
10.5	*If 10.3 and 10.4 are discordant, report Vironostika ELISA results.*	[_0_] Negative	[_1_] Positive	
10.6 patients age in months > 18mo	[_0_] No	[_1_] Yes		
	If no for 10.6 report results for HIV viral DNA	[_0_] Negative	[_1_] Positive	
10.7 Patients HIV status	[_0_] Negative	[_1_] Positive		

9.0 Treatment outcome				
9.1 What was the outcome of inpatient treatment at day5?	[_1_] Patient discharged	[_2_] Patient died	[_3_] Patient absconded	[_4_] Patient's whereabouts unknown
9.2 Indicate nights of in-patient treatment received				

APPENDIX 3 - INFORMATION AND CONSENT FORM

Your child's illness

Your child has features suggesting they have severe or very severe pneumonia severe enough to indicate that admission is necessary.

We are doing a study on severe or very severe pneumonia and would like to explain this to you and ask your permission to include your son / daughter.

Who is the study being done by?

The study is being done by doctors from the University of Nairobi and the Kenyatta National Hospital with help from researchers at the Kenya Medical Research Institute (KEMRI).

Why are we requesting to include your child?

The study is on severe forms of pneumonia. We are asking if we can study all children with these forms of severe pneumonia coming to KNH. We wish to improve our understanding of this disease.

Why are we doing the study and what is this study about?

Severe pneumonia is one of the most common reasons why children get admitted to hospital and it can be very serious. We are trying to understand what are some of the causes of this illness, what makes it hard to treat some children with this illness and get information that may help us provide better treatment for this illness in the future.

What will the study involve for my child if I agree?

If you are happy for your child to be involved we would like to ask you questions about the child's illness, examine them carefully and record the information about your child's illness.

    ☐ *If there is wheeze* – We will also try to treat your child with a medicine now that may help the breathing, it is possible if this medicine works that your child may not need admission.

We would also like to test for HIV in your child. There may be other blood tests your child needs to help us give the correct treatment, these are not part of the study but we will try and do all those tests needed now at the same time to avoid an extra needles. Testing for HIV is now a routine on admission to this hospital – it is recommended for all patients and should be done for all children not in the study too. I will explain this in more detail separately and ask if you agree to the test on your child.

    ☐*For HIV Exposed children* - HIV exposed children tend to suffer from a more severe cause of pneumonia that make the child very ill. If treatment is initiated early it could be life saving. We would also like to take a specimen of mucus from the nose using a soft plastic tube and sucking (demonstrate NPA device) that sometimes helps us find this form of pneumonia. You will not be charged for these tests.

After examining your child, doing the necessary tests and giving the recommended treatment, your child will be followed-up in the ward we will do this together with the ward doctors.

Are there any risks to my child participating?

Taking blood from the arm causes a small amount of temporary pain, but the amount taken is too small to affect your child's health and we will take the blood test for the study at the same time as any other routine blood tests that are needed. There may be some slight inconvenience to you because of the time taken to answer the questions and get the tests but this should not cause any harm.

Are there any benefits to my child participating?

Your child may benefit from the research. Identification of the exact organism causing the pneumonia will help in selecting the best antibiotic to treat the child especially if the child does not respond to the first line therapy. You will not be charged for tests that are part of the research. The research may also help us provide better treatment to children in the future.

What happens if I refuse to participate?

All participation in research is voluntary. You are free to decide if you want your child to take part. If you do agree you can change your mind at any time and withdraw your child from the research. This will not affect your child's care now or in the future.

Who will have access to information about me/my child in this research?

Information that is important to providing the right medical care for your child will be shared with the doctors looking after your child but all of the staff at KNH will try and ensure your medical records are kept confidentially. All the research records are stored securely and only people who are closely concerned with the research will be able to view information from participants.

Who has allowed this research to take place?

A committee from KNH has looked carefully at this work and has agreed that the research is important, that it will be conducted properly and that participants' safety and rights have been respected.

