

**PREVALENCE OF STREPTOCOCCAL PNEUMONIAE CARRIAGE IN THE
UPPER AIRWAY OF CHILDREN IN KENYATTA NATIONAL HOSPITAL**

**A Dissertation For The Partial Fulfilment Of A Masters Of Medicine In Paediatrics
And Child Health, University Of Nairobi**

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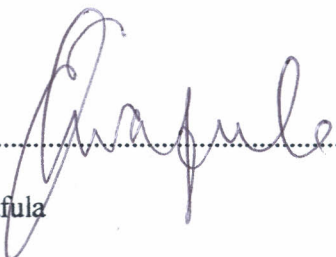
Declaration

This dissertation is my original work and has not been presented for the award of a degree in any other university.

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ABBREVIATIONS USED

1. WHO World Health Organisation
2. *S.pneumoniae, Pneumococcus* Streptococcus pneumoniae
3. *Hib* Haemophilus influenzae (*type b*)
4. *S.aureus* Staphylococcus aureus
5. KNH Kenyatta National Hospital
6. UON lab University of Nairobi laboratory
7. HIV Human Immunodeficiency Virus
8. CSF Cerebrospinal Fluid.
9. LRTI Lower respiratory tract infection
10. URTI Upper respiratory tract infection
11. KDHS Kenya demographic health survey
12. KAIS Kenya AIDS Indicator Survey

SUMMARY:

Background: A study of the pneumococcal colonisation of the upper airway of children is a useful way of determining the resistance patterns of the bacteria to the commonly used antibiotics in our country. Regular pneumococcal disease surveillance and resistance testing is rarely done in public hospitals in developing countries like ours, limiting our ability to effectively manage the invasive diseases it causes like Meningitis and Pneumonia. The findings of this study will be useful in guiding our current antibiotic regimes used in the treatment of Pneumococcal disease.

Objectives: To study the prevalence of upper airway carriage of *Streptococcus pneumoniae* for children less than five years and also obtain upper airway bacterial isolates to study the antibiotic sensitivity of pneumococcus.

Methods: This was a hospital-based cross-sectional survey, recruiting 250 patients aged 0-59 months from the paediatric filter clinic and the wards. The parents/guardians were required to give a written consent, fill in a questionnaire then oropharyngeal samples were taken (throat swabs) from their children. These samples were cultured for pneumococcus using standard lab methods and its antibiotic sensitivity tested.

Setting: Kenyatta National Hospital (KNH), it serves as a primary facility for its urban hinterland and is also a tertiary national referral hospital. Patients were recruited from the paediatric out-patient filter and emergency clinic (PEU).

Results: The prevalence of the upper airway colonisation of pneumococcus was 10.8%. The range of age of carriage was 2-60 months with a median age of 13 months and a mean of 16.8 months. Amoxicillin and Cotrimoxazole showed complete resistance against all the isolates tested. The other Penicillin antibiotics also showed high resistance. Ceftriaxone, Gentamicin and Chloramphenical showed no resistance with Erythromycin and the other Cephalosporin antibiotics also showing high sensitivity.

Conclusion: The oropharyngeal carriage rate of pneumococcus in children under five at KNH is 10.8%. In this study Cephalosporin antibiotics have a high sensitivity against pneumococcus compared to Penicillin antibiotics and Cotrimoxazole which have high resistance rates. Chloramphenical, Erythromycin and the Aminoglycosides; Gentamicin and Amikacin also showed high sensitivity rates.

Recommendations: Cephalosporins, Aminoglycosides and Chloramphenical should be used as the first-line antibiotics in the treatment of suspected pneumococcal disease in this hospital. Macrolides like Erythromycin also showed efficacy against pneumococcus. Penicillin antibiotics and Cotrimoxazole should not be used in the treatment of pneumococcal disease. Regular surveillance for antibiotic resistance should be done in this hospital and treatment guidelines should be changed according to the current trends.

BACKGROUND AND LITERATURE REVIEW.

Epidemiology of Pneumococcal Disease.

Pneumococcus remains the leading cause of bacterial community-acquired pneumonia and meningitis causing mortality in children less than 5 years in the developing world ⁽¹⁾ and accounts for 1 million deaths worldwide in children ⁽¹⁾. In Kenya, Current World Health Organisation (WHO) estimates on pneumonia are at 19.9% of total childhood mortality and an incidence rate estimated at 0.3-0.4 episodes per child year ⁽²⁾. This translates to a mortality of 30,000 children /year in Kenya ⁽²⁾. The following studies will illustrate the burden of pneumococcus in Kenya and Africa as a whole.

A study on infant and child morbidity and mortality at a rural district hospital in Eldoret Kenya ⁽³⁾ 4,720 admissions were studied. Pneumonia was the leading cause of death with a 20.4% mortality rate and the second leading cause of admission after Malaria.

Various studies done in Kenya reflect the morbidity and mortality associated with pneumococcus mainly by KEMRI and its affiliate NetSPEAR (The Network for Surveillance of Pneumococcal diseases in the East African Region) at their Kilifi centre for geographic medicine research. These studies are mainly to collect baseline data in preparation for the pneumococcal vaccine introduction in our country.

A study done at Kilifi District Hospital by the Kenya Medical Research Institute (KEMRI) on bacteraemia in hospitalised children, Berkeley et al ⁽⁴⁾ obtained blood cultures from 19,339 children below 13 years admitted there over a 4 year study period from August 1998 to July

2002. *S.pneumoniae* was the leading bacterial isolate (26%) in children below 1 year, had the highest incidence rate in under fives (111/100,000) and was also the leading pathogen in the children who died over 2 months of age (49%) with an overall 8.7 % mortality rate. (3).

Similar studies in Africa also confirm pneumococcus as the leading cause of invasive bacterial infection (5). In comparison in the developed world the incidence rate in children below 2 years is 44.4/100,000 in Europe (6) in the post-vaccine era and 166.9/100,000 during pre-vaccine period in USA(7,8).

