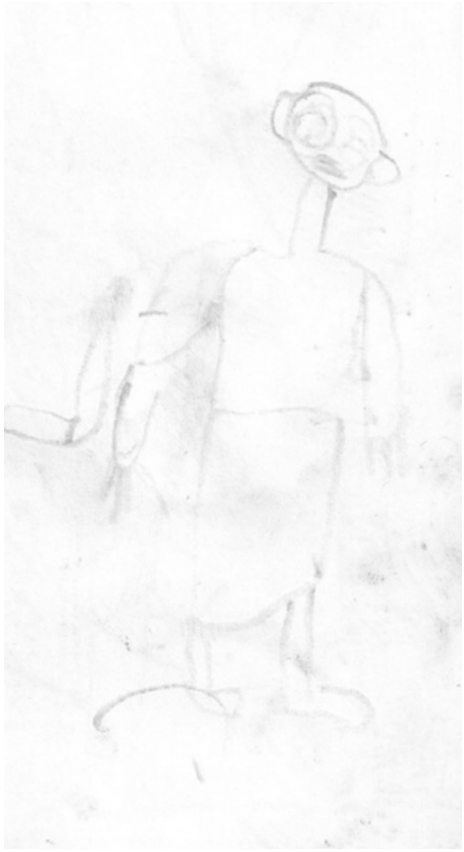
Pneumococcal carriage in Tanzanian children with respiratory tract infections



Master thesis by Josefine Blomqvist

Programme in medicine

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By student Josefine Blomqvist

Supervisors Susann Skovbjerg, Sia Msuya and Balthazar Nyombi

Sahlgrenska Academy

UNIVERSITY OF GOTHENBURG

Programme in medicine

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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 children with respiratory tract infection in the Moshi area in Tanzania, a region in which the pneumococcal vaccine was introduced in January 2013, and to relate the carriage rate to social factors and health status of the children.

Methods: During October and November 2013, 187 randomly selected children under two years of age with symptoms of a respiratory tract infection (chills, cough, running nose, rapid or difficult breathing or sore throat) were included in the study. The children were recruited from three different health clinics in the Moshi area. A nasopharyngeal sample was taken from the children and a questionnaire was given to the parents. The samples were cultured at the clinical laboratory at Kilimanjaro Christian Medical Centre (KCMC) in Moshi, where isolates of pneumococci were identified and tested for antibiotic susceptibility.

Results: The carriage rate of pneumococci in the nasopharynx among the children was 39%. One fourth of the pneumococcal isolates had reduced susceptibility against penicillin, while only 5.5% and 1.4% were non-susceptible against ceftriaxone and ampicillin, respectively. While almost all isolates were non-susceptible against co-trimoxazole (97%), the rate of pneumococci with reduced susceptibility against, erythromycin, tetracycline and clindamycin were 32%, 15% and 5.5%, respectively. No resistance was detected against quinolones. Carriage of pneumococci among the 187 children was not related to social factors (living conditions, parents' level of education, breastfeeding etc.) or health status (antibiotic use, previous illness, vaccination status etc.).

Discussion: The carriage rate is consistent with other studies in this area before the pneumococcal conjugate vaccination was initiated. The rather high rates of resistant 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 are needed in the Moshi area and may lower the resistant rates.

Background

Children mortality rate is still high in many parts of the world and the leading cause of death is pneumonia (1). Every year there are about 120 million episodes of pneumonia in children under five years of age and children under two are especially vulnerable. Pneumonia causes about 1. 3 million deaths in children under five years of age, the majority occur in developing countries. The pneumonia incidence is greatly associated with poverty and 43% of the deaths from pneumonia occur in the Sub-Saharan Africa (2).

Three important risk factors for developing pneumonia in children are: Under nutrition, suboptimal breastfeeding and zinc deficiency (3). These risk factors are shared with other childhood diseases such as diarrhea (3). Patients with immunodeficiencies including HIV is another vulnerable group for developing pneumonia (4). The HIV positive children are especially susceptible to bacterial pneumonia and infections with antibiotic resistant bacteria are also more common (2). Other risk factors for developing pneumonia include low-birth weight, cooking food on solid fuel, crowding, low maternal education, limited access to care and passive care-seeking behavior (3).

A study from north-eastern Tanzania in 2012 identified factors that are associated with a more severe form of pneumonia. These factors included young age, malnutrition, low parental

education and low socioeconomic status (5). Other important host-factors that strongly predict child survival in general are; living in rural areas, poor quality of water and bad sanitation (6, 7).

The most common bacteria that cause pneumonia is *Streptococcus pneumoniae* (the pneumococcus) (8), but other bacteria such as *Haemophilus influenzae* and viruses such as Influenza viruses and respiratory syncytial viruses (RSV) may also cause the disease (3). The pneumococcus is also the leading cause of meningitis and sepsis in children and accounts for about 11% of all childhood deaths worldwide. The highest rate of pneumococcal mortality is in Africa (8). Worldwide, *S. pneumoniae* is estimated to account for about 18% of the severe cases and 33% of the deaths from pneumonia each year (2).

An infection with *S. pneumoniae* cannot occur without the preceding colonization of the nasopharyngeal mucosa (9, 10). The colonization is a dynamic process when it comes to duration of carrying different strains (4). However, carriage in itself is not a risk factor for disease, especially not in children (9). Humans are the natural host for the pneumococci (11). Together with other bacteria such as *Moraxella cattarrhalis* and *Haemophilus influenzae*, *S. pneumoniae* constitutes the commensal flora of the nasopharynx in children. Nasopharyngeal carriage also enables horizontal spread between people in the society (4). The children are often asymptomatic carriers and therefore constitute the reservoir for these bacteria in the population. The bacteria are spread mainly in the child population (12), and the colonization rate varies greatly between different populations (9). The carriage rate of the pneumococcus increases during the first years of life, reaching a peak in children aged 2-3 years, after which it declines in older children (10).

Infection with *S. pneumoniae* is a complex process involving bacterial virulence, viral interplay and host-specific factors (9). The bacteria spread through aerosol droplets and close contact. From time to time the bacteria succeed in escaping the immune system, are aspirated and cause pneumonia (4). Pneumonia often starts with a viral infection in the upper respiratory tract, which usually gives symptoms of a common cold, such as cough and running nose. The infection then proceeds downward to the lungs enabling a secondary bacterial infection, pneumonia, which can be much more severe and even lethal (1).

Predictors of carriage are crowding, family size, number of siblings, income, smoking and previous antibiotic use (4). Recent antibiotic may lead to higher rates of carriage and especially carriage of antibiotic resistant strains (4), although other studies show lower rates of colonization following antibiotic treatment (13-15). The carriage rate is also higher in HIV positive children (13). The carriage proportion also increases during a viral respiratory tract infection, and symptoms like cough and coryza are positively associated with carriage (4, 13). The carriage rate also varies with season and is highest during the cold season, which could be a confounder for crowding, since people tend to stay inside more during the cold (16). In low-income countries both the carriage of the bacteria and its disease manifestations are more common than in high-income countries (9).