What if I have any questions? Please feel free to ask any questions about the study. If there is any part of this form that you do not understand, be sure to ask questions about it. You can also contact those who are responsible for the care of your child and this research:

UNIVERSITY OF NAIROBI,

DEPARTMENT OF PAEDIATRICS AND CHILD HEATH.

(Investigator) Dr Digolo. A. Lina

(Supervisors) Prof Mboori Ngacha, Dr Laving

If you agree to participate in this study please sign the consent form attached

Information and consent form

I, being a guardian of _____ (name of child) have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily. I understand that I can change my mine at any stage and it will not affect me/my child in any way.

☐ I agree to allow my child to take part in this research and for the collection of clinical data.

If NPA is to be requested

                ☐ I agree to a NPA being performed

Parents/guardian's signature: _____   Date:

_____

Parent/guardian's name: _____   Time:

_____

I certify that I have followed all the study specific procedures in the SOP for obtaining informed consent.

Designee/investigator's signature: _____   Date:

_____

Designee/investigator's name: _____   Time:

_____

Only necessary if the parent/guardian cannot read:

I* attest that the information concerning this research was accurately explained to an apparently understood by the parent/guardian and that informed consent was freely given by the parent/guardian.

Witness' signature: _____   Date:

_____

Witness' name: _____   Time:

_____

*A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.

Thumbprint of the parent as named above if they cannot write:

_____

APPENDIX 4 - NASOPHAREGEAL ASPIRATION SOP

1. PURPOSE

    1.1. **Purpose:** This SOP describes the nasopharyngeal aspirate (NPA) procedure for collecting nasopharyngeal epithelial cells.

        1.1.1. NPA is a procedure of collecting dry nasal specimen. .

2. ABBREVIATIONS AND TERMS

    2.1. NPA – Nasopharyngeal Aspirate

    2.2. SOP – Standing Operating Procedure

    2.3. QA – Quality Assurance

3. SPECIMEN

    Nasopharyngeal epithelial cells commonly referred as "nasal mucous specimen"

4. EQUIPMENT AND MATERIALS:

    4.1. Laboratory Request Form

    4.2. Feeding Tube (40 cm); recommended sizes as per age group;

        4.2.1. Infants (1-12months) – Gauge 8,

        4.2.2. Children (1- 5 yrs) – Gauge 10

    4.3. Suction pump – electric or foot pump (pressure 100mmHg if using electric pump)

    4.4. Mucous specimen trap and tubing to pump

    4.5. a pair of clean Gloves

    4.6. Disposable paper towel

    4.7. Normal Saline in bijou container

    4.8. Marker pen

5. RESPONSIBILITIES:

    5.1. It is the responsibility of the principal investigator to do this procedure.

6. PROCEDURES:

6.1. Preparation:

        6.1.1. Ensure all materials required are available and the Foot/electric pump is in good working condition and all tubes are properly connected.

        6.1.2. Confirm that the parent/guardian has signed informed consent form for the pneumonia study.

6.1.3. Ensure patient's privacy in the sample collection area; either beside or admission room.

6.1.4. Explain the procedure to the parent/guardian demonstrating the equipment and the materials to be used.

6.2. Procedure

6.2.1. Wash hands thoroughly and dry using disposable hand towel.

6.2.2. Put on a clean pair of gloves.

6.2.3. Connect up FG tube to mucus specimen trap and to the pump.

6.2.4. Position patient on his/her back (supine position) with head held still and arms immobilized - request assistance from the caretaker.

6.2.5. Without applying suction, gently insert the feeding tube into the nostril, aiming downwards. NB: The depth of the insertion necessary to reach posterior pharynx is equivalent to the distance between nose and the ear.

6.2.6. Once the catheter is inserted to the correct position, apply suction and slowly remove catheter using a rotation movement. The catheter should remain in the pharynx for no longer than 10 seconds.

6.2.7. Aspirate approximately 4mls of sterile normal saline to flush the feeding tube in an effort to clear the nasal specimen to the mucus specimen trap connected.