A community-based observational study done by KEMRI at Kilifi to assess the incidence rates of bacteremia in the rural community was also done around the same period as the above study by Brent et al(9). Pneumococcal bacteremia incidence rates were at 436/100,000 in children under 5 years old, 4 times more than what was previously estimated.

In young infants, pneumococcus is still the leading cause of bacteremia as shown in a study done by KEMRI over a 2 year period up to April 2002 at Kilifi district Hospital (10). 1,080 children less than 90 days old were studied for the causes and outcome of infant admissions in a rural hospital. Severe infection was the most frequent cause of admission. Pneumococcus accounted for the highest number of the invasive isolates from blood and Cerebrospinal Fluid (CSF) at 15%.

A necropsy study by Hatimy, 2000, University of Nairobi (UoN) Department of Paediatrics, on children dying of severe pneumonia in KNH, *S.pneumoniae* was among the top 3 isolates cultured from the lung aspirates and the leading cause of pneumonia in well-nourished children (11).

Pneumococcus has also been shown to be a leading cause of pyogenic meningitis in children in Kenya. Pyogenic meningitis is associated with a high morbidity and mortality even though it accounts for a lower number of paediatric admissions than Pneumonia. Earlier studies done at Kenyatta National Hospital published in 1995, CSF cultures rates from 40 children and 52 adults showed pneumococcus followed by *N.meningitidis* at 45% and 14% respectively were the leading cause of meningitis (12). Further studies on CSF cultures from other hospitals in Nairobi showed the same 2 organisms as the leading isolates (13, 14).

A study at a rural Kenyan district hospital on the incidence and outcome of pyogenic meningitis, 1.3% of all admissions was due to meningitis, 88% of them being under five years old. Majority of the CSF isolates in children above 3 months were pneumococcus and the overall mortality rate was 30 % (15).

Risk Factors for Carriage And Disease.

Various factors have exacerbated the burden of pneumococcal disease on children in the developing world. HIV and Severe Malnutrition have been shown to independently increase the risk of infection and mortality by invasive pneumococcal disease. Age less than 24 months, season, acute respiratory infections, household transmission and recent antibiotic use increase the risk of upper airway carriage of pathogenic serotypes of pneumococcus.

HIV:

Pneumococcal invasive disease has been shown to have significantly higher incidence rates in African children with HIV. A study done in South Africa on severe lower respiratory tract infections (LRTIs) in HIV-positive children, pneumococcal incidence rate was significantly

higher in HIV-positive children below 2 years and the case-fatality was higher in comparison to the sero-negative children (16). It was also shown to be the leading cause of bacteremia at 46.7% of the positive blood cultures.

A case-control study in Zimbabwe on causes of bacteremia in HIV- positive children also showed pneumococcus was one of the leading bacterial isolates. HIV-positive patients under 6 months old had a higher mortality rate and overall among cases and controls, bacteremic patients were more likely to die than non-bacteremic patients (17).

More studies in South Africa further illustrate the heavy burden of pneumococcus on HIV-positive patients. A study on adults and children below 13 years in Soweto showed the incidence of *S. pneumoniae* bacteraemia was 36.9-fold increased in HIV-seropositive children but the outcome was not significantly different from seronegative controls (18). They also compared the pneumococcal incidence rates over a decade of the HIV pandemic between 1986 and 1997. The rates were noted to double over that period and this was attributed mainly to HIV (18).

As a risk factor in pneumococcal upper airway colonisation, HIV sero-status has no impact. In Kenya, Rusen et al in a study published in 1997 reviewed a cohort of seropositive infants against seronegative controls and found no association between carriage and HIV status when asymptomatic but significant when having a respiratory illness (19). Similarly studies in South Africa, United states of America and Brazil showed no association (20,21,22).

Antibiotic-resistant pneumococci are more prevalent in HIV positive children. In South Africa, HIV positive children with invasive pneumococcal disease had a higher disease

burden, more resistant isolates and higher mortality in the more advanced HIV-stage group (23, 24).

A Romanian study on HIV positive children versus negative controls was done and showed no difference in the susceptibility patterns of nasopharyngeal isolates but high multi-drug resistance in both groups (25). Similarly in Gambia no difference was observed (26).

Malnutrition:

An early study in South Africa showed the association between pneumococci and malnutrition. 75 children with pneumococcal bacteraemia were studied. 50 had malnutrition and 34 of the 50 had severe malnutrition. severe malnutrition was also a risk factor for mortality in these patients (27). It has also been shown to be the leading bacterial cause of pneumonia in malnourished children (28).

In Uganda, a study on aetiology of severe pneumonia in children, pneumococcus was the leading pathogen and patients with severe malnutrition were at a higher risk of death (29).

In Mozambique invasive pneumococcal disease was significantly higher in patients with severe malnutrition than the controls (30).

Age:

In the studies by Brent et al, Roca et al, Usen et al in their studies on invasive pneumococcal disease (IPD) in Kenya, Mozambique and Gambia respectively, showed infants and children less than 24 months had a higher incidence of IPD (9,29,30).

Acquisition and carriage has been linked to age. Longitudinal studies on infants done in Gambia, Southern India, Alabama USA and Bangladesh all show early acquisition and carriage of pneumococcus by 1-2 months of age^(31,32,33,34).

Season:

The rainy season in the tropics like Kilifi Kenya and winter in temperate areas like Alabama USA is linked with higher carriage rates^(35,33). With regard to invasive pneumococcal disease in Africa no association has been shown⁽²⁹⁾ while in temperate regions like USA there is a link^(36,37).

Acute respiratory tract infections:

Upper airway carriage of pneumococcus was noted to be higher during acute upper and lower respiratory infections irrespective of a viral or bacterial aetiology. Studies to support this were done in Papua New Guinea, Israel and Brazil^(38, 39, 40). Osman Abdullahi et al also showed in their study in Kilifi a positive association between an episode of coryza and carriage⁽³⁵⁾.

