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Pneumococcal carriage in healthy Tanzanian toddlers

-A cross sectional study in Moshi, Tanzania

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Abstract

***Background*:** Pneumonia is the leading cause of death in children worldwide and the most important pathogen causing the disease is the bacterium *Streptococcus Pneumoniae* (the pneumococcus). This study aimed to determine the carriage rate and the resistance pattern of pneumococci in healthy children below 2 years of age in Moshi in northern Tanzania. In this region the pneumococcal conjugate vaccine was introduced into the child vaccination programme in January 2013. A second aim was to determine if there were any correlations between carriage of the bacteria and health status or socio-economic factors.

Methods: During October and November 2013, 150 randomly selected healthy children were included in the study. The children were recruited from three different health clinics in Moshi. A nasopharyngeal sample was taken from the children and a questionnaire was given to the parents. The samples were then cultured for pneumococci and the resistance pattern of the bacteria was determined at the clinical laboratory at Kilimanjaro Christian Medical Centre (KCMC) in Moshi.

Results: The carriage rate of pneumococci in the nasopharynx among the children was 29%. The rate of pneumococcal strains with reduced susceptibility against penicillin was 37 %, the rates against ceftriaxon and ampicillin were 2% for each. The rate of pneumococci with reduced susceptibility against co-trimoxazole, tetracycline and erythromycin were 95%, 33% and 10% respectively. All isolates were sensitive to norfloxacin and clindamycin. The only factors significantly more common in children carrying pneumococci than in children without pneumococci were malaria and among children aged 0-6 months exclusively breastfeeding.

***Discussion*:** The rather high rates of non-susceptible bacteria, as shown in this study, confirm that penicillin or co-trimoxazole should not be used as empirical treatment of pneumonia in this area, while amoxicillin could still be the first drug of choice.

Conclusion: More restrictions on the antibiotic use may be needed in the Moshi area.

Background

Streptococcus pneumoniae, also known as the pneumococcus, is a bacterium that can cause a wide range of infections, from local manifestations such as otitis media and sinusitis to invasive disease such as sepsis or meningitis.(1)

It is also the most common cause of pneumonia, which is a major global health problem and the leading cause of death in children under five years of age.(2) In the year of 2000 the estimated number of serious pneumococcal diseases to occur globally was 14.5 million, leading to about 826 000 deaths in children aged one month to five years.(3)

The pneumococcus is a gram-positive encapsulated diplococcus. The capsular polysaccharides on the surface of the bacteria are the primary factor of virulence and are also the basis from which serotyping is made, resulting in 40 serogroups and 93 serotypes.(1) All of them have varied ability to cause disease and the distribution of serotypes varies by regions and age.(1)

The pneumococcus frequently colonizes the upper respiratory tract, especially in children, and the human nasopharynx is the only natural reservoir for it. *S. pneumoniae* is transmitted between individuals through respiratory droplets, and nasopharyngeal colonization is a first step of pathogenesis.(1) Children are often asymptomatic carriers and invasive disease does usually not occur without foregoing colonization with the homologous strain.(1)

Studies show that nasopharyngeal carriage rates of *S. pneumoniae* varies widely between different countries and populations. In for example Kenyan, Turkish and Venezuelan the carriage rates in healthy children below five years of age were 23, 28 and 57 % respectively.(4-6) In a study from Kampala, Uganda there was a carriage rate of 62 % in the healthy study population (7), and in another study done in Dar es Salaam 2010 the carriage was 35 % when a population of 300 healthy children under five years of age were sampled.(8)

Pneumococci resistant to antibiotics are an increasing problem all over the world. Pneumonia caused by penicillin-resistant *S.pneumoniae* has been reported to be more lethal in hospitalized adult patients than infection due to penicillin-susceptible strains.(9)

Due to over-prescription and misuse of antibiotics this problem is even greater in low-income countries and the reduced efficacy of antibiotics may be a greater challenge for resource-limited countries since the drugs with the highest levels of resistance often is the cheapest antibiotics.(8)

Adequate antibiotic therapy at the early stage of disease is very important for successful outcome, and penicillin or amoxicillin are the first choice of drugs. Amoxicillin is now the first line choice because of the high rates of resistance of the cheap and previously much used trimethoprim-sulphamethoxazole (Co-trimoxazole).(10)

Investigations of antibiotic susceptibility of *S. pneumoniae* in hospitalized children with pneumonia is often very difficult, due to sampling limitations and because the children many times already have been given antibiotics. Instead, studies investigating the rate of nasopharyngeal carriage and penicillin susceptibility of pneumococci in the healthy child population provide data of the spread of resistant strains in the whole community.(8)

In the study from Dar es Salaam more than two-thirds of the isolates were penicillin-non-susceptible pneumococci (PNSP) and multidrug-resistance (resistance to three or more of the antibiotics tested) was found in 17 % of the isolates.(8)

A study done on healthy children in Brussels during 2006-2008 showed that children with lower socio-economic status were more likely to carry antibiotic-resistant *S. pneumoniae.(11)*

In Rombo district in northern Tanzania the carriage and resistance of *S. pneumoniae* was studied in healthy children below seven years after Azithromycin administration for trachoma control in 2000. The carriage rate found was only 11 %.(12) This was a unexpectedly low

carriage rate and a comparative study using the same methods in children of similar age was done on a local sugar plantation where the carriage rate instead was 50 %.(12) In the study from Rombo district the rates of *S. pneumoniae* with reduced susceptibility to penicillin and co-trimoxazole was 21 % and 42, % respectively.(12)

Similar resistance rates were shown in a study from Malawi where 21%, of the isolates from children under five years visiting health clinics were nonsusceptible to penicillin, 46% were nonsusceptible to co-trimoxazole and 22% to tetracycline.(13)

In the study from Uganda previously mentioned 84 % was PNSP, and resistance to co-trimoxazole and tetracycline was 84 % and 29 % respectively.(7)

Streptococcus pneumoniae stands for a major part of the pneumonia cases worldwide and was in the year of 2010 responsible for 33 % of the childhood pneumonial deaths in low- and middle-income countries.(14) A high proportion of deaths due to pneumonia occur early in life, 81 % of the incidents are in children under 2 years of age.(10)

Pneumonia is an acute respiratory tract infection affecting the lungs. When a healthy person breathe the small alveoli in the lungs are filled up with air but when suffering from pneumonia the alveoli are instead filled up with pus in the affected area leading to difficulty to breath and limits oxygen uptake.(2)

Malnutrition, time of breastfeeding, pre-existing illnesses such as HIV and measles, indoor pollution and living in crowded homes are all factors that can increase a child's susceptibility to pneumonia.(2) All of this are more common in the sub-Saharan countries and these countries have the highest burden of deaths from pneumonia in the world.(10) Fifteen countries in the world, all in Asia and sub-Saharan Africa, are responsible for 65 % of the

total episodes of pneumonia and 64 % of the severe cases, and Tanzania is one of these countries.(10)

In year 2010 pneumonia was responsible for 15 % of the deaths among children under five years of age in Tanzania.(15)

To lower the number of invasive pneumococcal disease in children vaccines are now introduced in many countries.