Early diagnosis and antibiotic treatment can prevent many deaths from pneumonia (17), however, in many countries, treatment of pneumococcal infections has become more challenging due to high rates of *S. pneumoniae* resistant to penicillin (7). Resistance is driven by the global spanding misuse of antibiotics which in many low-income countries is apparent by the wide-spread over the counter selling of antimicrobials. The resistance was earlier contained in microorganisms found in hospitals (nosocomial infections) but is now prevalent

in the community and even carried among healthy individuals. The resistance seems to be higher in urban settings rather than rural (18). The first line treatment of pneumonia has earlier been either penicillin or co-trimoxazole (trimethoprim-sulphamethoxazole). These antibiotics were both cheap and had few side-effects (7), but now since the rate of pneumococci with reduced susceptibility against penicillin and co-trimoxazole is high in many low-income countries the first line drug is instead amoxicillin (ampicillin) (2). The increasing problem with antibiotic resistance is especially challenging for low-income countries that may not afford the more expensive second-line drugs (18). In a study from Dar es Salaam in 2012 more than two thirds of the pneumococci isolates from healthy children were non-susceptible to penicillin. High resistance rates to several other antibiotics were also shown, especially co-trimoxazole (83%). The multi-drug resistance was 17% among the isolates, defined as resistance to three or more antibiotics (11).

Infection as well as carriage of the pneumococcus can be prevented through vaccination (9). Many countries now use the pneumococcal conjugate vaccine in their general child vaccination programmes (19), which has decreased the incidence of invasive pneumococcal disease (13). Not only the vaccinated individual but also other persons in the vaccinated community are protected, through so called herd immunity (4, 12). There are 94 different serotypes based on differences in the pneumococcal capsule (9, 20). The 13-valent vaccine covers 13 of these serotypes, namely: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F (12). The vaccine is designed to cover the serotypes most often isolated from invasive disease (9) and also the serotypes that most frequently carry antibiotic resistance (21). In general; the serotypes that are carried asymptomatically are carried for a longer time than the serotypes that more frequently cause disease (9). The serotype distribution differs geographically which makes it very important with local studies. In Dar es Salaam the most prevalent serotypes

found in the nasopharynx of children are 19F, 6B and 9V. Globally, these serotypes are the most common ones to colonize children (11).

In countries using the pneumococcal conjugate vaccine, the pneumococcal serotypes present in the vaccines have decreased among the disease isolates (22), but at the same time the prevalence of non-vaccine serotypes have increased (13). Immunization also protects the community from pneumococcal resistance by decreasing the circulation of resistant strains, but also through a decreased over-all use of antibiotics (21). Many low-income countries are about to include the pneumococcal conjugate vaccine in the vaccination programme of the children (23). Tanzania introduced the pneumococcal conjugate vaccine, PCV 13 in January 2013. The outcome is at present uncertain since the vaccine is designed for high-income countries that may have another distribution of serotypes (7, 23). Further studies on serotype distribution on both carried and invasive strains in low-income countries are therefore necessary (4). Several pathogens that cause pneumonia are vaccine-preventable, including; *H. influenzae* type B, influenza virus and measles. Out of these, *S. pneumoniae* is the most important pathogen (2). Since preventable, it is a reasonable approach if trying to accomplish the Millennium Development Goal 4, which strives at reducing the under five mortality by two thirds before 2015 (3, 8).

In a study from Kenya in 2012 the carriage prevalence rate was 66%. Among the serotypes found, only 59% were serotypes covered by the Pneumococcal Conjugate Vaccine, PCV 13. This raises the question of whether vaccine-introduction is a benefit or not. The outcome depends on the circulation of non-vaccine serotypes and their ability to colonize the nasopharynx and from there cause invasive disease (13).

Most studies conducted in developing countries earlier are hospital-based, which creates an unrepresentative study population (2). It is easy to both over- and underestimate the true incidence of childhood pneumonia. Studies on pneumonia risk factors in low- and middle Income Countries are scarce and the risk factors are often not independent which will lead to an over-estimation of each risk factor. On the other hand is it easy to under-estimate the disease burden caused by *S. pneumoniae* due to limited access to the pathogen, difficulties in transporting the specimen and culturing limitations (8). There are great difficulties in determining the etiological agents from the infection site (9). The lung, the site of infection, is a closed organ only in contact with the surroundings through the naso- and oropharynx. Sampling from the sputum or the nasopharynx is a possibility, but the result is complicated since the pathogens causing pneumonia are also common in the nasopharynx of children, causing no symptoms at all (3). However, pneumococcal disease is often preceded by carriage of a homologous strain (10), and the asymptomatic colonization with pneumococci gives an idea of the invasive strains circulating in the community and the rate of antibiotic resistance (11). Techniques like PCR may be a help to detect pneumococci in respiratory samples, but is also problematic since you tend to find many different pathogens, which leads to difficulties in establishing the causative agent. The availability to PCR methods is also highly restricted in low-income countries.

Many studies look before and after immunization with the Pneumococcal Conjugate Vaccine to determine how big the disease burden for each etiological agent is. These trials are not without problems, since the introduction of a new vaccine often comes with big resources, which of course contribute to an improved health in general (3). About 12 % of the pneumonia cases become severe cases. This is a 25 % reduction from 2000 till 2010, a trend seen in many Low-and middle-income countries (3). This is supported by the fact that

exposure to the most important risk factors also decreased during that period of time. This is probably due to the improved overall quality of life in many of these countries. However, the proportion of severe cases increased which could mean that the most vulnerable in the society remains in poverty and more effort needs to be directed towards that group (3). Combating pneumonia includes reducing the risk factors, immunization and case management. Since it is more common in children younger than two, interventions should be focused on improving vaccination-programs, breastfeeding and childhood nutrition (2).

Tanzania

The Democratic Republic of Tanzania, situated in eastern Sub-Saharan Africa has a population of 43 million people. Tanzania has had a fast population growth, due to a high fertility rate and a declining mortality rate. Almost half of the population is under 15 years of age and only 4% are over 65 years. The mean number of births per woman is 5.4 children and the birth rate is highest in rural areas, among the lowest wealth quintile and among the women with the lowest education. The life-expectancy at birth is only 51 years (17). Despite an economical growth the past years, Tanzania remains a poor country with more than 75% living in rural households. Tanzania also remains highly aid-dependant and developmental funding constitutes 14% of the country's total GDP (24).