6.2.8. Disconnect the FG tube from the Mucus specimen trap and suction pump, then discard the feeding tube and tie knot in the mucus trap tubes to prevent leakage.

6.2.9. Label the mucus trap with child's serial number, name and date and time of specimen collection

6.2.10. Dispose off gloves and wash hands.

6.3. *Specimen transportation and storage*

6.3.1. Fill in the laboratory request form; remember to include date and time of collection.

6.3.2. Take the sample to KEMRI Lab (Micro).

APPENDIX 5 - PCP SOP

TITLE: The detection of Pneumocystis carinii oocysts using indirect immunofluoresent test.

1.0 PURPOSE:

1.1 Detect If is an indirect immunofluoresent kit for the detection of P. carinii oocysts in induced sputum.

2.0 DEFINITIONS:

2.11 NPA- nasopharyngeal aspirate

2.2 RPM-Revolutions per Minute

2.3 FITC-Fluorescein Isothiocyanate

3.0 SPECIMEN:

3.1 NPA

4.0 EQUIPMENT/MATERIALS/REAGENTS:

4.1 Precision pipettes

4.2 Centrifuge

4.3 Distilled water

4.4 Wash bottle

4.5 Analar Acetone

4.6 Analar Ethanol

4.7 Humidified Incubator @ 37°C

4.8 Coverslips

4.9 Fluorescent microscope

4.10 Pipette tips

4.11 Mucolytic agent

4.12 Slides

4.13 Vortexer

4.14 Mounting medium

5.0 METHODOLOGY

5.1 PRINCIPLE

5.1.1 When specimens are fixed on slides and enzyme digested, then treated with murine anti- P. carinii antibody and fluorescently labeled with anti-mouse antibody and viewed using a fluorescent microscope, oocysts show a medium bright to bright apple green fluorescence.

5.2 PROCEDURE

5.2.1 All manipulations should be done in a safety cabinet (category 2) and discarding of any materials in a disinfectant (virkon).

5.2.2 Inactivate any HIV that maybe present in the samples by adding an equal volume of absolute ethanol and incubate for 10 minutes at room temperature before processing.

5.2.3 Specimen should be treated by addition of mucolytic agent for 10mins at room temperature.

5.2.4 Label slide with sample number.

5.2.5 Centrifuge specimen for 15 min at 3000 x g, and wash the pelletable material in distilled water, repeat once or twice ensuring that the pellet is fully resuspended between washes.

5.2.6 Resuspend the final pellet in a small amount of distilled water such that the density is not excessive and vortex.

5.2.7 Spread 10-20ul over the entire area of one or more slide wells, evaporate to dryness at 37°C.

5.2.8 Fix specimen by overlying 1-2 drops of analar acetone, allow to evaporate at room temperature.

5.2.9 Rinse preparation with distilled water.

5.2.10 Overlay the dried specimens with 20ul dilute enzyme ensuring the entire well is covered.

5.2.11 Incubate slides for 30 min in a humidified incubator at 37°C.

5.2.12 Rinse slides with distilled water by running a stream of water over the surface of the wells but not directly at the specimen.

5.2.13 Wick and air dry the slides.

5.2.14 Add 15ul of anti-P. carinii antibody and incubate in a humidified chamber for 15 minutes at 37°C

5.2.15 Rinse wells with distilled water, wick and air dry.

5.2.16 Add 15ul FITC-conjugated anti-mouse antibody to specimens and incubate for 15 minutes at 37°C.

5.2.17 Rinse wells, wick and air dry.

5.2.18 Place a drop of mounting medium onto the well in use and apply a cover slip, invert onto an absorbent tissue and press gently to remove excess mounting medium and air bubbles.

5.2.19 Examine specimens for medium bright to bright apple green oocysts which maybe evenly or unevenly labeled. Cellular debris and other material fluoresce red due to Evans Blue.