There are two different kinds of vaccines against *Streptococcus pneumoniae,* the polysaccharide vaccine and the conjugate vaccines. The polysaccharide vaccine includes the 23 serotypes most commonly known to cause disease but does not give a long-lasting protection and is therefore not recommended for small children.(16) The conjugate vaccine is available in three forms, PCV7, PCV10 and PCV13 covering seven, ten and thirteen serotypes respectively. PCV13 is the newest of them all and it covers for serotype 1,3,4,5,6A,6B,7F,9V,14,18C,19A, 19F and 23F.

Since it is a conjugate vaccine it will activate T-cells and give a long lasting protection also in children under two years of age.(16)

Studies done after the introduction of the vaccines have showed a reduced rate of invasive pneumococcal diseases caused by the serotypes included in the vaccines.(17, 18) However a rise in carriage of the serotypes not included in the vaccine has also been seen,(19) and a shift towards other disease-causing serotypes has been observed.(17, 19) In one study done in the United States after introduction of the PCV7 the percentage of invasive pneumococcal disease 2006-2007 caused by serotypes covered by the PCV7 was 2 % and the number of invasive pneumococcal disease caused by the additional serotypes included in the PCV13 was 63 % in children under five years of age.(17, 18)

Studies done after the introduction of PCV13-vaccine have also shown direct and indirect effect of the vaccine. A shift towards colonization with other serotypes has been observed, although these are not commonly causing disease. (19) The indirect effect, or herd immunity, means that even non-vaccinated individuals also get protection of the vaccine through diminished spread of the disease-causing serotypes.

Introduction of the vaccines also affect the spread of resistant bacteria. After the introduction of PCV7 and PCV9 reduced levels of penicillin non-susceptible pneumococci were found both in vaccinated and unvaccinated individuals, and a reduction in antibiotic use was also seen.(20) The serotypes causing disease in children are also the most common ones to be antibiotic resistant and there was an increase in intermediate resistant serotypes not included in the PCV7 or PCV9 but in the PCV13.(20)

These studies mainly come from western countries and the indirect effect of the vaccine in developing countries where carriage of pneumococci is highly prevalent and the distribution of serotypes may be different needs to be further investigated.(21)

In January 2013, Tanzania introduced the PCV13 into their child vaccination program. According to the demographic health survey the total vaccine coverage in the whole country was 75 % in 2010. In the northern region the coverage was 80 %.(22)

In Tanzania the under-5 mortality rate declined with 41 % from 137 deaths per 1000 live births in 1992-1996 to 81 deaths in 2006-2010. It differs a lot between regions though, the Northern region has an under-5 mortality rate at 58 deaths per 1000 live births compared to the Southern Highlands or the Lake region with a rate at 102 and 109 respectively.(22) There are also several socio-economic factors that make a variance in mortality rates. For example if the mother has no education versus secondary school or higher the rates are 97 and 73 deaths

respectively, and if you compare the lowest and highest wealth quintile the rates are 103 and 84 deaths per 1000 live births, respectively.(22)

Although a considerable decrease in childhood mortality have been seen, both in terms of all-cause mortality and specific for pneumonia, more effort still needs to be done to reach the forth millennium goal (reduce the child mortality with two thirds between 1990 and 2015).(10) Despite the number of deaths is declining a larger proportion of deaths is seen in sub-Saharan Africa.(23)

There are many challenges and obstacles for low-income countries to combat on the way to achieve these goals, for example under-developed and weak health care systems cannot easily scale up antibiotic coverage for those children who need it most because of the relatively low rates of access to health care.(24)

The PCV13 is now included in the vaccination-program in many countries in order to decrease the number of cases of pneumonia and deaths from it. When evaluating the outcome it is important to investigate the carriage rates of pneumococci, the serotypes circulating in the population before and after the introduction, and if there is any differences in the rates of resistant bacteria. This is especially important in countries where the disease-burden is high and there is little research on the field.

Aim

- To investigate the colonization rate and antibiotic susceptibility of *S. pneumoniae* in the nasopharynx of healthy Tanzanian children.
- Relate the carriage rate to social factors and health status of the children.

Methods

This was conducted as a cross-sectional study during October and November 2013 in Moshi urban district, a town with approximately 200 000 inhibitants by the foot of Kilimanjaro in northern Tanzania. Children below 2 years of age coming for routine care such as immunization and growth monitoring, seeking healthcare or accompanying a relative were included in the study. The children were recruited at one Health centre, Pasua, and two dispensaries, Bondeni and Njoro, nearby Moshi. The health facilities were chosen and approved by Dr. Benjamin Sana at Moshi Municipal Council.

The parents were asked questions from a questionnaire containing questions about health status, previous illnesses, neonatal problems, breast feeding, socio-economic status, living conditions, vaccinations, antibiotic treatment etc. The weight and length of the children were also noted. The questionnaire was constructed together with our supervisors and was before the sampling began tested on two children with parents at Kilimanjaro Christian Medical Centre (KCMC). The questioning was done in Kiswahili by the two experienced Community Health nurses Celina Mayo and Bertha Kiwale, who also took a nasopharyngeal sample from each child.

Of all 351 questioned, 13 parents/guardians refused to participate; leaving a total of 338 healthy children or children with upper respiratory tract infections that was sampled and interviewed. All 338 children were interviewed and sampled regardless of symptoms and the separation between healthy children and children with symptoms (described in another student report) was done afterwards.

The samples taken by blue-capped E-swabs (Copan diagnostics, Murrieta, USA), were transported cooled from the health facilities to the microbiological laboratory at Kilimanjaro Christian Medical Centre in Moshi. The samples were immediately inoculated onto blood

agar plates with 5 % sheep blood where after the E-swabs were frozen in a -20°C freezer. The plates were incubated in 5 % CO_2 in 34-36˚C for two days, and checked after each day. 5 % CO_2 atmosphere was obtained by closed jars supplied with CO_2 paper sachets (GasPak EZ CO_2 Container System, Becton Dickinson and Company (BD), Sparks, MD) and CO_2 indicators (BD). Suspected pneumococcal isolates (α-haemolytic, greyish colonies with depressed centres) were subcultured on blood agar plates and then re-incubated in 34-36˚C with 5 % CO_2 overnight. A colony from this pure subculture was cultured on Muller-Hinton agar plates (supplied with 5 % defibrinated sheep blood and 20 mg/L β-NAD (Applichem, GmbH, Darmstadt, Germany). One Optochin-disc and one Oxacillin-disc (both from Oxiod, Hampshire, UK) were put onto the plates. An inhibitory zone around the Optochin-disc of ≥ 14 mm served as identification of *S. pneumoniae.* Colonies of *S. pneumoniae* were then frozen at -20˚C in cryotubes containing 1,0 ml of STGG.(25) The STGG freezing medium consisting of skim milk powder (BD Difco, USA), tryptone soya broth (Oxoid), glucose, glycerol and distilled water.