In 2010 the under-5 mortality rate was 81 per 1000 live births which is a big progress towards reaching the forth Millennium Development Goal of reducing the under five mortality rate by two thirds before 2015. In Tanzania the most common cause of death in children is malaria, followed by pneumonia (25). There are big differences within the country, with the lowest figures in the northern zone with an under five mortality rate of 58 per 1000 live births (17). The current general child vaccination programme includes vaccinations against diphtheria, pertussis, tetanus, polio and measles. BCG against tuberculosis is also given at birth. Since

2009 vaccination against *H. influenzae* type B and Hepatitis B are included in the general child vaccination programme. The latest addition is the Pneumococcal Conjugate Vaccine PCV13 which was introduced in January this year. Currently, the first dose is given when the child is six weeks old, the second at ten weeks and the third and final dose is given when the child is 14 weeks old. In the Kilimanjaro region 94% of the children aged 1-2 years have got all the basic vaccinations based on either the child's health cards or the mothers' verbal report (17). The country is divided into 21 regions and 113 districts. The health care is divided into dispensaries and health centres at the lowest level, followed by district hospitals, regional hospitals and finally at the highest levels the national referral hospitals. The health care often lacks medical equipments and drugs as well as health care staff. Half of the health care spending comes from out of pocket payment and this is a heavy burden for people, especially on the poorest (26). As stated earlier the incidence of pneumonia and its disease manifestations vary greatly between regions and is highest in Africa and South-east Asia.15 countries account for almost two thirds of the total incidence as well as mortality in pneumonia and Tanzania is one of those countries (2).

Knowing of the local resistance rates and serotype distribution is crucial when determining empirical treatment and designing vaccines, especially in low-income countries where studies are scarce but the disease burden is heaviest. The increasing antibiotic resistance must be frequently monitored and local studies looking at the resistance rates are needed (27). This study is highly topical since Tanzania has recently introduced the pneumococcal conjugate vaccine, PCV 13 in their general child vaccination programme and the outcome is at present uncertain. Few studies have been conducted in the Moshi area before, and they have all shown high resistance rates to several antibiotics which is a big threat against successfully treating the children of this region.

Aim

- To investigate the colonization rate and antibiotic susceptibility of *S. pneumoniae* isolated from the nasopharynx in Tanzanian children with symptoms of upper respiratory tract infection.
- To investigate the relation between the pneumococcal carriage rate to social factors and health status of the children.

Method

During October and November 2013, 188 randomly selected patients below two years of age seeking health care with symptoms of respiratory tract infection were included in the study. This study was done in parallel to another student project investigating healthy children. The total sample size from the two studies was 338 children. The children attended the health clinics for routine care such as immunization or growth monitoring or attending out patient department (OPD)-clinic, care and treatment clinic (CTC) or for a few other reasons. The children were recruited at Bondeni and Njoro Dispensaries and Pasua Health Centre, in the Moshi area, which were randomly selected and approved by the Municipal Director Dr. Benjamin Sana (see appendix). The town of Moshi is situated in the northern zone and in the Kilimanjaro region of Tanzania and has a population of around 180,000 people. Translation and assistance at the clinics was given by experienced community health nurses from Kilimanjaro Christian Medical Centre (KCMC), the largest referral hospital in the northern zone. The questions were asked in English and then translated to Kiswahili. The inclusion criteria were presence of at least one of the following symptoms with or without fever: chills, rapid or difficult breathing, cough, running nose and sore throat. The study was based on voluntary consent and only 13 parents/guardians refused to participate.

A questionnaire was handed out to the parents/guardian to fill out (see appendix). It contained questions about health status, socio- economic conditions, previous illness, pregnancy problems, breast feeding, vaccinations, antibiotic treatment etc. The weight and height of the child was noted. The questionnaire was tested on two mothers of toddlers before the sample collection began.

A nasopharyngeal sample was taken by a blue-capped E-swab (Copan diagnostics, Murrietta, CA) from the children, and transported cooled to the Microbiological Laboratory at KCMC in Moshi. The samples were cultured for the isolation of *Streptococcus pneumoniae* on sheep blood agar plates incubated over night in 34-36 °C, CO_2, in 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).The remaining sample was then frozen and kept in the freezer (-20°C) at KCMC. Suspected pneumococci were identified by positive optochin test (diameter >14 mm) (Optochin 5 µg, Oxoid, Hampshire, UK).

Colonies with pneumococci was suspended in 2 ml phosphate buffered saline to McFarland 0.5. Isolates of pneumococci were then tested for antibiotic susceptibility against oxacillin (1µg), trimethoprim-sulphamethoxazole (1.25-23.75 µg), norfloxacin (10 µg), tetracycline (30 µg), erythromycin (15 µg) and clindamycin (2 µg) (all from Oxoid) by disc diffusion tests on a Muller Hinton agar supplied with sheep blood and NAD (AppliChem, GmbH, Darmstadt, Germany). The agar plates were incubated over night in 34-36 °C CO_2.The oxacillin-discs was used for screening of non- susceptibility against penicillin. Pneumococcal isolates with reduced sensitivity against oxacillin (diameter <20 mm) according to the disc diffusion test was tested by minimal inhibitory concentration, (MIC)-determination against penicillin, ampicillin and ceftriaxone (all 0.016-256 µg/mL, all from Biomérieux, Marcy I´Etoile

France). The isolates were then frozen (-20 °C) at KCMC in STGG storage medium (27), consisting of skim milk powder (Difco, BD Diagnostics, Sparks, MD), tryptone soya broth (Oxoid), glucose (Sigma Aldrich, Saint Louis, MO), glycerol and distilled water.

The breakpoints used for the disc diffusion testing and the MIC-determination were from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) published in January 2013 (28). Pneumococci with a MIC of >0.06 mg/L were defined as having reduced susceptibility against penicillin. For further breakpoints see table 1.

Table I. Antibiotic susceptibility breakpoints

Antibiotic	**Sensitive**	**Resistance**
Ampicillin	MIC ≤0.5 mg/L	MIC>2 mg/L
Benzylpenicillin	MIC ≤0.06 mg/L	MIC>2 mg/L
Ceftriaxone	MIC ≤0.5 mg/L	MIC>2 mg/L
Clindamycin	≥19 mm	<19 mm
Co-trimoxazole	≥18 mm	<15 mm
Erythromycin	≥22 mm	<19 mm
Norfloxacin	≥12 mm	<12 mm
Tetracyclin	≥25 mm	<22 mm

The field work was preceded by a course in global health at the University of Gothenburg during September 2013. Further education on the topic was given by Swedish SIDA in Härnösand during one weekend in May. Preparation for the laboratory work was done by a one week's attendance at the Microbiological laboratory at University of Gothenburg.

Statistical methods

Data analyses were done using the SPSS version 21.0. Comparisons between groups were performed using Fisher´s exact test. P values <0.05 were considered statistical significant.

Ethical considerations

The parent or guardian was informed that participation in the study was voluntary. They were informed that whether or not the patient chose to participate, the treatment at the clinic would not be affected. The parent or guardian willing to participate in the study then signed a consent form prior to the interview and sample collection. No social security number was noted, so the patients will not be able to be identified. The consent form will not be a part of further analysis. The study was carried out in accordance with existing ethical guidelines. Ethical clearance was obtained from College Research Ethics and Review Committee (CRERC) in Moshi, Tanzania (see appendix).