Antibiotic exposure:

Recent use of antibiotics within a month of sampling has been associated with a higher carriage rate of antibiotic-resistant pathogenic strains especially to penicillin and Cotrimoxazole^(41,42) and those causing invasive disease⁽⁴³⁾. Abdullahi et al in Kenya found antibiotic use as a risk factor for carriage. Overall carriage rates of pneumococcus are not affected by antibiotic exposure.

Household transmission: Household transmission of pathogenic pneumococcal strains that both invasive and non-invasive upper respiratory tract infections has been demonstrated. In

Gambia a study to investigate household transmission showed that siblings of patients with IPD had an 8.5% concordance of serotypes that caused the IPD in their siblings (44). Another study in Gambia showed children were carriers more than adults and odds of carriage were higher if there were other household carriers. household transmission was from children to other members (45). Hendley et al demonstrated that pneumococcal carriage was higher in adults with children in the household than adults without children (46). In Brazil, a study in an urban slum community also showed carriage of similar clonal types among household members (47).

Pathogenesis of carriage to disease:

Pneumococci are gram-positive capsulated bacteria. There are 90 known serotypes of pneumococci based on the capsular polysaccharides yet less than 20 are pathogenic to humans. Pneumococcal disease is a result of the immune response to the cell wall components and the virulent factors mentioned below especially pneumolysin.

The capsule is a protective mechanism to evade phagocytosis. The serotypes are either of transparent or opaque variants. opacity is based on the cell wall polysaccharide content. Transparent variants have been shown to be colonisers and more potent CNS pathogens while opaque ones cause bacteremia and septicaemia (48).

The mechanism by which pneumococci become pathogens after colonisation is still unclear and only a small inoculum is required to cause disease (49). Gillespie and Balakrishnan (50)

wrote a review of the pathogenetic mechanisms based on various studies done to understand the disease process which is acquisition, adhesion, invasion, inflammation, septicaemia, shock and mortality/morbidity This is summarised below.

After acquisition by the aerosol route from carriers, pneumococci adhere to the upper airway epithelial cells via surface adhesins on pneumococcal cells (51). The host recognises these foreign antigens and mounts an immune response (52). The cytokines produced during this response upregulate **platelet activating factor (PAF) receptors** which the bacteria also uses to bind to pharyngeal, endothelial and lung cells via the surface adhesins to disseminate within the body (53). **Neuraminidase**, another virulent factor, is an enzyme that cleaves sialic acid on cell walls exposing more receptors of PAF enhancing adhesion (50).

After dissemination, the pneumococci are able to invade tissues by production of the enzyme **hyaluronidase** which degrades connective tissue and also allows the pneumococci to breach the blood-brain barrier (54). **Pneumococcal surface protein A (PspA)** binds lactoferrin which is used by the bacteria to acquire iron and multiply (55).

Pneumolysin is a bacterial toxin that is responsible for the inflammation and invasiveness of pneumococci and attributed to mortality (56). It is cytolytic to bronchoalveolar epithelial cells and to the pulmonary endothelium. It enhances blood-stream invasion by its cytotoxicity to the alveolar capillary barrier. Pneumolysin also disrupts immune chemotaxis and opsonophagocytosis enhancing dissemination and invasion. It depletes complement factors crucial in overcoming the infection (55, 57).

Pneumolysin also activates **phospholipase A** that breaks down cell wall membranes of the lung and cause further damage. The released fatty acids and lysophosphatides are also directly cytotoxic. Arachidonic acid metabolites are potent neutrophil chemotaxins that will enhance inflammation and damage to lung tissue once produced and unregulated⁽⁵⁰⁾.

Autolysin is an enzyme anchored on the pneumococcal cell surface that is involved in cell autolysis. It breaks down the cell wall and releases this products which are able to potentiate intense inflammation⁽⁵⁸⁾. Cell lysis also releases the intracellular toxin pneumolysin.

In the CNS once the pneumococci breach the blood-brain barrier, Autolysin and pneumolysin trigger an immune response and astrocytes and CNS macrophages release cytokines⁽⁵⁹⁾. The cytokines enhance inflammation and cause cerebral oedema and meningitis. Release of nitric oxide and oxygen free radicals cause neuronal damage and vasoconstrictive immune mediators cause cerebral ischemia and further damage.

The risk of mortality and adverse neurological sequelae is high in meningitis due to these mechanisms. Overwhelming infection and dysregulation of host immune response results in septicæmic shock ,organ failure and death.

Diagnosis:

To diagnose pneumococcal disease, isolates are acquired from normally sterile sites like CSF, blood and lung aspirates. The methods used to acquire the specimens are invasive and associated with risks to the patient. Blood cultures have been shown to have a low yield of 10-30%^(4,9) but Lung aspiration for culture is the gold standard in aetiological diagnosis of pneumonia but it is an invasive and risky procedure^(60,61). It requires a highly skilled and

experienced clinician to minimise the risks thus has limited use in routine epidemiological studies.

Pneumococci that cause acute Lower Respiratory Tract Infections(LRTIs) and invasive disease are similar to those that colonise the upper respiratory tract⁽⁴⁹⁾. They cause disease as explained in the pathogenesis above⁽⁵⁰⁾thus sampling of the upper airway is less risky, simple and accessible source of bacteria for microbiologic tests of anti-microbial resistance⁽⁶²⁾.

Upper respiratory tract carriage peaks during the cold/rainy season but is found throughout the year^(33,35). Pneumococci are also carried in the upper airway for a long duration of upto 4 months and are acquired by all infants at least once in their lifetime^(33,35,63). This further enhances its suitability as a sampling site in children.

Antimicrobial susceptibility of pneumococcus:

To effectively treat pneumococcal disease, effective antibiotics must be used. In Kenya and other parts of Africa emerging penicillin and multi-drug resistance has been shown in studies in Uganda, Gambia and South Africa^(64,65,66,67).