A few numbers of identified colonies were suspended in 2 ml sterile Phosphate buffered saline to McFarland 0.5. Using a sterile cotton swab, a fraction of the suspension was inoculated on Muller-Hinton agar plates for testing of antibiotic susceptibility against Trimethoprim-sulphamethoxazole (1,25-23,75 µg), Erytromycin (15 µg), Clindamycin (2 µg), Norfloxacin (10 µg) and Tetracycline (30 µg) (all from Oxiod).

The Oxacillin disc was used for screening of penicillin non-susceptibility and isolates with a zone-diameter of < 20 mm were further tested with E-tests for minimal inhibitory concentration (MIC) determination for benzylpenicillin, ampicillin and ceftriaxone (all 0.016-256 µg/mL from Biomerieux, Marcy l'Etoile, France).

Pneumococcus with MIC > 0.06 mg/l was classified as having reduced susceptibility to penicillin and if MIC was > 2 mg/l the isolates were classified as resistant.(26) The breakpoints used for the disc-diffusion tests and MIC-determination were from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) from 2013. (26) (Table I and II).

Table 1: Breakpoints according to EUCAST on disc diffusion tests

Antibiotic	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Clindamycin	≥ 19		< 19
Erytromycin	≥ 22	19 – 21	< 18
Norfloxacin	≥ 12		< 12
Oxacillin	≥ 20		
Tetracycline	≥ 25	22 – 24	< 21
Trimethoprim-sulphamethoxazole	≥ 18	15 – 17	< 14

Table II: Breakpoints according to EUCAST on MIC tests

Antibiotic	Sensitive (mg/L)	Intermediate (mg/L)	Resistant (mg/L)
Ampicillin	≤ 0.5	0.5 – 2	> 2
Benzylpenicillin	≤ 0.06	0.06 – 2	> 2
Cefotaxim	≤ 0.5	0.5 – 2	> 2

Statistical methods

The data-analysis was done using cross-tabulations in SPSS version 21. Fisher exact test was used for comparisons between groups, p-values ≤ 0.05 were counted as significant.

Ethics

The parents were informed that participation of the child in the study was voluntary. They were also informed that whether or not they chose to participate, the treatment at the clinic would not be affected. The children in the study are not able to identify. The parents had to sign a consent form before the start of the questioning and sampling.

Ethical approval was obtained from Kilimanjaro Christian Medical College and University Ethical Committee; this is presented in the appendix.

Results

Recruited children

150/338 children had no symptoms of respiratory tract infection and were therefore included in this study. Half of the children (79/150) were recruited at a dispensary (Bondeni, n=26 and Njoro, n=53) and the other half from a health centre (Pasua Health centre, n=71).

The cause for visiting was growth monitoring for 66 children, vaccination for 65 children, OPD (sick children seeking care at "out patient department") for 8 children and other reasons mostly accompanying a family member) for 11 children. All children lived in areas classified as urban or semi-urban, none was rural. Other characteristics of the study population are shown in table III.

49 % of the children had been treated with antibiotics within the last three months before the study (Table IV). In terms of current or previous diseases no child had had measles and only three children had unknown HIV-status, whereas 37% (56/150) of the children had had diarrhoea and 21% (32/150) malaria. Almost all children were breastfed (Table IV). Of the children aged 0-6 months (n=68) the breastfeeding was exclusive in 37 %, predominantly in 40 %, whereas 24 % of the children were mix fed.

Table III. Study Population

	Healthy children n=150 (range)
Age (months) median	7 (0-24)
Weight (kg) median	8.0 (2.0-16.0)
Length (cm) median	65 (42-87)
Gender (boys: girls)	82:68

Nasopharyngeal carriage of pneumococci:

In the nasopharyngeal samples collected from the healthy children pneumococci was isolated in 29% (43/150).

The relation between pneumococcal carriage and social factors or health status is shown in table IV. Malaria was significantly more common in children carrying pneumococci than in children without pneumococci (44% versus 12%, $p<0.0001$). No other disease, including previous respiratory tract infection, was associated with pneumococcal carriage. Among the 68 children aged 0-6 months mixfeeding was significantly more common in the children without pneumococci compared to children with pneumococci ($p=0.015$), whereas exclusively breastfeeding tended to be more common in children with nasopharyngeal carriage of pneumococci compared to children without pneumococci ($p=0.081$). No statistical significance was seen for any other of the factors investigated in relation to pneumococcal carriage.

Table IV Correlation between pneumococcal carriage and social factors

	Pneumococcal carriage		p-value
	Yes n = 43 (%)	No n = 107 (%)	
Preterm[a]	0 (0)	6 (5.6)	0.18
Breastfed	41 (95)	99 (93)	n.s
Antibiotic use			
<1 week	2 (4.7)	7 (6.5)	n.s
≥1 week-4 weeks	11 (26)	26 (25)	n.s
>4 weeks-12 weeks	9 (21)	19 (18)	n.s
Recent respiratory tract infection[b]	5 (12)	22 (21)	n.s
Hospitalized[c]	2 (4.7)	7 (6.5)	n.s
Diarrhoea	39 (36)	17 (40)	n.s
Malaria	19 (44)	13 (12)	< 0.0001

Siblings	25 (58)	63 (59)	n.s
Siblings < 5 years	10 (23)	24 (23)	n.s
Four or more persons per room	7 (16)	13 (12)	
Anyone smoking	7 (16)	15 (14)	n.s
Firewood[d]	3 (7.0)	8 (7.5)	n.s
Vaccinated against pneumococci			
No	19 (44)	43 (40)	n.s
Yes partial	8 (19)	19 (18)	n.s
Yes fully	16 (37)	45 (42)	n.s
Education mother			
No school	0 (0)	3 (2.8)	n.s
Primary school	34 (79)	67 (63)	0.057
Secondary school	9 (21)	36 (34)	n.s
University	0 (0)	1 (1.0)	n.s
Education father			
Primary school	25 (58)	60 (56)	n.s
Secondary school	17 (40)	35 (33)	n.s
University	1 (2.3)	12 (11)	0.11
	n = 16 (%)	n = 52 (%)	p-value
Breastfeeding 0-6 months			
Exclusive	9 (56)	16 (31)	0.081
Predominatly	7 (44)	20 (39)	n.s
Mixfed	0 (0)	16 (31)	0.015

[a] Meaning children born 14 days or more before estimated date.