5.3 INTERPRETATION OF RESULTS

5.3.1 **Positive result**:

Made when five or more oocysts show typical fluorescence in the stained fixed specimen.

5.3.2 Negative result:
Made when there are no fluorescent oocysts in the entire specimen.

5.3.3 Equivocal result:
Made when one to five oocysts are observed

5.4 QUALITY CONTROL
5.4.1 Viewing slides by second person to blind first
A second person should examine the slides without knowing the results of the first person then compare the results. Results found to differ should be reassed and consensus obtained.

5.4.2 Checking procedure and quality of staining.
Make sure the stain does not dry on the slide by ensuring the incubator is humidified. Proper washing of the slides should be done with distilled water whenever required.

5.4.3 Equivocal result procedure.
If two people get equivocal results on the same slides it should be reported as equivocal.

If one says it's equivocal and the other a different result then the two should come to a consensus on which result to give to the clinician.

6.0 APPENDICES:
6.1 Reagent preparation
6.1.1 0.1% Dithiothreitol solution
0.1g in 100ml of water
6.1.2 Stock Enzyme reconstitution
Add 200ul of 0.001M HCl to the lyophilized enzyme
7.1.2.1 Working enzyme
1 part reconstituted enzyme + 9 parts enzyme diluent

APPENDIX 6 - BLOOD SAMPLING SOP

1.0. PURPOSE:

To outline the procedure of obtaining blood samples

2.0. SPECIMEN

Blood

3.0. EQUIPMENT/ MATERIALS/ REAGENTS

- Antiseptic solution
- Strapping (adhesive straps)
- Dry and wet Cotton swabs
- 2 pair of clean gloves
- Tourniquet
- Scissors
- Slint
- Blood sample bottles (purple top, BD vacutainer green top, culture bottle, malaria slides, I-stat cartridge, optimal kit, heamocue)

4.0. METHODOLOGY

- Explain the procedure to the child's mother, to ensure cooperation.
- Perform a thorough vascular access assessment
- Prepare and gather all necessary equipment
- Identify potential site to be used.
  - Forearm
  - Back of the hand or wrist
  - Scalp
  - Long saphenous (anterior to medial malleolus.)
- Obtain assistance from other health care personnel for patient immobilization
- Wash hands with anti- microbial soap
- Put on gloves
- Apply the tourniquet about 6-8 inches above the selected site to distend the veins
- Clean the skin with antiseptic solution and allow it to dry.
- Grasp area below the proposed site, using thumb to firmly stabilize the vein and soft tissue.
- Place the bevel of the catheter in an upward position between thumb and index finger
- Hold the catheter at 45 angles above the skin surface. Pierce the skin and underlying tissue to reach the vein

- Lower the shaft of the needle until it is almost flat with the skin surface and move needle tip directly over vein.
- Enter the vein slowly; verify entry by flashback of blood.
- Advance the needle and catheter assembly approximately 3-5mm further to ensure entry of the catheter into the lumen of the vein
- Push the needle into the vein until the hub meets the skin
- Apply digital pressure over catheter tip and withdraw the needle
- Using a syringe withdraw 7-12mls of blood and distribute accordingly into the sample bottles.(*refer to blood sample aliquot chart*)
- Using saline or water for injection   Flush the cannular gently to confirm that it is in the vein.

  NB: On flushing lines. The smaller the syringe used, the greater the pressure exerted on the fluid in the line. Avoid using 1ml syringes to flush a blocked line-the line may rupture or damage tissues by infiltration.
- If the cannular is satisfactorily inserted, tape it in place.
- Immobilize the extremity with a splint
- Instruct the parent to notify a member of the nursing staff if the arm or site becomes swollen, painful, if there is leakage from the site or bleeding
- Label the dressing with date and your initials
- Document on the nurses' notes:
  - Date and time of insertion
  - Site of insertion

APPENDIX 7- STANDARD OPERATING PROCEDURES FOR RAPID HIV TESTING

1.0 Introduction
Rapid testing involves a series of two serological tests done to determine the HIV status of a patient who, in the case of PIDTC has issued informed consent and undergone pre-test counseling either directly or through an a legal proxy.