Results

Characteristics of study population: One child was excluded from the study due to being treated for a respiratory tract infection but having no current symptoms, leaving 187 children (median age 8.5 months, range 1-24 months) for the analysis. The characteristics of the included children are described in Table 2. The main causes for seeking health care were out patient department (OPD)-clinic, growth monitoring and vaccination, only two came for the care and treatment clinic (CTC). 12 children came for other reasons and this mostly meant accompanying mother or a sibling. Regardless of which reason the children visited the health clinics for; they all had at least one symptom of a respiratory tract infection and were included in the study. The majority had running nose and cough (Table 2). 65 children had yet another symptom where the most common was abdominal discomfort, teeth eruption, diarrhea,

vomiting, skin rash, eye problem and loss of appetite. The children lived in either urban or semi-urban areas. The parents mainly spoke Kiswahili, but some also spoke English (data not shown). The majority was under one year of age (65%) and one third was under six months of age (33%).

Table II. Study Population

Recruited children	n (%)
Age (months) median, range	8.5, 1-24
Weight (kg), median, range	8.5 , 3.4–14
Length (cm) median, range	67, 49-89
Gender (girls: boys)	94: 93
Health clinic	
Pasua	121 (65)
Bondeni	24 (13)
Njoro	42 (23)
Reason for visit	
Vaccination	36 (19)
Growth Monitoring	69 (37)
OPD-clinic[a]	68 (36)
CTC[b]	2 (1.1)
Other reason	12 (6.4)
Symptoms	
Fever	48 (26)
Chills	3 (1.6)
Rapid or difficult breathing	14 (7.5)
Cough	128 (68)
Running nose	159 (85)
Sore throat	14 (7.5)
Other	65 (35)

[a] Out patient department
[b] Care and treatment clinic

Type of area	
Urban	7 (3.7)
Semi-urban	180 (96)
Education of mother	
No schooling	1 (0.5)
Primary school	124 (66)
Secondary school	57 (30)
University	5 (2.7)
Education of father	
No schooling	1 (0.5)
Primary school	101 (54)
Secondary school	72 (39)
University	10 (5.3)

Nasopharyngeal carriage: The nasopharyngeal carriage rate of pneumococci in the children was 39% (73/187), and most of the isolates were found in children less than one year of age (Table 3). The carriage rate did not differ between the three health clinics, Pasua, Bondeni and Njoro (36%, 46% and 43%, respectively). Almost no relation was found between pneumococcal carriage and social factors or health status (Table 3). 67% of the mothers and 55% of the fathers had primary school as the highest education and no relation was found between low education and pneumococcal carriage (data not shown). One father and one mother had never been to school. A majority slept three or less in one room and 17% slept more than three people in the same room, but this was not associated with carriage. Almost all children were breastfed (Table 3). Among the children aged 0-6 months (n=61) 9 were exclusively breastfed, and this factor tended to be associated with more frequent carriage of pneumococci (p=0.070). On the other hand, predominantly breastfeeding was more common in children without pneumococci than in children with pneumococcal carriage (66% versus 30%, p=0.0093). Half of the children (n=98), 47% of pneumococcal carriers and 56%

of non-carriers, had received antibiotics within the past three months (data not shown). Previous respiratory tract infection was slightly more common in children without pneumococci than colonized children, but the difference was not significant.

Table III. Correlation between pneumococcal carriage and social factors and health status

	Pneumococcal carriage		p-value
	yes	no	
	(n =73), *n* (%)	(n=114), n (%)	
Age			
<1 year	51 (70)	70 (61)	n.s
≥1 year	22 (30)	44 (39)	n.s
Preterm birth[a]	6 (8.2)	13 (11)	n.s
Breastfed	68 (93)	103 (90)	n.s
Breastfed 0-6 months[b]			
Exclusively	6 (26)	3 (7.9)	0.070
Predominantly	7 (30)	25 (66)	0.0093
Mixfed	10 (43)	10 (26)	n.s
Family member with symptoms[c]	24 (33)	38 (33)	n.s
Antibiotic use			
<1 week	10 (14)	28 (25)	0.093
≥1 -4 weeks	17 (23)	26 (23)	n.s
>4 -12 weeks	17 (23)	36 (32)	n.s
Recent respiratory tract infection[d]	21 (29)	45 (39)	0.16
Previous episode of diarrhea	34 (47)	55 (48)	n.s
Previous episode of malaria	25 (34)	32 (28)	n.s

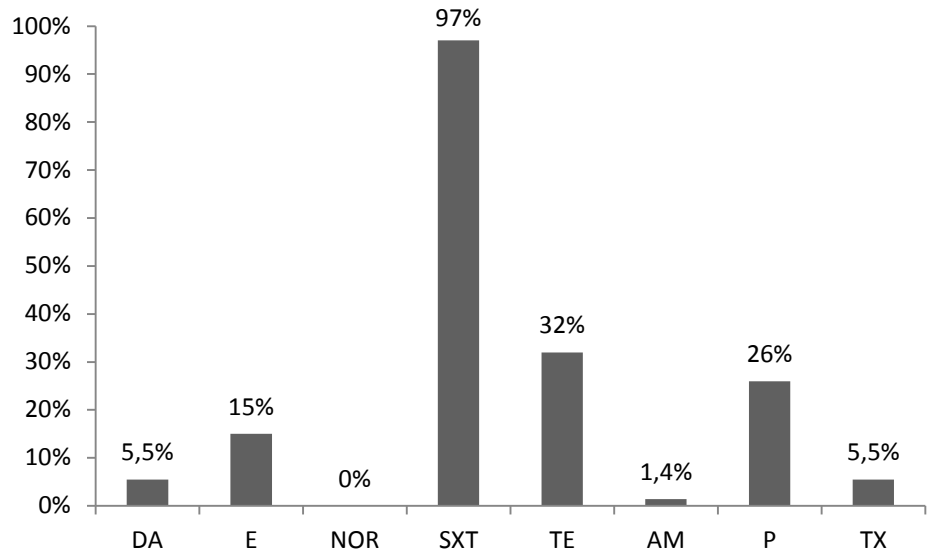
Hospitalization[e]	5 (6.8)	5 (4.4)	n.s
Siblings	39 (53)	66 (58)	n.s
Child<5 years[f]	16 (22)	31 (27)	n.s
No. of people sleeping in the same room			
1-2	10 (14)	9 (7.9)	n.s
3-4	59 (81)	100 (88)	n.s
>4	4 (5.5)	5 (4.4)	n.s
Smoking of parent	10 (14)	17 (15)	n.s
Indoor cooking without chimney	10 (14)	13 (11)	n.s
PCV 13 vaccination			
Partial	7 (9.6)	13 (11)	n.s
Full	37 (51)	44 (39)	0.13

[a] Birth more than two weeks before calculated date
[b] If the children aged 0-6 months were breastfed, yes(n=23), no (n=38)
[c] Family member sharing one or more of the symptoms defined in the inclusion criteria
[d] Defined as cough with fever, rapid or difficulty breathing or pneumonia during the past three months
[e] Admittance to hospital the past three months
[f] Number of people below the age of five in the household other than the index patient
ns= non significant

Antibiotic susceptibility: A package containing laboratory materials such as antibiotic discs and strips was sent from the University of Gothenburg to Moshi before the study started. However, the package was delayed approximately three weeks during transportation. Because of the delay new antibiotic discs and strips were brought and tested in parallel with the old ones for the first ten samples of pneumococci. The result showed no significant difference between the new and old discs or strips and no difference at all in the classifications of strains into Sensitive, Intermediate and Resistant according to the EUCAST breakpoints (See appendix). Thus, the strips and discs that had been delayed were of good quality and were further used in the study.