In East Africa, a study by a pneumococcal surveillance group (NetSPEAR), collected data between 2003-2007 on pneumococcal serotypes and antibiotic susceptibility from CSF and blood culture isolates from the East African region⁽⁶⁸⁾. None of the isolates were resistant to Penicillin, resistance was low to Chloramphenicol, Erythromycin, Amoxicillin and Cefotaxime. Cotrimoxazole resistance increased during the study period from 19% and 27% in 2003 to 69% and 60%in 2006 in CSF and blood cultures respectively⁽⁶⁸⁾.

Prevention

Vaccination has been shown to prevent pneumococcal disease and reduce carriage of vaccine serotypes_(69,70). The initial vaccines to be developed were 23-valent polysaccharide unconjugated vaccine with low immunogenicity in the highest risk group; less than 2 year olds ₍₇₁₎. Newer conjugated vaccines to either diphtheria (PCV 7) or haemophilus toxoid (PCV 10) were later developed with efficacy in this age group_(69,70).

The pneumococcal conjugate vaccine (PCV7) commonly in use, consists of the 7 common serotypes of pneumococci that cause disease in paediatric populations. These are 6B,9V,14,18C,19F,23F. The 9 valent type has in addition serotypes 1 and 5, the ten valent all the above plus serotype 7F and the newest a 13 valent type has all the above plus serotypes 3,6A and 19A₍₇₂₎.

Kenya is set to include PCV7 in its national immunisation programme early in the year 2010. USA was the first to introduce the vaccine on a large scale in the year 2000. Studies done there reflect a reduction in the burden of invasive disease caused by the vaccine and related serotypes but not in upper airway carriage _(73,74).

The study by NetSPEAR showed that coverage of vaccine serotypes in the region is between 50% for PCV 7 to 79% for PCV 13 vaccines₍₆₈₎. Berkeley's study showed coverage of 61% in children less than 2 years for PCV 9 serotypes₍₄₎ and Abdullahi's study showed a coverage of 47% for PCV 7 serotypes₍₃₅₎. This implies that invasive pneumococcal disease in the region can be reduced by half by the heptavalent vaccine(PCV7).

The importance of *S.pneumoniae* in the morbidity and mortality of children cannot be more emphasized as already illustrated. The studies tabulated below include studies in our region and other developing countries that show prevalence and antimicrobial resistance patterns of pneumococci. They are few and most were done over ten years ago.

TABLE 1: Studies In Developing Countries On Pneumococcus Prevalence.

Study author	Study area	Sample Size and population	Sample type	Prevalence of pneumococcus
Berkley et al,1998-2002 ⁽⁴⁾	Kilifi	N=16,570 1132 isolates	Blood	26%
Wachira N,1996 ⁽⁷⁵⁾	KNH	2m0-59m0 N=322	Nasal swabs	39%
Frederiksen, Henrichsen, WHO centre,1986 ⁽⁷⁶⁾	Zambia	2-59 mo N=194	Throat swabs	19%
Oguzkaya et al 2006 ⁽⁷⁷⁾	Turkey	5-6 yr olds N=683	Throat swabs	4.2%
Kanungo et al,2000 ⁽⁷⁸⁾	India	5-10 yr olds N=520	Throat swabs	24.3%

Dr Nancy Wachira's study done in 1996 showed the nasopharyngeal carriage of pneumococcus at 39.1%. None of the known risk Factors had an influence on the carriage rate. All the isolates were sensitive to Penicillin, Amoxicillin/Clavulanate and Cefuroxime. Resistance was highest to Erythromycin (5.6%), Chloramphenical (3.2%) and Ceftazidime (0.8%).

In conclusion, the current study aimed to establish the prevalence of oropharyngeal carriage and the current antibiotic susceptibility pattern 13 years later .

STUDY JUSTIFICATION .

Upper airway carriage is an important step in the pathogenesis of pneumococcal disease as shown above. The organisms in the pharynx are the same ones that disseminate to cause disease. They are also important in the transmission of the pneumococcus via the aerosol route (44,45). Introduction of the vaccine is expected to lower the carriage rates which will impact on disease transmission(74). Baseline data in the pre-vaccine era will be useful in monitoring the trends.

Measures to reduce the burden of pneumococcus morbidity and mortality as illustrated by these studies in our country include the use of effective antibiotics in treatment. Emerging pneumococcal resistance to the inexpensive drugs used as our primary antibiotics has been reported in Kenya (65,68). It is thus necessary to study the current antibiotic sensitivity patterns in our Hospital .

Antimicrobial resistance routine surveillance is rarely done in public health institutions in this country, like Kenyatta National Hospital. In this department, 2 studies have been done, in

1986 and 1997^(75,79), both showed antimicrobial resistance to common antibiotics in use in our hospital.

Widespread availability of oral and parenteral antibiotics and abuse, especially in urban areas in our country may have increased antibiotic resistance^(41,80). Knowledge on antibiotic sensitivity will thus guide in rational antibiotic use.

STUDY UTILITY:

Findings of this study will guide in the choice of current antibiotic use in management of suspected pneumococcal disease in our unit.

STUDY OBJECTIVES

PRIMARY OBJECTIVES:

1. To determine the prevalence of the oropharyngeal carriage rate of *Streptococcus pneumoniae* in children aged 0 months to 59 months.
2. To determine the antibiotic sensitivity pattern for *Streptococcus pneumoniae*.

METHODOLOGY

This was a hospital based cross-sectional survey. The study took place within Kenyatta National Hospital's Paediatric Emergency and Filter unit . Collection time was between 8am and 3 pm to allow sample processing. Thereafter the samples collected were processed at the UoN paediatrics department lab within the Kenyatta Hospital Campus.

Children between the age of 0 months to 59 months presenting in our filter clinic , whose parents gave consent to participate in the study. Very ill patients requiring care as emergencies or as priority cases were first attended to by the primary physician prior to being recruited into the study.