2.0 Abbreviations
HIV – Human Immunodeficiency Virus

PIDTC – Provider-initiated Diagnostic Testing and Counseling

ELISA – Enzyme-linked Immunosorbent Assay

3.0 Equipment/Materials
3.1 Disposable latex gloves

3.2 Spirit swabs

3.3 Sterile lancet

3.4 Determine HIV-1/2 (Inverness Medical) testing kit

3.5 SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc.) testing kit

3.6 Chase buffer

4.0 Procedure
After pre-test counseling for HIV and having obtained verbal consent to test:

- Glove and wash hands to remove glove powder.
- Swab patient's finger with 3 different spirit swabs and prick it using a sterile lancet.
- Wipe away first drop of blood and allow another to gather.
- For Determine Assay, drip 2 drops of blood (50ul) onto the test pad and apply 1 drop of chase buffer. Allow the test to develop for 15 minutes and then interpret the results. A positive result is indicated by the appearance of 2 lines on the test strip and a negative by the appearance of one on the proximal portion. Any other result is invalid and the test is repeated.
- For SD Bioline Assay, a 20ul capillary pipette is provided, immerse the open end in the blood drop and release pressure to draw blood into the capillary pipette to the black line. Add the drawn specimen into the sample well and add 4 drops (120ul) of assay diluent. As the test begins to work purple color is seen moving across the results window at the centre of the test device. Interpret the result in 5-20 minutes. The presence of only the control line (C) within the results window indicates a negative result. The presence of 2 lines as control line (C) and Test line 1(1) within the results window indicates a

positive result for HIV 1. Presence of control line (C) and test line 2 (2) indicates a positive result for HIV 2. The presence of 3 lines; control line (C), test line1 and test line 2 indicates a positive result for HIV 1and/or HIV 2. The absence of a control line (C) within the results window indicates an invalid result.

A test result is considered positive when both tests are positive and negative when both tests are negative. A discordant result will be repeated using ELISA based Vironostika HIV Uni-Form II Ag/Ab test (Sensitivity = 100% and Specificity = 99.9%) manufactured by BioMerieux, Boseind Netherlands.

Standard Operating Procedures for Dried Blood Spot Collection and Handling

1.0 Introduction
Children less than 18 months of age who test positive for HIV 1 or 2 antibodies will undergo a DNA PCR test to confirm the presence of HIV viral DNA in whole blood. The procedure through which this is performed is detailed below.

2.0 Abbreviations
HIV – Human Immunodeficiency Virus
DNA – Deoxyribonucleic Acid
DBS – Dried Blood Spot
PCR – Polymerase Chain Reaction

3.0 Materials/Equipment
3.1 Disposable latex gloves
3.2 Spirit swabs
3.3 Sterile lancet
3.4 DBS plate

4.0 Procedure
The DBS card is clearly labeled with the appropriate identification number. The circles are not be touched with fingers or anything else.
- Warm the area of the body that is to be pricked. (For an infant aged 1-4months and less than 6kgs use the heel of the foot. For infants aged 5-10 months and less than 10kgs use the toes preferably and for larger infants use the finger).
- Wear gloves and wash hands.
- Position baby with the foot downward.
- Clean area to be pricked and dry for 30seconds.
- Prick area using a lancet.
- Wipe away first drop of blood and allow a large drop of blood to collect.

- **Fill the entire circle with a drop. Fill at least 2 circles.**
- **Clean the foot and allow it to dry and clot in free air. Do not apply a bandage.**
- **Do not touch or smear the DBS, allow it to air dry horizontally for at least 3 hours.**
- **Transport the specimen to the laboratory where PCR will be performed to determine the presence/absence of HIV viral DNA.**

APPENDIX 8 - STANDARD OPERATING PROCEDURES FOR CLINICAL SPECIMEN TAKING, TRANSPORTATION AND LABORATORY PROCESSING OF BLOOD CULTURES

1.0 Introduction

Blood cultures are indicated as routine investigation for management of children in East Africa if they have suspected meningitis, severe pneumonia or very severe pneumonia.