All pneumococcal isolates were tested for antibiotic susceptibility with disc diffusion test and, when reduced sensitivity against oxacillin, with MIC-determination. The resistance rates against the eight different antibiotics tested for the 73 pneumococcal isolates are showed in Figure 1. 26% (19/73) of the isolates had reduced susceptibility against penicillin (MIC>0.06 mg/L), however no sample was resistant (MIC>2 mg/L). One single isolate (1.4%) was resistant to ampicillin (MIC: 8.0 mg/L). Four isolates had reduced susceptibility against ceftriaxone (MIC >0.5 mg/L), two of these were resistant (MIC: 8 and 32 mg/L, respectively). Co-trimoxazole, erythromycin, clindamycin, norfloxacin and tetracycline were tested according to the disc diffusion test. All but two pneumococcal isolates (n=71, 97%) had reduced susceptibility against co-trimoxazole, 70 of these were resistant. Pneumococci with reduced susceptibility against tetracycline, erythromycin and clindamycin were 32%, 15% and 5.5%, respectively.

Figure 1: Frequency of pneumococci with reduced susceptibility (I/R) against different antibiotics. Total specimens 73



DA= Clindamycin
E= Erythromycin
NOR= Norfloxacin
SXT= Trimethoprim- sulphamethoxazole
TE= Tetracycline
AM= Ampicillin
P= Benzylpenicillin
TX= Ceftriaxone

No reduced susceptibility was detected against norfloxacin, which is a detector of quinolone resistance. 16 strains (22%) were multi- resistant, that is resistant to three or more antibiotics.

Discussion

Carriage of *s. pneumoniae* has been studied worldwide, but these studies are scarce in low-income countries and are often limited to hospital-based studies. In this study, 187 children, below two years of age with respiratory tract symptoms attending health centres in the Moshi area have been sampled for assessment of nasopharyngeal pneumococcal carriage. The resistance pattern has been investigated and the carriage rate has been correlated to social factors that are known to be risk factors for pneumococcal carriage in children. The children were randomly selected from three different health clinics, meaning that not all children attending the health clinics were sampled for pneumococci, but among those that were asked, only 13 parents/guardians refused to participate. Most of the samples (121) were collected from the health centre, Pasua, compared to the two dispensaries. However, no difference in the carriage rate was detected between the health clinics, suggesting that the carriage rate and resistance pattern found in this study reflects the true carriage and resistance rate in the Moshi region. This is also the first study of its kind since the introduction of the pneumococcal conjugate vaccine, PCV13, in this region.

The nasopharyngeal carriage rate among the children with symptoms of a respiratory tract infection found in this study was 39%. This is less than a study from 2013 conducted at Kilimanjaro Christian Medical Centre, KCMC, which showed a carriage rate of 56% among children born to HIV-positive mothers between 2005-2009 (29), it is also less than the study from Kenya which showed a carriage rate of 66% among healthy children (13), however, it is consistent with the study from Dar es Salaam, which showed a carriage rate of 35% among

healthy children (11). In 2011, a longitudinal study was conducted at a sugar plantation in the Moshi region. Samples were taken from 80 healthy children below five years of age. The children were then followed over a 12 month period. The carriage rate (from naso- or oropharynx) of pneumococci at the start of the study was 29% (30), which is somewhat lower than the carriage rate found in this study. All previous studies from the region were done before the introduction of the pneumococcal conjugate vaccine which could explain why the carriage rate found in this study is lower than in many of the other studies. It could also be explained by the fact that the children in this study were young (median age 8.5 months) and the pneumococcal carriage is known to be higher in older children (10). However, a majority of the pneumococcal strains isolated in this study were from children less than one year of age. The differences could also reflect the fact that the pneumococcal carriage rate varies greatly between regions and it is therefore crucial with local, continuous monitoring.

Almost no relations were found between pneumococcal carriage and social factors or health status of the children which might be due to the small sample size. The only significant relation was found between the predominantly breastfed children under six months and pneumococcal carriage. 30% of the pneumococcal carriers had been predominantly breastfed compared to 66% among the non-carriers (p=0.0093). However, the groups were small, and the statistical comparisons were many, so it cannot be excluded that the statistical significance was found by chance. There was a tendency that recent respiratory tract infection was less common among children that carried pneumococci compared to non-carriers (29% versus 39%, p=0.16); Perhaps the children with a recent respiratory tract infection had been treated with antibiotics which could lower the carriage rate of pneumococci in the nasopharynx, Antibiotic use within the past week also led to a tendency of lower carriage rate; 14% had received antibiotics within the past week in the pneumococcal group compared to 25% in the

non-carriers group, the same tendency was seen if antibiotic treatment had been given between four and 12 weeks ago (23% compared to 32%). This is consistent with other studies that also show lower rates of pneumococcal carriage after antibiotic use (14, 15), however, in this study, these relations were not significant. In the thesis by N. Oriyo, antibiotic use showed no effect on the pneumococcal carriage (30).

A strange association (although not significant) was seen between full PCV 13 vaccination (meaning receiving all three doses of the vaccine) and pneumococcal carriage in this study. 51% of the carriers had received full vaccination compared to 39% of the non-carriers. This is a surprising finding since pneumococcal vaccination is expected to lower the rate of pneumococcal carriage in the child population. Either the study is conducted too soon after the introduction of the vaccine, or the children carry serotypes not included in the 13-valent vaccine. Further analyses of the serotypes of the isolated strains will reveal the serotype distribution in the Moshi children and investigate the covering rate of the vaccine.