Children whose parents declined to participate in the study or any child with a contraindication to a throat swab e.g. bleeding per oral were excluded from the study. Transfer-in patients from other hospitals and patients who were currently on treatment and had received over 24 hours of antibiotic treatment were also excluded.

Ethical consideration

1. Approval for the study was sought from the scientific and ethics committee of KNH and UoN.
2. No extra cost was charged to the patient for the samples taken and laboratory work.
3. The clinical procedure was safe; no harm was done to the participants during the study.
4. Patients were not denied care at the hospital if they declined to participate in the study.

SAMPLE SIZE:

Using Fischer's formula with a prevalence rate of 20-40%, based on the tabulated studies in our region (4, 75-78), Sample size is between 256-384 persons with a mean of 320 patients.

$$N = \frac{p(100-p) Z^2}{d^2} \cdot 1-\alpha-2$$

N=sample size

p=prevalence from similar studies shown in table 1(4, 75-78).

d=required precision of the confidence interval set at 5%

$Z^2 \cdot 1-\alpha-2$ =square of the standard normal deviate corresponding to a confidence interval of 1- α (1.96)

SAMPLING METHOD

The sampling frame was based on patients meeting the eligibility criteria, selected from our paediatric filter/emergency unit irrespective of the clinical diagnosis. A triaged patient who received emergency/priority care and once stabilised for admission to the wards was also recruited and sampled. Consecutive sampling was used until the sample size was achieved.

Recruitment of patients was done using a Questionnaire. Parents were explained to the study and informed written consent was requested. The Questionnaire included the patient's bio-data and clinical data plus the lab data form. The lab data form was detached and sent with the oropharyngeal sample to the lab. Oropharyngeal samples were obtained after filling in the

questionnaire. To maintain quality of the samples processed a maximum of 20 patients were sampled per day.

MATERIALS AND LAB METHODS

To obtain the oropharyngeal sample, a polyester- tipped swab was inserted under direct inspection into the posterior pharynx and rotated 180°, avoiding the tongue and buccal Mucosa. The swab was then placed in a container with a transport media (Stuart's Media) and transported to the lab. The swabs were placed onto blood agar plates in a 5% carbon dioxide atmosphere for incubation for 24 hours. The agar plates were examined for colonies of *S. pneumoniae*. Colony morphology, α -haemolysis and optochin sensitivity was used to detect the bacteria according to standardised lab methods for pneumococcal isolation. After isolation, antibiotic sensitivity using disc diffusion method against the common antibiotics used in our setup was done.

DATA ANALYSIS

Analysis was done using SPSS (Statistical Package For Social Sciences)

Data was summarised in form of frequency tables, graphs and bar charts. Tests of association, student's t-test for continuous variables and Chi-square for discrete variables was used determine significance of variations noted.

RESULTS

The study was carried out over a 2 month period within the paediatric filter and emergency unit of Kenyatta National Hospital. Shown below are findings from the study.

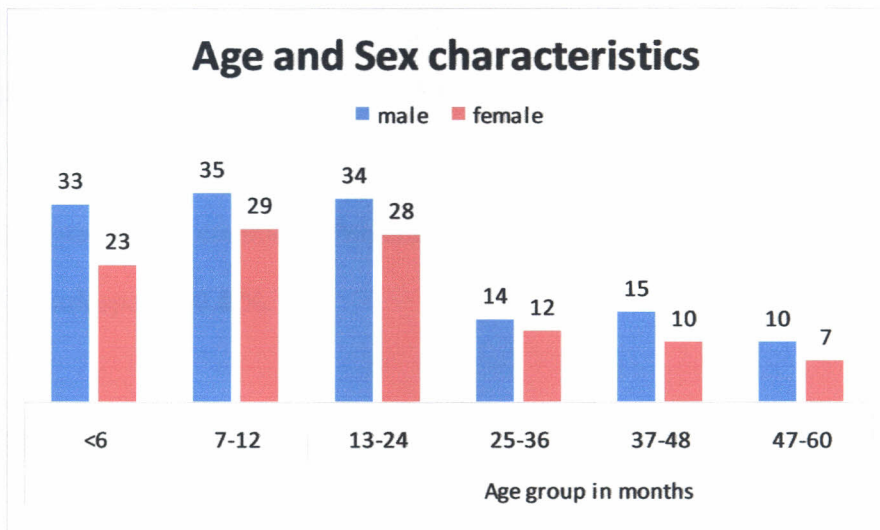
General description of the study population.

A total of 250 patients were sampled. Majority were male 141(56.4%) vs. female 109(43.6%). 182(73 %) were children below 2 years with a median age of 13 months and an inter-quartile range of 8-27 months. 154(84.6%) of the under 2 year olds were still breastfeeding. 76(30.5%) had siblings below the age of 5 years in the same household. 84 %(210) had completed immunisation as per KEPI schedule vs. 15.6 %(39). Only 1 patient was not immunised but was a newborn 2 days old. 56 %(140) had a median Z-score, 83(31.2%) - 1-SD, 19(7.6%) -2-SD and 8(3%) were \leq 3-SD. Only 53.8% (134) were done a rapid HIV test with only 3 positive results. This is summarised in table 2 below.