[b] Defined as cough with fever, rapid or difficulty breathing or pneumonia during the past three months

[c] Admittance to hospital the past three months

[d] Using firewood for cooking indoors without chimney

n.s = non significant

Antibiotic susceptibility:

The first package of antibiotic-discs and E-tests was delayed in Dar es Salaam for approximately three weeks and was therefore not stored cooled properly according to the manufactory's instructions. For that reason new, small packages of discs and E-tests were

transported by passenger plane to Moshi. To investigate if there was any difference in the function of the antibiotic discs and strips due to the delay, the old and new discs and E-tests, respectively, were both tested on the first ten pneumococcal isolates. No significant difference was found between the old and new material and no difference at all regarding classification of the pneumococcal strains into Sensitive, Intermediate and Resistant according to the breakpoints presented in Table I and II. The results from the dubbeltesting are presented in Table V and VI.

Table V: Disc-diffusion dubbeltesting

Namn	Optochin	Oxacillin	Oxa-SIR	SXT	SXT-SIR	E	E-SIR	DA	DA-SIR	NOR	NOR-SIR	TE	TE-SIR
1	25	29	S	23	S	29	S	24	S	20	S	32	S
1 new	25	28	S	23	S	29	S	25	S	20	S	32	S
2	15	15	R	8	R	12	R	23	S	19	S	19	R
2 new	15	16	R	6	R	15	R	22	S	19	S	20	R
5	22	9	R	12	R	26	S	22	S	21	S	14	R
5 new	21	9	R	13	R	27	S	23	S	21	S	13	R
7	17	28	S	21	S	28	S	29	S	18	S	33	S
7 new	17	28	S	24	S	27	S	28	S	18	S	36	S
9	15	25	S	9	R	30	S	26	S	21	S	36	S
9 new	15	25	S	8	R	29	S	29	S	22	S	36	S
10	20	27	S	6	R	35	S	29	S	25	S	39	S
10 new	20	28	S	6	R	34	S	29	S	25	S	38	S
14	18	26	S	19	S	29	S	26	S	20	S	34	S
14 new	18	25	S	20	S	28	S	33	S	20	S	34	S
16	25	13	R	6	R	31	S	29	S	19	S	33	S
16 new	26	13	R	6	R	31	S	29	S	25	S	34	S
28	18	24	S	6	R	23	S	22	S	17	S	38	S
28 new	18	23	S	6	R	23	S	23	S	18	S	38	S
30	15	21	S	6	R	30	S	25	S	18	S	33	S
30 new	15	21	S	6	R	32	S	26	S	18	S	33	S

Table VI: MIC dubbeltesting

	MIC P	MIC AM	MIC CT
35	0.125	0.19	0.25
35 new	0.125	0.19	0.25
39	0.19	0.125	0.094
39 new	0.19	0.125	0.094
47	0.25	0.19	0.125
47 new	0.25	0.19	0.125
58	0.19	0.125	0.064
58 new	0.19	0.125	0.064
61	0.25	0.125	0.094
61 new	0.25	0.125	0.094
63	0.25	0.25	0.008
63 new	0.25	0.25	0.008
87	0.94	0.16	1.5
87 new	0.94	0.16	1.5
93	0.032	0.016	0.125
93 new	0.032	0.016	0.125
101	0.032	8	32
101 new	0.034	8	32
106	0.023	0.125	8
106 new	0.023	0.125	8

Antibiotic susceptibility of the pneumococcal strains:

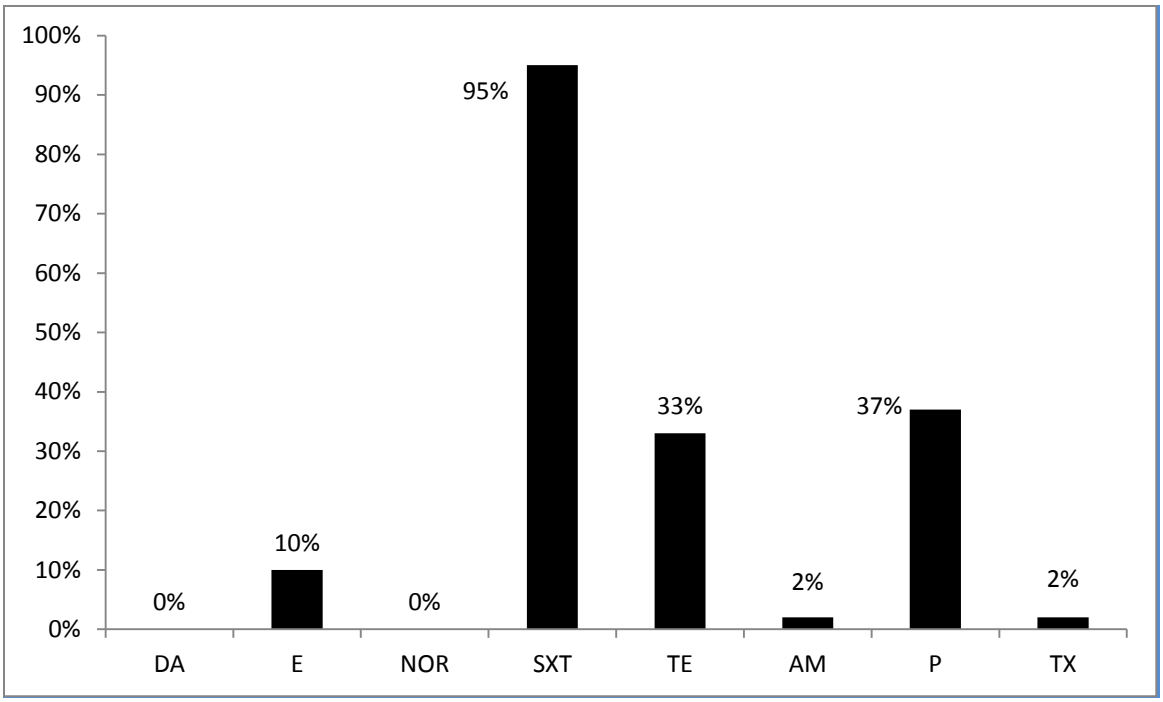
Of the 43 *Streptococcus pneumoniae* strains 16 had a zone-diameter for Oxacillin < 20 mm and were further tested with Minimal Inhibitory Concentration (MIC) for benzylpenicillin, ampicillin and ceftriaxone. All 16 samples (37 %) had MIC > 0.06 mg/L for penicillin and were thus classified as PNSP (penicillin non susceptible pneumococci), no one was resistant (MIC > 2 mg/L). Only one isolate had reduced susceptibility against ampicillin and one isolate against ceftriaxone, MIC 0.94 and 1.5 mg/L respectively.