The rate of pneumococci with reduced susceptibility against penicillin was high (26%) and high resistance to several other antibiotics was also shown. Almost all of the strains were resistant to co-trimoxazole. In the Dar es Salaam study from 2012; the pneumococci with reduced susceptibility against penicillin were 68% (MIC≥0.064 mg/L). The resistance rate in the Dar es Salaam study is much higher than the 26% found in this study. One could speculate if this difference could be due to an early effect of the PCV 13 vaccination, since the Dar es Salaam study was conducted before the introduction of the vaccine. Or, the difference in the resistance rate could be explained by the fact that Dar es Salaam is a much bigger city with a population of several million citizens compared to the 180,000 in Moshi and antibiotic resistance is known to be a bigger problem in urban settings (18). The antibiotic use is

probably higher and hence resistant strains could be more widely spread in a city like Dar es Salaam than in a smaller town like Moshi. However, for some antibiotics the resistance rates were higher in Moshi compared to Dar es Salaam. The resistance rate against co-trimoxazole, tetracycline and erythromycin in the Dar es Salaam study was 83%, 10% and 6%, respectively, while the rates in this study was 97%, 32% and 15%. The multi-drug resistance in this study was 22% which is similar to the number found in the Dar es Salaam study (17%). Despite the different study populations (healthy children in the Dar es Salaam study and sick non-hospitalized children in this study), these rather large differences tell us how much the antibiotic resistance rates can vary, even within a country and local, frequent monitoring are needed to know which treatment is effective.

It should be noted that none of the strains with reduced susceptibility against penicillin were resistant, all were intermediate. Pneumococci that are intermediately resistant to penicillin are still susceptible to penicillin administered parenterally so called benzylpenicillin or penicillin G. This means that patients with pneumococcal disease can still be treated with penicillin at hospitals where parenteral administration is possible.

In the thesis by N. Oriyo, 88% of the colonized children carried a resistant strain, and 77% carried a multi-resistant strain. 25% of the children carried a strain resistant to penicillin and 74% carried a strain with intermediate resistance. However, the resistance rate was highest against co-trimoxazole (82%) (30). These are higher figures for the pneumococci non susceptible to penicillin, but lower for the co-trimoxazole resistance compared with this study. A study from Gothenburg Sweden from 2012 shows completely different figures. The carriage rate among healthy toddlers was 45%, but the resistance rates were much lower than those found in this study (10). Pneumococcal isolates with reduced susceptibility against

penicillin was only 4%, although the breakpoint used for the pneumococci non-susceptible to penicillin was MIC≥0,125 mg/L, which is a higher limit than used in this study. The resistance rate against co-trimoxazole was 9,8%, but among the clinical isolates the resistance rates were higher (17% against co-trimoxazole and 9,3% against penicillin) (10). The study from Gothenburg shows the importance of not only testing clinical samples, but also testing children at health clinics seeking for all kinds of reasons, which might give a more accurate picture of prevalence of resistant strains in the community. In this study the resistance pattern found among the isolates is likely to reflect the prevalence of resistant *S. pneumoniae* in the Moshi region. The resistance rate is especially high against co-trimoxazole since this has been the cheapest antibiotics available and since it has been used as prophylaxis against opportunistic infections in the HIV-infected children (11, 29). The antibiotic also shares some components with an earlier used medicine for malaria (pyrimethamine-sulphadoxine) (30). These factors together explain the wide-spread resistance against this antibioticum.

In the thesis by N. Oriyo many of the children were treated with antibiotics during the follow up time, often asymptomatically for coughs and running nose. The most common antibioticum prescribed was amoxicillin, followed by erythromycin (30). In this study, the same pattern was noticed. More than half of the children had received antibiotics within the past three months prior to interview and many times the antibiotics was given to treat mild symptoms such as running nose and cough. Surprisingly, among the children that had received antibiotics during the past week, 68% had got it prescribed by a doctor. The most prescribed antibiotics were amoxicillin and ampicillin followed by erythromycin, co-trimoxazole/septrim and penicillin. Fortunately, the resistance rate against ampicillin was low (1,4%) but higher against erythromycin (15%). The high resistance rates found in this study can be explained by the widespread overuse of antibiotics, as compared with the Gothenburg

study in Sweden, where only 25% of the children had received antibiotics within the past six months and hence much lower resistance rates were found (10). Overuse of antibiotics in Moshi may danger the low resistance rates of pneumococci against amoxicillin, the antibioticum that is nowadays the first drug of choice against pneumonia.

The HIV/AIDS epidemic has been especially prevalent in the Sub-Saharan Africa. In Tanzania 6% of adults between 15-49 years old are infected with the Human Immunodeficiency Virus, HIV (17) and almost 15,000 children die each year due to AIDS (25). In this study only four children had unknown HIV-status. The mother was HIV positive and if the virus had been transmitted to the child was currently unknown which meant that the four children were on treatment with ART and had prophylaxis with co-trimoxazole.

A majority (91%) of the children in this study were breastfed, but only 15% of the children less than six months of age were exclusively breastfed; many parents had started giving water, soup or porridge to the children. This is even less than the figures from Tanzania demographic and health survey from 2010 which states that 97% of the children in Tanzania are breastfed, but only half of the children are breastfed exclusively the first six months as recommended by the WHO. The median duration of breastfeeding is 21 months (17). The mothers probably breastfeed their children longer and more often exclusively in the poorer areas of Tanzania. The children in the Moshi area have in general good access to food and the need to breastfeed longer is not as big as in the more rural parts of Tanzania.

Almost half (48%) of the children had at least one previous episode of diarrhea and almost a third (30%) had had malaria. This was not associated with pneumococcal carriage. These factors are not by themselves known risk factors for pneumococcal carriage, but many times

parents treat diarrhea and sometimes even malaria with antibiotics which can have impact on the pneumococcal carriage. It also shows that diarrhea and malaria are common among small children in the Moshi region.

Strengths and weaknesses: One can discuss how representative this study population is for the Moshi area. The children were randomly selected, which means that not all children attending the three health clinics during October and November 2013 were included in the study. The health clinics were open Monday to Friday and most patients attended them during 9am- 2pm. The sample collection was done during that time. A few patients might have come to the clinics before 9am or after 2pm and some of the parents/guardians may not have known about the study and left before they could be asked to participate. However, I do believe that during the days of sample collection, we enrolled most of the children less than two years of age attending the health clinic. In addition, most of the children came for vaccination or growth monitoring and the vaccination coverage in the Moshi region is 94%. I therefore argue that the results in this study are representative for the Moshi region.

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 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 and the material in the sent packages was double tested and found to be of good quality.

The sample size in this study is 187 children. However, together with another student project, the sample size is 337 and further sample collection are planned to be conducted during the following year which will increase the study population further. This may give more accurate figures of the carriage and antibiotics rates, and relations to risk factors not found in this study may be revealed.

All of the 187 e-swabs and the 73 pneumococcal strains found in this study are currently kept in a freezer at KCMC in Moshi. The samples and the e-swabs will be transported cooled to Gothenburg for further analysis, including real time PCR. In that way, other bacteria and viruses could be found and serotyping of the pneumococcus will be performed.

Conclusion

High resistance rates were discovered in this study. Penicillin and co-trimoxazole should not be used as empirical treatment for pneumonia, while amoxicillin and ampicillin can still be the first drugs of choice. Both the carriage and resistance rates will probably be influenced by the PCV 13 vaccination, however the effect of the vaccine is probably too early to be seen. The antibiotic use is excessive and further regulation on a more rational antibiotic use is needed in the Moshi region and may lower the resistance rates.