Table 2: General Characteristics Of The Study Patients

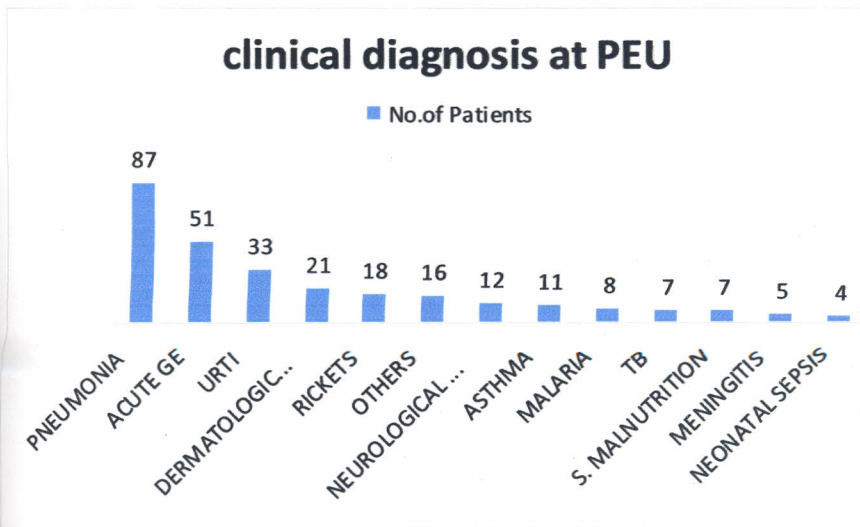
Characteristic		Frequency (n=250)	Percent (%)
Sex	Male	141	56.4
	Female	109	43.6
Z-score (weight for height)	Median	140	56
	-1-SD	78	31.2
	-2-SD	19	7.6
	≤ 3SD	8	3.2
	Missing	5	2.0
Age group in months	<6	56	22.4
	7-12	64	25.6
	13-24	62	24.8
	25-36	26	10.4
	37-48	25	10.0
	49-60	17	6.8
Rapid test status for HIV	Negative	131	52.4
	Positive	3	1.2
	Not done	116	46.4
Immunisation status as per KEPI schedule	Incomplete	39	15.6
	Completed	210	84.0
	Non-immunised	1	0.4
Antibiotic use within 14 days Of sampling		82	32.8
Average duration of antibiotic Use		7.78 days	

Figure 1: age and sex characteristics. (N=250)



In all age groups males predominated and majority of children were less than 24 months old. The clinician's diagnosis is summarised in figure 2. majority had acute respiratory infections, pneumonia and URTI collectively accounting for 136 patients(54%) of the total sample. The others are shown below:

Figure 2. Clinical diagnosis at PEU (N=250)



82(32.8 %) patients had a history of antibiotic use within the last 14 days prior to sampling. The average duration of use being 7.78 days. Majority of those on antibiotics were on orals, penicillins mainly, at 16 %(40/250). Cotrimoxazole 8.8%,cephalosporins 3.2%,Macrolides 2.4%,others like Tetracycline and Flagyl 0.4% and unknown type 2%.Parenteral antibiotic use wasn't as common. Penicillins and Aminoglycosides constituted 3.2% each while Cephalosporins 0.8 %.

Table 3: Patients On Antibiotics And the Type Used.

(n=82)

ANTIBIOTIC USED	Oral (%)	Parenteral (%)
Penicillins	40(16)	8(3.2)
Cephalosporins	8(3.2)	2 (0.8)
Aminoglycosides	(0)	8(3.2)
Cotrimoxazole	22(8.8)	(0)
Macrolides	6 (2.4)	(0)
Others	1(0.4)	(0)
Unknown type	5 (2)	(0)

General Description Of Those With Positive Oropharyngeal Carriage.

The oropharyngeal carriage rate was 10.8% (27/250 isolates). Most of the patients were male, 18 and females 9. 21 patients were less than 24 months of age and 6 above 24 months. The median age was 13 months and an inter-quartile range of 6.5-20.5 months.

5 of the patients were not up to schedule in their immunisation. 10 of the patients had siblings less than 5 years old. Only 1 patient had a Z-score above 3-SD indicating severe malnutrition. 8 of the patients did not have a rapid test done for HIV. The others were negative. This is summarised in table 4 .

Table 4: General Characteristics Of Those With Positive Oropharyngeal Carriage.

Characteristic		Frequency (n=27)
Sex	Male	18
	Female	9
Z-score (weight for height)	Median	12
	- 1-SD	10
	- 2-SD	4
	≤ 3SD	1
Age group in months	0-24	21
	24-60	6
Rapid test status for HIV	Negative	19
	Positive	0
	Not done	8
Immunisation status as per KEPI schedule	Incomplete	5
	Completed	22
	Non-immunised	0
Sibling below 5 years		10
Antibiotic use within 14 days Of sampling		15
Average duration of antibiotic Use		7 days

Their clinical diagnosis was as follows;8 patients were diagnosed with Acute Gastroenteritis,7 with Pneumonia,3 URTI,1sickler,1 febrile illness,1 septic arthritis,1 malaria,1 meningitis,1TB,1 asthma,2 dermatitis and 2 neurological disorders.

Table 5: Clinician’s Diagnosis At PEU In Patients With A Positive Throat Culture(N=27)

DIAGNOSIS	pneumococcus present(%)
PNEUMONIA	7(25.9)
ACUTE GASTROENTERITIS	8(29.6)
URTI	3(11.1)
DERMATOLOGIC DISORDERS	2(7.4)
RICKETS	0(0)
OTHERS	3(11.1)
NEUROLOGICAL DISORDERS	2(7.4)
ASTHMA	1(3.7)
MALARIA	1(3.7)
TB	1(3.7)
SEVERE MALNUTRITION	1(3.7)
MENINGITIS	1(3.7)
NEONATAL SEPSIS	0(0)

15 patients had used antibiotics with an average of 7 days of use. 6 were on Cotrimoxazole, 4 on a Penicillin, 1 a Cephalosporin, 1 Metronidazole, 2 on parenteral drugs, 1 on Crystalline penicillin and the other on a combination of a Cephalosporin and Aminoglycoside. none on a Macrolide. 1 patient did not know the medication(unknown).