Cotrimoxazole, erythromycin, clindamycin, norfloxacin and tetracycline were tested according to the disc diffusion test. The rate of pneumococci with reduced susceptibility to trimethoprim-sulfametoxazole (Cotrimoxazole) was 95 %. The rates for tetracycline and erythromycin were 32 % (where 12 isolates were resistant and two isolates had intermediate

susceptibility) and 10 % (where two isolates were resistant and two isolates had intermediate susceptibility) respectively. No isolates with resistance against norfloxacin and clindamycin were found.

Multidrug-resistance, meaning reduced susceptibility to three or more antibiotics, occurred in 12/43 isolates giving a rate of 28 %. The susceptibility testing results are presented in figure I.

Figure I: Rates of pneumococcal isolates with reduced susceptibility (intermediate or resistant) against antibiotics



DA= Clindamycin
E= Erythromycin
NOR= Norfloxacin
SXT= Cotrimoxazole/ trimethoprim-sulphametoxazole
TE= Tetracycline
AM = Ampicillin
P = Benzylpenicillin
TX = Ceftriaxone

Discussion

Carriage of *Streptococcus pneumoniae* has been studied all over the world, but few studies have been conducted in low-income countries, especially after the introduction of the pneumococcal conjugate vaccine. This study investigated the carriage rate in 150 healthy children below two years of age at three different health facilities in Moshi, northern Tanzania after the introduction of the conjugate vaccine. Isolated pneumococci were further tested for antibiotic susceptibility in order to investigate the spread of resistant bacteria in the society, and the carriage was also related to social factors and health status. No significant difference in carriage rate was noticed between the three health clinics which suggest that the carriage rate and resistance pattern found in this study reflects the true carriage and resistance rate in the Moshi region.

The children included in this study had a nasopharyngeal carriage rate at 29 %. This is lower compared to the studies on healthy children from Kenya and Uganda which showed a carriage rate at 57 % and 62 % respectively,(4, 7) but in line with the studies on healthy children from Turkey, Venezuela and Dar es Salaam which showed a carriage rate at 23 %, 28 % and 35 % respectively.(5, 6, 8) The relatively low carriage rate in the present study could be explained by the fact that the included children were quite young (median 7 months). The carriage rates of pneumococci increases during the first year of life and is the highest in children at 2-3 years of age.(27)

Among the 43 pneumococcal isolates there were rather high rates of reduced susceptibility to antibiotics. More than one-third of the isolates had reduced susceptibility against penicillin. This is lower than the 68 % in Dar es Salaam but higher than the 21 % found in Rombo district in northern Tanzania.(8, 12) The rate of pneumococci with reduced susceptibility

against cotrimoxazole, tetracycline and erythromycin were 95%, 33% and 10% respectively in this study, which is even higher than the numbers from Dar es Salaam where the numbers were 83 %, 10 % and 6 % respectively.(8) Also the multidrug resistance is even somewhat higher in the Moshi region compared to Dar es Salaam, 28 % compared to 17 %. This confirms that co-trimoxazole or per-oral penicillin should not be used as empirical treatment of pneumonia in this area, while amoxicillin could still be the first drug of choice. The majority of the isolates with reduced susceptibility for penicillin had intermediate resistance and this means that high doses of intravenous penicillin still can be used as treatment for hospitalised patients.

Interestingly, when pneumococcal carriage was related to social factors and the health status of the children, significantly more children with pneumococci had had malaria compared to children without pneumococcal carriage (44% vs 12%). Malaria is still a lethal threat against children in Tanzania and low-income countries in general, but is not known to increase the carriage of pneumococci. Malaria was earlier treated with a medicine that shares some components with co-trimoxazole (pyrimethamine-sulphadoxine).(28) Notably, almost all isolates in this study was non-susceptible against co-trimoxazole.

Diseases known to increase the risk of pneumococcal carriage are HIV and measles (2) but none of the children included in the study had a history of measles or known HIV diagnosis. 21 % of the non-carriers had a history of respiratory tract infection during the last three months versus 12 % of the carriers, although the difference was not significant one can speculate if the children with respiratory infection had been treated with antibiotics recently and therefore carried pneumococci to a lower extent.

A few other studies have been performed in Moshi investigating pneumococcal carriage in relation to risk factors.

One study investigated carriage in children to HIV-infected mothers born at KCMC between 2005 and 2009. The colonization rate was 56 % and having siblings less than 10 years of age was a significant risk-factor for carriage whilst hospitalization was negatively associated with pneumococcal carrige.(29) Another longitudinal study was performed at a sugar-plantation in Moshi 2011, where naso- and oropharyngeal samples were taken from 83 healthy children under five years of age and those found to carry pneumococci were followed over a 12-month period. In this thesis the carriage rate was the same as in this study i.e. 29 %.(28) The pneumococcal carriage was higher in children with siblings but antibiotic use did not affect the rates.(28)

When comparing the group of pneumococcal carriers to the children not carrying pneumococci in the present study, there was no difference in proportion of children with antibiotic treatment within the last three months (52 % compared to 49 %), anyone smoking in the household (16 % compared to 14 %) or living in a crowded home -in this study counted as living four or more persons per room in the household (16 % compared to 14%). A smoking member of the household and living in crowded homes are known risk factors for carriage of *S. pneumoniae* (1) but previous antibiotic use has in studies from Kenya and Malawi been shown both to decrease carriage and to increase the rates of non-susceptible pneumococci.(4, 13) Notably as many as half of the children had received antibiotics during the last three months. We observed during the study that many of the children had got antibiotic for questionable reasons, for example due to running nose only. In contrary to our beliefs the antibiotics were often prescribed by a doctor and not bought over counter. All the children in this study that had received antibiotics the last week had got it prescribed.

Interestingly, within the population of breastfeeding children 0-6 months (in total 68 children of whom 16 carried pneumococci) mixfeeding, i.e. a combination of food and breastfeeding, were more common in children not carrying pneumococci than children with pneumococci, and exclusively breastfeeding tended to be more common among pneumococcal carriers than children without pneumococci. This is in contrast to other studies where exclusive breastfeeding has been found as a protective factor for pneumonia.(10, 14)

Of the 150 infants included in the study 59 % were partially or fully vaccinated against pneumococci. However no significant difference in carriage rate was observed between the vaccinated and non-vaccinated children. It will be interesting to see how the carriage rate and spread of bacteria with reduced susceptibility to antibiotics is affected when the vaccine-coverage will be even higher in the future.

Although not statistically proven there was a higher percentage of mothers going up to primary school level in the group of carriers and a higher percentage of mothers going up to secondary school in the group of non-carriers. This could be an indication of the fact that lower socio-economic status increases the carriage-risk(11), and there was also a tendency of higher percentage of the fathers going to university in the non-carrier group, which may indicate that higher education is more common among the parents of children not carrying pneumococci.

Strengths and weaknesses

The number of 150 healthy children included in the study makes up a decent sample size, although the number of found pneumococci is too small to really correlate to social factors.