2.0 Materials/Equipment

1 pair medium size non-sterile gloves

1 PedsPlus BacTec blood culture medium

1 Sterile 5mls syringe

1 Sterile 23g butterfly

1 Sterile 23g needle with 25 mm shaft

1 Sterile 21g needle with 35 mm shaft

2 Sealed alcohol wipes

1 Specimen information form

1 Piece of clean dry cotton wool

1 Appropriately-filled laboratory request form

3.0 Procedures

3.1 **Venesection**: timing, method, volume, safety considerations, transport to the laboratory and out-of-hours handling.

3.1.1 Timing of venesection

Venesection for blood culture is performed in the casualty department or admitting centre before administration of antibiotics in hospital. Other blood tests, e.g. malaria slide, full blood count, may be taken at the same time, through the same needle.

3.1.2 Culture media for blood

The standard medium for blood culture will be the BacTec PedsPlus/F aerobic medium.

3.1.3 Method of venesection

- Explain the procedure to the parent or guardian.
- First label the bottle with the patient's details and layout the materials from the supplied kit.
- Check that the broth culture bottles are not cloudy or turbulent before using them. If one is cloudy, replace it with a new clear bottle.
- Select an arm and apply a tourniquet.
- Clean the skin with one of the supplied alcohol wipes or with a hospital supplied spirit swab or tincture of iodine. If the skin is very dirty use water and cotton wool to cleanse it before applying the alcohol.
- After the alcohol has dried insert the needle bevel-upwards into the vein and withdraw the required volume of blood.

- Loosen the tourniquet. Place the cotton wool ball over the puncture site and remove the needle carefully whilst gently pressing on the cotton wool.
- Ask the mother or a nurse to press the cotton wool until the venepuncture site has sealed with a clot. Attend to the aliquoting of the specimen immediately.

3.1.4 Volume of blood

The BacTec PedsPlus/F culture vials are optimized to receive 3mls of blood with a range up to 5mls. The guiding maximum venesection volume under KEMRI is 2mls/Kg body weight. Children under 60 days are specifically excluded from this study and children over 60 days can be reliably expected to be ≥2 kg. Therefore it is recommended that 3mls of blood for culture be taken from every child regardless of age or weight.

3.1.5 Method of inoculation of cultures

BacTec bottles are supplied with a slight negative pressure that sucks in the blood from the syringe. If the blood specimen is to be divided into several aliquots (e.g. blood slide, EDTA tube) then the BacTec bottle is inoculated last. However it is important to inoculate the BacTec bottle within a minute of taking the blood, before the blood clots.

- Immediately prior to inoculating the BacTec bottle, wipe the top with a clean alcohol wipe and change the needle from the 23g blue needle used for drawing blood to a new sterile 21g green needle.
- Plunge it through the rubber stopper and allow the blood to be pulled into the culture bottle.
- Safely dispose of the two contaminated needles in a puncture proof container. Do not re-sheath the needles.
- Gently agitate the bottle until the blood has dispersed evenly in the medium.

3.1.6 Transport to the laboratory

The specimen is placed together with the completed form in the plastic bag from the blood culture kit. The bag should be transported to the microbiology laboratory as soon as possible after venesection and handed to a laboratory technologist. Timely transportation of specimens to the laboratory is the responsibility of the clinician taking the specimen. Blood should be delivered to the laboratory within 6 hours of being drawn, or within 4 hours of being drawn when the ambient temperature is <18°C. Whilst awaiting collection the blood culture broth should be stored at room temperature. **Under no circumstances should it be placed in a refrigerator.**

3.1.7 Out of hours sampling.

Out of working hours the BacTec bottle should be placed in an accessible laboratory incubator at 35°C and processed or loaded into the BacTec incubator at the beginning of the next working day.