Populärvetenskaplig sammanfattning

Lunginflammation orsakar globalt sett mer än en miljon dödsfall hos barn under fem år varje år. Den viktigaste bakterien som orsakar sjukdomen är *Streptococcus pneumoniae* (pneumokocken). Bakterien finns i övergången mellan näsa och svalg (nasofarynx) hos barn utan att nödvändigtvis orsaka sjukdom. Det finns ett antal kända riskfaktorer för både

bärarskap av bakterien och för sjukdom, bl.a. låg ålder, rökning, trångboddhet, matlagning inomhus utan skorsten och HIV. *S. pneumoniae* har tidigare behandlats med penicillin, men en alltmer utbredd resistens mot penicillin och andra antibiotika ses över hela världen. Lunginflammation och antibiotikaresistens är särskilt stora problem i låg-inkomstländer. Den här studien syftade till att undersöka hur många barn som bär pneumokocken i nasofarynx och hur vanligt det är med antibiotikaresistens samt om det finns något samband mellan sociala faktorer och bärarskap i Moshi i Tanzania. Studien är aktuell pga. att Tanzania infört vaccination mot pneumokocker i januari 2013.

Den här studien genomfördes på tre olika hälsocentra i Moshi-regionen i Tanzania. 187 barn under två år med minst ett symptom på en övre luftvägsinfektion (hosta, rinnande näsa, förhöjd andningsfrekvens, ansträngd andning eller halsont) och vars föräldrar gick med på att delta, inkluderades i studien. En pinne fördes in i näsan och ett så kallat nasofarynx-prov togs. Ett frågeformulär gavs till föräldrarna med frågor om hemförhållanden och barnens hälsotillstånd. Nasofarynx-pinnen togs med till laboratoriet på sjukhuset Kilimanjaro Christian Medical Centre och odlades ut enligt standard- metoder. De pneumokocker som hittades testades för känslighet mot flera olika antibiotika.

39% av barnen bar pneumokocken i nasofarynx. 26% av pneumokockerna hade nedsatt känslighet mot penicillin och nästan alla pneumokocker var resistenta mot trim-sulfa (septrim). Resistensen var låg mot ampicillin, ceftriaxon och clindamycin. Mot erytromycin och tetracyklin var den 15%, respektive 32%. Nästan inga samband hittades mellan sociala faktorer och bärarskap av pneumokocken.

Bärarskapsfrekvensen i den här studien stämmer överens med andra studier från Moshi-regionen. Resistensen var hög mot flera olika antibiotika och den här studien bekräftar att trim-sulfa och penicillin inte längre ska användas som förstahandsbehandling mot lunginflammation, men att ampicillin (som används mycket i Moshi-regionen) fortfarande kan användas. Fler restriktioner av antibiotika-användningen behövs i Moshi och kan leda till sänkta resistensnivåer i Moshi-regionen. Effekten av den nyligen införda vaccinationen är i nuläget för tidig att se, men man kan förvänta sig en minskad bärarskapsfrekvens och kanske även en minskad antibiotikaresistens.

Acknowledgements

I would like to sincerely thank all persons that took part in this study and to the parents of the children who volunteered to participate in this study! My supervisor Susann Skovbjerg gave me excellent guidance. Translation and assistance at the clinics was given by the community Health nurses Celina Mayo and Bertha Kiwale at Kilimanjaro Christian Medical Centre (KCMC) and the car that took us there was driven by Dawen Kileo. The work at the Clinical laboratory was mainly performed and supervised by Lab-researcher Margaretha L. Sariko together with student Nancy Kassam and was supervised by Dr Balthazar Nyombi, Head of the Clinical Laboratory at KCMC. The clinical part of the study was supervised by Dr Sia Msuya, Assistant Head, Department of Community Medicine at KCMC. Last, but not least I would like to thank my friend and study partner Fredrika Johansson for good collaboration and company.

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Appendices

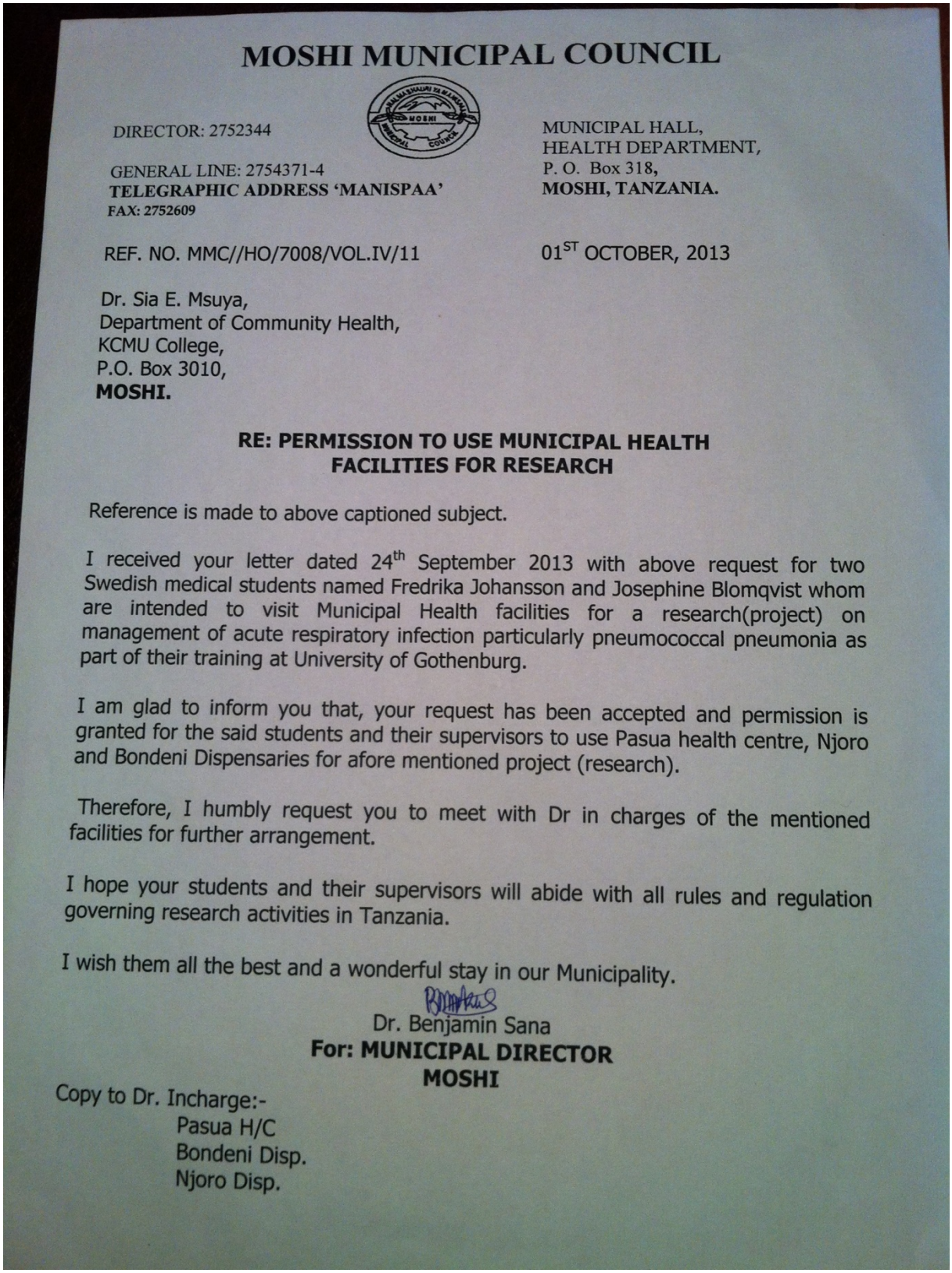
The e-test double testing

Sample number	Penicillin	Ampicillin	Ceftriaxone
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