This study has some weaknesses. The study is based on information recalled by parent, this is of course not without problem and can cause recall bias, since parents tend to forget, for instance recent antibiotic use, name of antibiotic, recent respiratory tract infection etc. However, the parents had some time to think through the questions before answering and many of the questions were asked repeatedly but in different ways, which could help the parents to remember. The questioning of the parents was also done in English but translated to the parents in Kiswahili, by the community health nurses, which of course may lead to misinterpretations.

Despite some limitations at the clinical laboratory, for instance frequent power cuts and the delay of the laboratory package sent from University of Gothenburg, the laboratory work proceeded smoothly.

Conclusions

The findings in this study confirm that co-trimoxazole or per-oral penicillin not should be used as empirical treatment of pneumonia in this area, while amoxicillin could still be the first drug of choice. The high resistance rates against penicillin and co-trimoxazole, and the high rate of previous antibiotic treatment of the children suggests that more restrictions on the antibiotic use are needed in the Moshi area.

Populärvetenskaplig sammanfattning på Svenska

Lunginflammation är ett stort hälsoproblem i världen och låginkomst-länder är särskilt drabbade både av sjukdoms- och dödsfall. Några som är extra sårbara för att drabbas är små barn och lunginflammation är den sjukdom som dödar flest barn under fem års ålder i världen. Det finns olika virus och bakterier som orsakar lunginflammation men en bakterie som orsakar många sjukdomsfall är *Streptococcus pneumoniae* eller pneumokocken som den också kallas. Denna bakterie kan även ge andra infektioner såsom öroninflammation eller hjärnhinneinflammation. År 2000 beräknades pneumokocken orsaka nästan en miljon dödsfall. Som behandling mot lunginflammation finns olika sorters antibiotika, där framförallt penicillin är ett viktigt läkemedel. Ett ökande problem i världen är bakterier som har resistens mot olika antibiotika och pneumokocker med nedsatt känslighet för exempelvis penicillin kan både ge mer allvarliga och svårbehandlade infektioner.

Denna studie gick ut på att undersöka bärarfrekvensen av *S. pneumoniae* i bakre svalget hos friska barn under två års ålder i Moshi i norra Tanzania, ett land där lunginflammation står för femton procent av dödsfallen hos barn under fem år. Förutom att undersöka hur många barn som bar på bakterien undersöktes även känsligheten för olika antibiotika hos de pneumokocker som hittades. Genom ett frågeformulär undersöktes levnads- och hälsofaktorer som kan öka risken för bärarskap; såsom trångboddhet, vedeldning inomhus, sjuk i mässling eller HIV, tidigare antibiotikaanvändning och så vidare.

Bland de 150 barn som inkluderades i studien var det 43 stycken som bar på bakterien. Bland de pneumokocker som hittades var det många som hade nedsatt känslighet för penicillin och nästan alla var resistenta mot ett annat vanligt antibiotikum som kallas trim-sulfa. Detta innebär att mer riktlinjer för antibiotikaanvändning behövs i i Moshi-regionen samt att vaccinet mot pneumokocker som nu är inkluderat i barnvaccinations-programmet i Tanzania kan göra stor nytta.

Acknowledgments

This study was made possible thanks to many people involved. I want to thank my supervisor Susann Skovbjerg for excellent guidance, and my friend Josefine Blomqvist for great collaboration and company. The clinical part of the study was supervised by Dr Sia Msuya, Assistant Head, Department of Community Medicine at KCMC. The laboratory work was supervised by Dr Balthazar Nyombi, Head of the Clinical Laboratory at KCMC. I appreciate all the support and advice from them which made this study possible.

A special thanks to the community Health nurses Celina Mayo and Bertha Kiwale at Kilimanjaro Christian Medical Centre (KCMC) for translation and assistance at the clinics. Big thanks also to Dawen Kileo who drove the car that transported us to all the health facilities.

The work at the Clinical laboratory was mainly performed and supervised by Lab-scientist Margaretha L. Sariko together with student Nancy Kassam, I would like to express my sincere gratitude to them and all the people at the laboratory.

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Appendix

Appendix 1. Patient Questionnaire

Consent Form

Hello. My name is ……..a researcher/nurse/clinician working at KCMC/ KCMU-College. We are conducting a study in collaboration with the office of DMO, Moshi Municipality, KCMU Co/KCMC and Gothenburg University. The study is looking at prevalence of *Streptococcus pnemoniae* carriage, sensitivity patterns to common drugs and associated risk factors among children less than 2 years in the district. This bacteria/bug is the most cause of pneumonia in children. Rapid treatment of pneumonia once a child has symptoms is vital and drugs given need to be sensitive/ effective. However there is a problem that the bacteria develop resistance to the common drugs that are used by doctors/clinicians in our setting. This study therefore wants to collect sample from the child nose. This will be processed at the laboratory at KCMC to show if the child is carrying the bacteria and if that type of bacteria responds to the normal used drugs. The results from this study will help the government, the district and health providers to adapt/ change treatment of pneumonia/ IMCI taking into account the sensitivity pattern of drugs.

Your permission for the child to participate is very important as the success of this study depends much on you, by giving us your sincere answers. I guarantee the information which will be provided to me will be confidential and used by members of the study alone and your name or the name of the child will not appear in any data or report.

Participation is voluntary and you can withdraw to be part of this study at any time, without affecting your right of services or care at this clinic.

I have been informed fully about my child participating in this research including the risks and advantages. I voluntary agree to participate fully in this study for the beneficence of child health as I have been informed

Interviewee signature…… Parent/Guardian Signature……..……

Date……..…. Date……

Patient Questionnaire

Pneumococci in healthy children and in children with respiratory tract infections in Moshi, Tanzania

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ID Number of participant:……..
1. Name of the facility……
2. Level 1. Health centre 2. Dispensary
3. Main reasons for attending at health facility; *tick the appropriate & multiple answers are possible*
   a. Vaccination of the child
   b. Growth monitoring
   c. OPD clinic (child is sick); *symptoms*.……..
   d. CTC clinic
   e. Others; *mention*.……..
4. Date of filling questionnaire……
5. Name of the person filling the questionnaire……

1. Date of birth of the child……
Age in months.....

2. Weight (Kgs).....

3. Length (cm).....

4. Gender Girl
Boy

5. Delivery on time (meaning term or preterm baby)?
Yes
No, Divergence (weeks).....
Unknown

6. Problems during delivery?
No
Yes, Which.....

7. Problems during neonatal period?
No
Yes, Which.....

8. Is the child still Breastfeeding?

0. No 1.Yes

If YES still breastfeeding; **(For children aged 0-5 months)**

	No	Yes
Have you started giving water to the child?.....		
Have you started giving cow's milk to the child?.....		
Have you started giving porridge to the child?.....		
Have you started giving juice/ soup to the child?.....		
Have you started giving semisolids e.g. *mtori*?.....		
Have you started giving solids e.g. *ugali* with stew?.....		