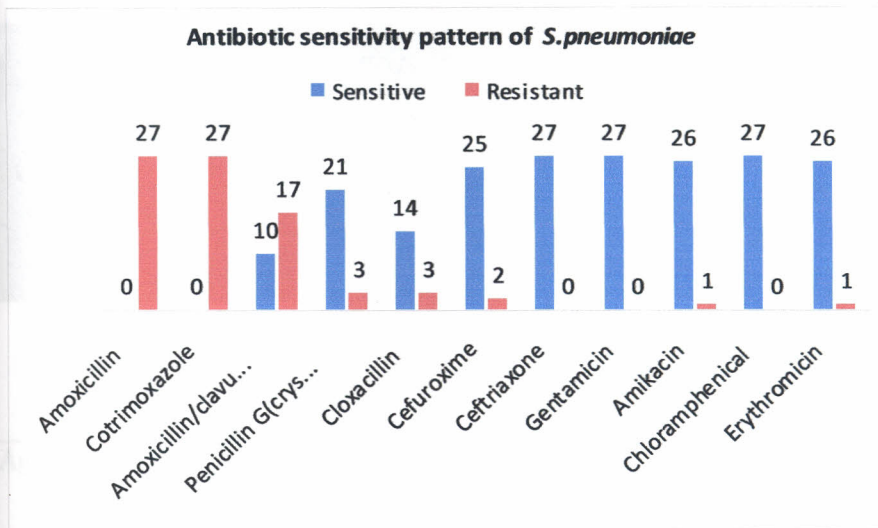
Table 6: Patients On Antibiotics And the Type Used.(N=15)

ANTIBIOTIC USED	Oral (%)	Parenteral (%)
Penicillins	4(14.8)	1(3.7)
Cephalosporins	2(7.4)	1(3.7)
Aminoglycosides	(0)	1(3.7)
Cotrimoxazole	6(22.2)	(0)
Macrolides	0(0)	(0)
Others	1(3.7)	(0)
Unknown type	1(3.7)	(0)

The antibiotic sensitivity profile is shown below. All isolates were resistant to Cotrimoxazole and Amoxicillin(100%).Resistance was also noted to other Penicillin antibiotics i.e. Amoxicillin/Clavulanate(63%) and less so to Crystalline Penicillin(12.5%) and Cloxacillin(17.6%). Chloramphenical, Ceftriaxone and Gentamicin showed no resistance while Cefuroxime, Amikacin and Erythromycin had resistance rates of less than 1%.

Figure 3: Antibiotic Sensitivity Pattern Of *S.Pneumoniae*.

(N=27)



None of the characteristics in table 2 like age,sex,immunisation status and siblings less than 5 years or the clinical diagnosis had a statistically significant association($p \leq 0.05$) with a positive throat culture from the univariate analysis done.

Table 7: Comparison between patients with carriage of pneumococcus and those without.

<u>Characteristic</u>		<u>Pneumococcus present</u> N=27 (%)	<u>Pneumococcus absent</u> N=189 (%)	<u>Risk</u>	<u>Risk Ratio</u>
Sex	Male	18(66.7)	105(55.5)	0.1475	
	female	9(33.3)	84(44.4)	0.0968	1.5
Age group in months	0-24	21(77.8)	135(71.4)	0.1346	
	24-60	6(22.2)	54(28.6)	0.1	1.3
Immunisation status as per KEPI schedule	incomplete	5(18.5)	30(15.3)	0.1428	
	completed	22(81.5)	159(84.1)	0.1215	1.17

Only the above characteristics carried a risk for carriage in the upper airway. Male children, children less than 24 months and those who had not completed their immunisation according to our national programme were at increased risk. Clinical diagnosis and antibiotic exposure were not risk factors for carriage.

DISCUSSION

The aim of the study was to determine the carriage rate of pneumococcus in the upper respiratory tract and use the isolates to determine the antibiotic sensitivity. This was achieved and the findings reflect a carriage rate of 10.8 % and a high penicillin resistance.

The study was carried out over a 2 month period from December 2009-January 2010. A sample size of 250 was achieved with majority of the children being less than 2 years old. The earliest colonisation of the oropharynx being noted at 2 months of age with a mean age of 13 months. None of the neonates had a positive culture. Gray et al in 1982 in Alabama USA carried out an epidemiologic study on infants 0-24 months and found a mean age of acquisition of pneumococcus of 6 months⁽⁶³⁾, and much earlier in Papua New Guinea and Gambia^(31,81).

In Kenya, a similar study done in Eldoret at a referral University Hospital in 2003, nasopharyngeal isolates were shown to also have a high Cotrimoxazole and Penicillin resistance⁽⁸²⁾. The carriage rate was at 35.9% and median age of colonisation was 6 months.

Osman Abdullahi's study in Kilifi showed a higher nasopharyngeal prevalence rate of 51-60% in the less than 5 years group but was influenced by season, recent antibiotic exposure and coryza⁽³⁵⁾.

In this study emphasis on β -lactam antibiotic resistance was made. Resistant strains were defined by the susceptibility to Penicillin (1mcg) disc. A zone of inhibition less than 20 mm was a resistant strain. Disc diffusion method is a validated cost effective screening tool for antibiotic susceptibility testing^(83,84)

Resistance was highest to amoxicillin and cotrimoxazole with 100% resistance noted to both drugs. Exposure to these 2 drugs may explain the pattern. The current paediatric national treatment guidelines recommend the use of cotrimoxazole and amoxicillin for the first-line treatment of Pneumonia⁽⁸⁵⁾.

This is reflected in the study population as most of the children on antibiotics were using either of these 2 drugs. In Malawi and Zambia ^(86,87) studies done on patients using Cotrimoxazole either as treatment or prophylaxis in HIV showed a higher risk of carriage with cotrimoxazole-resistant strains of pneumococci.

The implication of the findings of the current study is significant . It shows the need to change from our current first line antibiotics used in suspected invasive pneumococcal invasive disease like pneumonia and meningitis⁽⁸⁵⁾ to the other antibiotics that showed better sensitivity profiles as a 1st line treatment.

The Cephalosporins, Chloramphenicol and Erythromycin exhibited good sensitivity patterns and can be used as alternative drugs but are costlier to buy and have their associated side effects like gastrointestinal and allergic reactions with erythromycin and bone marrow toxicity with chloramphenicol especially in neonates.