The disc-diffusion double testing

Sample Number	Optochin	Oxacillin		SXT		E		DA		NOR		TE	
1	25	28,9	S	23	S	28,9	S	23,9	S	20,1	S	32	S
1 new	24,8	28,3	S	23,2	S	29	S	24,5	S	20	S	31,8	S
2	15,3	15,4	R	8	R	12,4	R	22,8	S	19,1	S	19,1	R
2 new	15,1	15,7	R	6	R	14,7	R	22	S	19	S	20	R
5	22	9,2	R	12,3	R	26,2	S	22,1	S	21	S	14,2	R
5 new	21	9,2	R	12,5	R	26,5	S	22,7	S	20,7	S	13,3	R
7	16,6	28,4	S	21	S	27,8	S	28,7	S	17,7	S	33	S
7 new	16,5	28,4	S	23,6	S	27,4	S	28,1	S	18	S	36,3	S
9	14,5	25	S	8,5	R	30	S	26	S	21	S	35,5	S
9 new	15	25	S	8	R	28,5	S	28,5	S	21,5	S	36	S
10	20	26,9	S	6	R	34,5	S	28,6	S	25	S	38,5	S
10 new	20	27,8	S	6	R	34	S	29,2	S	25	S	38,2	S
14	18,1	25,7	S	18,7	S	29,1	S	25,5	S	20	S	33,8	S
14 new	18,1	24,6	S	20,2	S	28,1	S	32,8	S	19,6	S	33,7	S
16	24,8	12,8	R	6	R	31,2	S	29,1	S	19,2	S	32,6	S
16 new	26,1	12,9	R	6	R	30,5	S	28,7	S	25,1	S	33,5	S
28	18,2	23,6	S	6	R	23	S	22	S	17,3	S	38	S
28 new	18,2	23	S	6	R	23,4	S	23	S	17,8	S	38,2	S
30	15	21	S	6	R	30,1	S	25,1	S	18,1	S	33,2	S
30 new	15,1	21	S	6	R	31,7	S	26,1	S	17,9	S	32,7	S

Municipal Council Permission



MOSHI MUNICIPAL COUNCIL

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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.

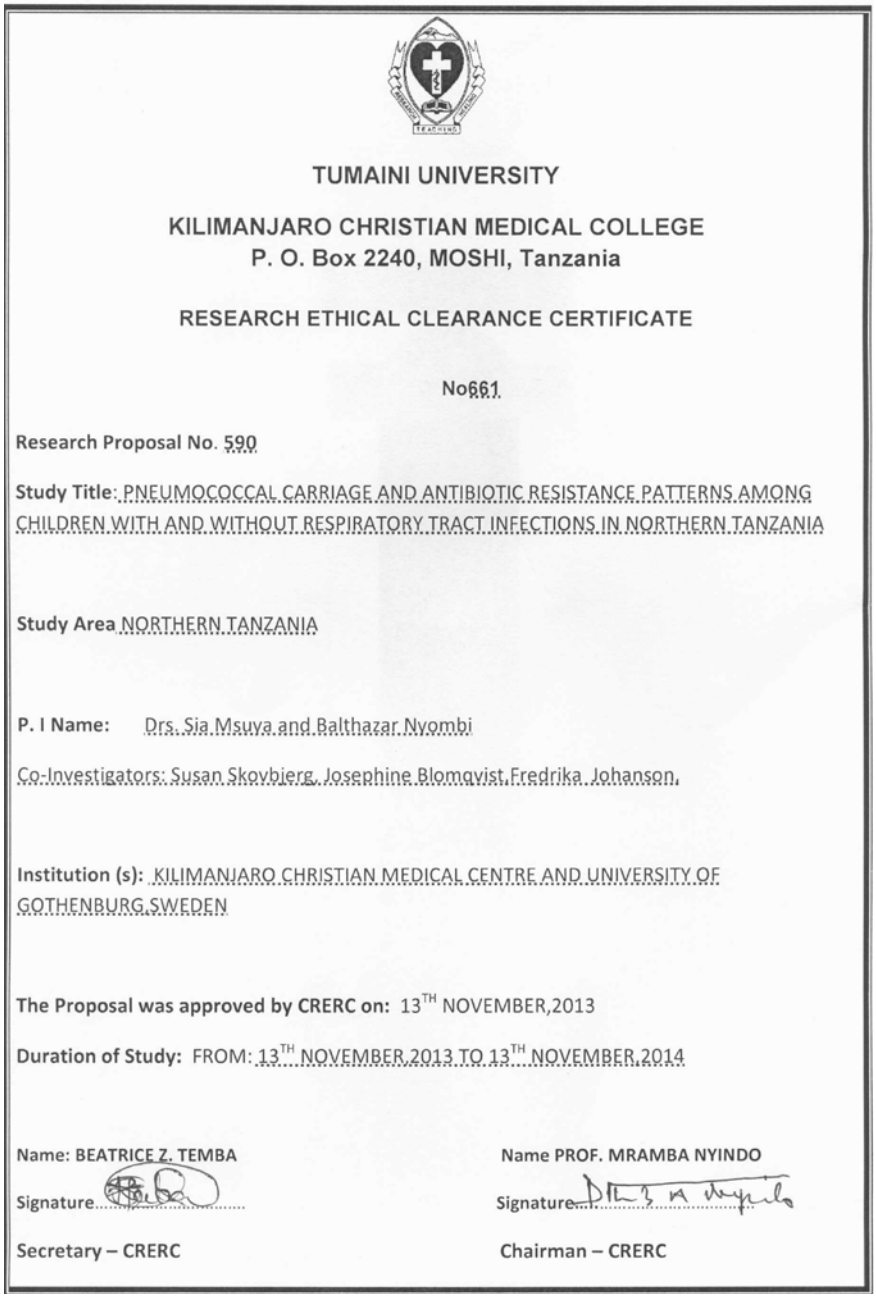
I hope your students and their supervisors will abide with all rules and regulation governing research activities in Tanzania.

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.

Ethical clearance



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

Patient Questionnaire

Patient Questionnaire

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

---

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!