After filling the questions above, please tick if the child is still EBF, predominantly or mixfed
Exclusive.....Predominantly BF.....Mix feeding……

IF YES still breastfeeding; **(For children aged 6 months or more)**
Have you introduced other foods? No Yes

IF **NOT** BREASTFEEDING:
Age of the child in months when breastfeeding was stopped…….....
The child has never been breastfed.....

9. Reason for clinical visit?.....

10. Does the child have the any of the following symptoms of respiratory tract infection today?

	No	Yes
Fever	_____	_____
Chills	_____	_____
Rapid or difficult breathing	_____	_____
Cough	_____	_____
Running nose	_____	_____
Sore Throat	_____	_____
Other:.....		

11. Sudden onset of symptoms?
No
Yes
Unknown

12. Has anyone else in your family had similar symptoms as your child in the last 14 days?
No

Yes
Unknown

13. Has the child used any antibiotics the week (7 days) before seeking medical care?

No
Yes, which.....

Prescribed.....Over the counter.....

14. Has the child used cough medicine?

No
Yes, which (or contents).....

15. Has the child used any antibiotics in the previous?

	No	**Yes**	**If yes; types**
One month prior to interview	_____	____	_____
Unknown			
Three months prior to interview	_____	____	_____
Unknown			

16. Has your child had any of the following respiratory tract infections (cough with fever, rapid breathing, difficulty in breathing, pneumonia) within the past 3 months?

No
Unknown
Yes, how many times:.....

Symptoms:.....

Antibiotic treatment given.....

17. Current or previous diseases	**No**	**Yes**	**When**
Asthma.....			
Gastrointestinal infections.....			
Measles.....			
Malaria.....			
HIV.....			
Lung tuberculosis.....			
Malformations.....			
Heart disease.....			
Other.....			

Details (symptoms, treatment, number of times):

.....

.....

18. Has the child been hospitalized within the past 3 months?

No

Yes, When.....

Why.....

19. Area where you are living?

Type	**Name of ward**
Rural area……	
Semi-rural area, Size.....	
Semi-urban, Size.....	
Urban area, Size.....	

20. Parents level of education?

Mother	Father
□ Never been to school	□ Never been to school
□ Primary school	□ Primary school
□ Secondary school	□ Secondary school
□ University	□ University

21. Parents current occupation?

Mother	Father
□ Employed. What.....	□Employed. What.....
□ Self employed. What.....	□Self employed.What.....
□ Unemployed	□ Unemployed
□ Student	□ Student
□ Other.....	.....Other.....

22. How many siblings does this child have?.....

23. In total how many people live in your household?.....

Out of those how many are children under five?.....

24. Number of rooms in your home?.....

25. Number of people sleeping in the same room?.....

26. Smoking of parents or other persons in the household?

Yes
No

27. How does your family cook food?

Electricity
Gas
Opem fire outside
Open fire with chimney
Open fire without chimney

27. Is your child vaccinated? *Please tick vaccinations the child has received*

	Birth	**6 weeks**	**10 weeks**	**14 weeks**	**9 months**	**18 months**	
BCG							
OPV							
OPV 1							
OPV 2							
OPV 3							
Heptavalent 1 (DPT, Hep B, Hemophilus B)							
Heptavalent 2 (DPT, Hep B, Hemophilus B)							
Heptavalent 3 (DPT, Hep B, Hemophilus B)							
PCV 13 – 1							
PCV 13 – 2							
PCV 13 – 3							
Measles							
Others:							
Others:							
Others:							

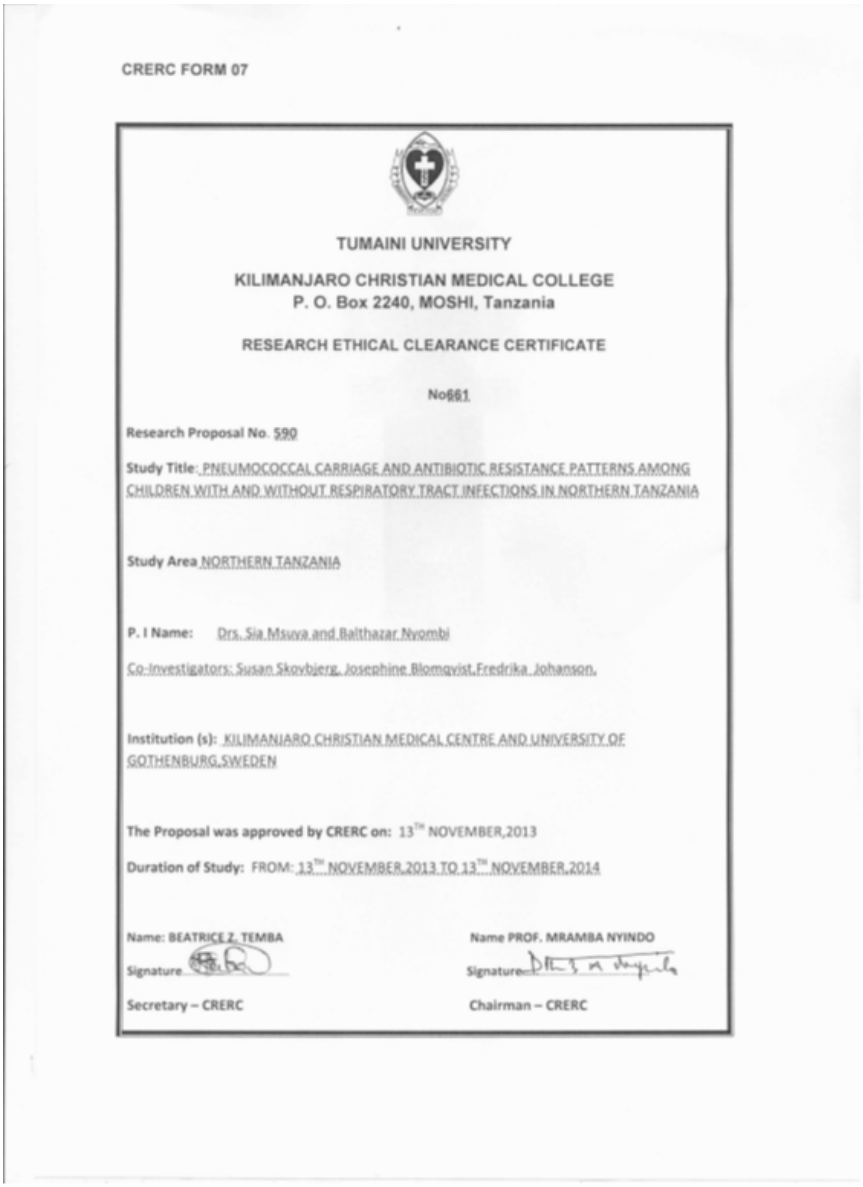
Unknown……..
Adequately immunized for age: No Yes

28. If the child has siblings < 1 year, have they been vaccinated against pneumococci?

Yes
No
Unknown

Thank you for your participation!