Worldwide ,Spain has the highest documented rates of pneumococcal penicillin resistance with the incidence increasing from 1979 to date⁽⁸⁸⁾ .In Africa ,penicillin resistance was initially demonstrated in South Africa by Appelbaum, Koornhof et al in 1977⁽⁸⁹⁾ and showed high level multi-drug resistance. Later in 1981, Koornhof et al⁽⁹⁰⁾ did a study on hospitalised children comparing new admissions and in-patients for more than 24 hours. Carriage of antibiotic

resistant pneumococci was related to a younger age, Recent exposure to a B-lactam antibiotic and hospitalisation longer than 24 hours. Furthermore in 1998 a study in Botswana using nasopharyngeal isolates showed similar resistance to Amoxicillin and Cotrimoxazole though no risk factors were identified (91) .

In East Africa, a study done in Uganda, 2000 (66) showed similar results to this one. 83% of the nasopharyngeal isolates (n=118) had intermediate-level penicillin resistance and high Cotrimoxazole resistance. 10.4% showed Chloramphenicol resistance and all isolates were susceptible to cefotaxime and erythromycin. The data from NetSPEAR similarly showed high resistance to Cotrimoxazole and minimal resistance to Amoxicillin, Erythromycin and Cefotaxime. No resistance was noted to Penicillin (68).

The studies done in Gambia and Kenya showed a predominance of males in carriage and invasive disease rates (). They also showed that age especially children below 24 months had a higher rate of upper airway carriage than older ones. Immunisation status has not been directly linked with increased risk except when associated specifically with pneumococcal vaccines.

The Question thus remains : Does anti-microbial resistance equate treatment failure? Various studies in Africa show that invasive disease by intermediate level Penicillin resistance can still be treated with the penicillins (92,93). High level resistance requires higher doses of Penicillin e.g. 90mg/kg/d of amoxicillin to treat otitis media (92,93). A study done in Tanzania showed antimicrobial resistance amongst other factors like infancy and malnutrition increased the risk of mortality in patients with bacteremia (94).

In conclusion ,Pneumococcal vaccination is one of the most effective ways of reducing the burden of resistant pneumococci. Though this study did not serotype the isolates, majority of the strains in the East African region are covered by the heptavalent and more so the novel 13 valent pneumococcal vaccine^(68). Introduction of the vaccine is expected to reduce carriage and spread of resistant serotypes and offer herd immunity^(95,96,97).

Study limitations:

The small number of isolates obtained limits generalisability of the results to the whole paediatric population that presents to our hospital. Secondly the degree of resistance was not established. The antibiotic discs used will only show inhibition zones but the minimum level of inhibition was not confirmed. Limited availability of single disks and multi disks with Cloxacillin and Penicillin-G resulted in a few of the samples not being tested for antibiotic susceptibility to the 2 drugs.

Resistant strains were also not stereotyped due to the high expense and technical limitation in our set-up. A Rapid test for HIV at the study area was done on half the study patients limiting ability to analyse and make recommendations for this important sub-group of patients.

Conclusions :

The prevalence of oropharyngeal carriage in children presenting to Kenyatta National Hospital is 10.8%. Antibiotic resistance of pneumococcus is highest to the 2 most commonly used drugs in our set-up, Amoxicillin and Cotrimoxazole .

Recommendations:

Cephalosporins, Aminoglycosides, Macrolides and Chloramphenicol are better suited in the treatment of pneumococcal infections than the penicillins. Cloxacillin is the best choice of the penicillins to use.

Amoxicillin and Cotrimoxazole are not recommended for treatment of pneumococcal infections. A follow-up study with larger number of isolates will be required to further substantiate this. Antibiotic resistance surveillance should be done regularly and guidelines for antibiotic use should reflect the changing patterns.

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CONSENT FORM

A study on the prevalence of *Streptococcus pneumoniae* in the upper airway of children at Kenyatta National Hospital.

I am a postgraduate student in the Paediatrics Department of the University of Nairobi, College of Health Sciences. I would like to request you and your child to take part in a study that is part of my degree work. The participation is purely voluntary and you can withdraw at any stage. No cost will be incurred by you.

The study will involve requesting you to answer a questionnaire; the data will be used for research purposes only. The results will be useful in determining the type of drugs we use to treat pneumonia and meningitis. I will need to also obtain a sample of a throat swab. The risk is minimal and I will explain the procedures prior to you're giving the consent.

I appreciate your consent by signing below.

I, the participant/guardian of the patient consent to Participate in this study the nature of which has been explained to me by Dr A.M Irungu

Guardian's signature:

Doctor's signature:

Clinical data :

Study no.....

1. Weight(kg):

2. Height(cm):

3. Length (cm):

4. Z-score : 1) median 2) -1-SD 3) -2 SD 4) \leq -3 SD

5. HIV status:

Rapid antibody test: 1) done 2) not done

1) negative 2) positive 3) indeterminate

6. Clinician's Diagnosis at PFC:.....

7. Antibiotic use within the last 14 days: 1) yes 2) no

8. Duration of antibiotic use: (no. of nights)

9. Type of antibiotic used:

Study no.....

Oral:

1) Penicillins

2) Cotrimoxazole

3) Macrolide

4) Cephalosporin

5) Others

6) Unknown

11. Parenteral:

1) Penicillins

2) Aminoglycosides

3) Cephalosporins

4) Others

Laboratory data:

Study no.....

Lab no:

12. Culture results

1) Negative

2) positive

13. Organism isolated

1) S.pneumoniae

2) H.influenzae

3) S. aureus

4) Others

5) None

14. Antibiotic sensitivity

	<u>Sensitive</u>	<u>Resistant</u>
Amoxicillin	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Augmentin	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Penicillin G	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Cotrimoxazole	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Cefuroxime	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Ceftriaxone	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Gentamicin	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Amikacin	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Chloramphenical	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>
Erythromicin	(0) <input type="checkbox"/>	(1) <input type="checkbox"/>

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