Appendix 2. Ethical approval



CRERC FORM 07

TUMAINI UNIVERSITY

KILIMANJARO CHRISTIAN MEDICAL COLLEGE
P. O. Box 2240, MOSHI, Tanzania

RESEARCH ETHICAL CLEARANCE CERTIFICATE

No661

Research Proposal No. 590

Study Title: PNEUMOCOCCAL CARRIAGE AND ANTIBIOTIC RESISTANCE PATTERNS AMONG CHILDREN WITH AND WITHOUT RESPIRATORY TRACT INFECTIONS IN NORTHERN TANZANIA

Study Area NORTHERN TANZANIA

P. I Name: Drs. Sia Msuya and Balthazar Nyombi

Co-Investigators: Susan Skovbjerg, Josephine Blomqvist,Fredrika Johanson,

Institution (s): KILIMANJARO CHRISTIAN MEDICAL CENTRE AND UNIVERSITY OF GOTHENBURG,SWEDEN

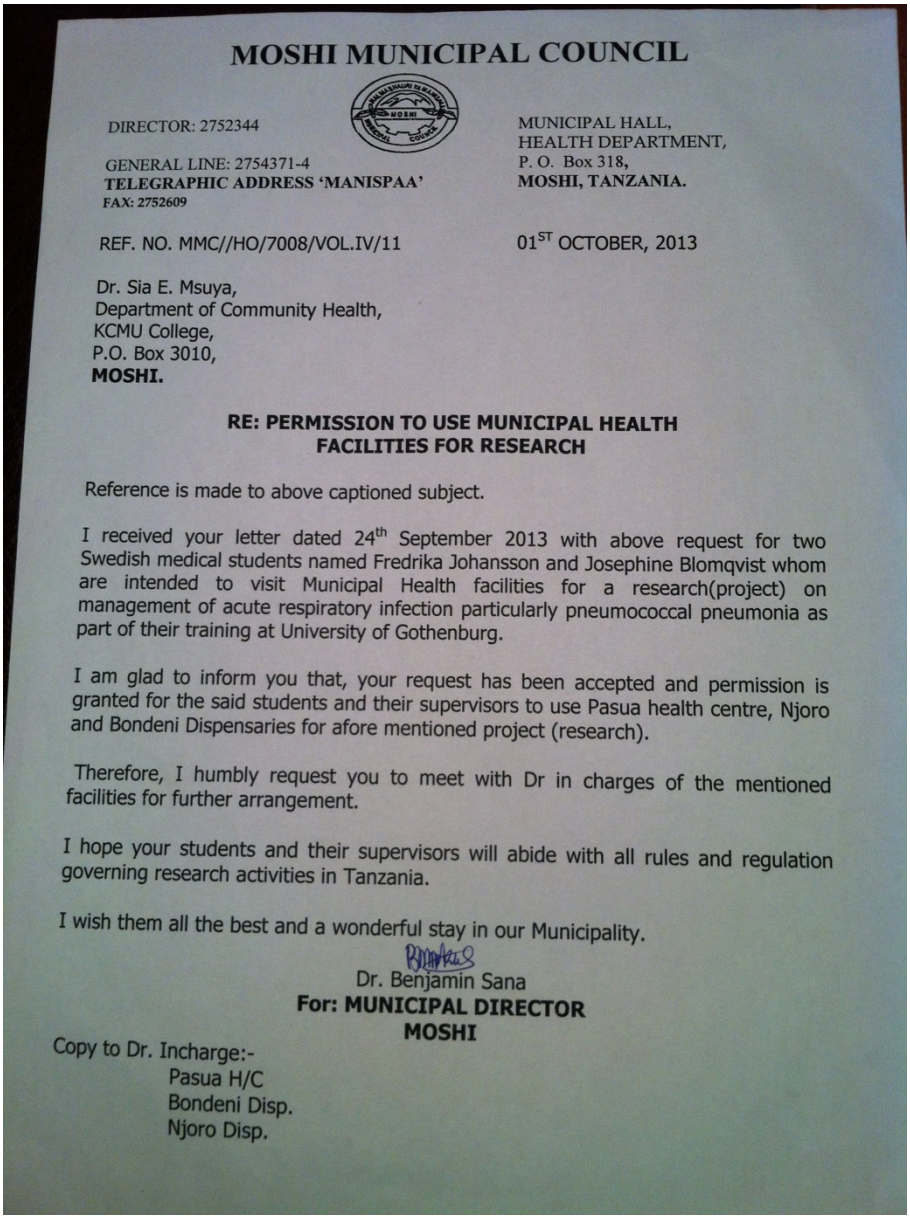
The Proposal was approved by CRERC on: 13TH NOVEMBER,2013

Duration of Study: FROM: 13TH NOVEMBER,2013 TO 13TH NOVEMBER,2014

Name: BEATRICE Z. TEMBA
Signature
Secretary – CRERC

Name PROF. MRAMBA NYINDO
Signature
Chairman – CRERC

Appendix 3: Permission from Moshi Municipal Council



MOSHI MUNICIPAL COUNCIL

DIRECTOR: 2752344
GENERAL LINE: 2754371-4
TELEGRAPHIC ADDRESS 'MANISPAA'
FAX: 2752609

MUNICIPAL HALL,
HEALTH DEPARTMENT,
P. O. Box 318,
MOSHI, TANZANIA.

REF. NO. MMC//HO/7008/VOL.IV/11

01ST OCTOBER, 2013

Dr. Sia E. Msuya,
Department of Community Health,
KCMU College,
P.O. Box 3010,
MOSHI.

RE: PERMISSION TO USE MUNICIPAL HEALTH FACILITIES FOR RESEARCH

Reference is made to above captioned subject.

I received your letter dated 24th September 2013 with above request for two Swedish medical students named Fredrika Johansson and Josephine Blomqvist whom are intended to visit Municipal Health facilities for a research(project) on management of acute respiratory infection particularly pneumococcal pneumonia as part of their training at University of Gothenburg.

I am glad to inform you that, your request has been accepted and permission is granted for the said students and their supervisors to use Pasua health centre, Njoro and Bondeni Dispensaries for afore mentioned project (research).

Therefore, I humbly request you to meet with Dr in charges of the mentioned facilities for further arrangement.

I hope your students and their supervisors will abide with all rules and regulation governing research activities in Tanzania.

I wish them all the best and a wonderful stay in our Municipality.

Dr. Benjamin Sana
For: MUNICIPAL DIRECTOR
MOSHI

Copy to Dr. Incharge:-
Pasua H/C
Bondeni Disp.
Njoro Disp.