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Factors associated with colonization of *Streptococcus pneumoniae* among under-fives attending clinic in Mwanza City, Tanzania

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Abstract
Background: *Streptococcus pneumoniae* is a known cause of severe invasive bacterial infection leading to morbidity and mortality among children in sub-Saharan Africa. Nasopharyngeal colonization of *S. pneumoniae* is a critical step towards invasive disease progression. The objective of this study was to investigate the magnitude of nasopharyngeal carriage of *S. pneumoniae* and its associated factors in Mwanza, Tanzania.
Methods: Children underfives attending Reproductive and Child Health (RCH) clinics in Mwanza, Tanzania clinics were enrolled and investigated for nasopharyngeal carriage of *S. pneumoniae.* Demographic and clinical data were collected using standardized data collection tool. Nasopharyngeal swabs were taken and processed as per standard laboratory procedures. *S. pneumoniae* isolates were identified using conventional methods. Antimicrobial susceptibility testing was performed using the disc diffusion method as described by Clinical Laboratory Standard Institute.
Results: Among 350 children enrolled in the study, 172 (49.1) were females and 309 (88.3%) were below 2 years of age. A total of 253 (72.3%) children had received at least one dose of pneumococcal vaccine (Prevanar 13) whereas 83 (23.7%) had used antibiotics at median duration of 5 days in the past 14 days. Out of 350 underfives, 43 (12.3%) were found to carry *S. pneumoniae* in their nasopharynx. Children with chronic diseases and those at school were 3.4 and 4.4 times more at risk to be carriers of *S. pneumoniae* than their counterpart group (OR; 3.4 (CI(1.0-11.6) 95%, p=0.05) and OR; 4.4 (CI (1.2-15.7) 95%, p=0.023), respectively. Number of children at home, positive HIV status and someone smoking showed association with *S. pneumoniae* carriage but the differences were not statistically significant. The resistance levels of *S. pneumoniae* to penicillin, co-trimoxazole and erythromycin were 40%, 88.2% and 41.7%, respectively. However all of the *S. pneumoniae* isolates were found to be 100% sensitive to ciprofloxacin.
Conclusion: A high nasopharyngeal carriage of penicillin resistant *S. pneumoniae* is observed in Mwanza, Tanzania despite a good coverage of pneumococcal vaccination. The carriage is significantly associated with schooling and presence chronic diseases. Continuous surveillance of penicillin resistant strains coupled with serotyping of the isolates is highly recommended to determine the influence of the pneumococcal vaccination.

Keywords: *Streptococcus pneumoniae*, penicillin resistant, colonization, children, Tanzania

Introduction

Streptococcus pneumoniae is a leading cause of serious community-acquired infections such as bacteraemia, meningitis and pneumonia in children aged less than 5 years (Jacobs, 2004; O'Brien *et al.*, 2009). It is also the most common cause of sepsis and bacteraemia in patients infected with the human immunodeficiency virus (HIV) (Kinabo *et al.*, 2013). Infections caused by *S. pneumoniae* are among the leading causes of morbidity and mortality among children in developing countries (Falade & Ayede, 2011). In such countries, pneumococci cause significant morbidity and mortality because of the limited access to adequate healthcare and the high

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prevalence of HIV infection (Blossom *et al.*, 2006). Children colonized with *S. pneumoniae* have been described to have threefold higher incidence of hospital admission compared to the non-colonized children (Faden *et al*., 1997).

Despite efforts aimed at reducing child mortality and morbidity in developing countries, a large number of individuals are still suffering from infections caused by these bacteria, especially young children, the elderly or patients with decreased immunity (Bogaert *et al.*, 2004). Studies have shown that *S. pneumoniae* is the most common bacteria isolated from blood and sputum samples of children with severe pneumonia (Nantanda *et al.*, 2008). Pneumococcal disease is commonly preceded by asymptomatic colonization, which is especially high in children (Bogaert *et al.,* 204; Kinabo *et al.*, 2013). In some cases colonization is followed by invasive pneumococcal diseases, and it has been shown that the streptococcal nasopharyngeal carriage prevalence in unvaccinated children is also high in Africa ranging from 7–90% (Joloba *et al.,* 2001a; Batt *et al.,* 2003; Blossom *et al.,* 2006; Dulpl, 2012; Kinabo *et al.*, 2013).

Tanzania introduced the pneumococcal conjugate vaccine (Prevanar 13) in December 2012. The introduction of this vaccine will likely be associated with selection of serotypes not prevented by vaccination; hence there is a need for continuous surveillance to provide susceptibility patterns of these strains. Moreover, antibiotic choices in Tanzania are significantly governed by cost rather than effectiveness and hence there is an urgent need to know the specific trends of antimicrobial resistance in pneumococcal disease in different parts of Tanzania. Since, there has been limited information concerning nasopharyngeal colonization and their antimicrobial susceptibility patterns, this study was carried out to determine magnitude of nasopharyngeal carriage of *S. pneumoniae* and its associated factors in Mwanza, Tanzania.

Material and Methods

Study area and design

This cross-sectional study was conducted at Reproductive and Child Health (RCH) clinics in Mwanza City in north-western Tanzania where underfives are attending for regular child health care and immunizations. The RCH clinics involved in this study were Buzuruga, Nyamagana, Makongoro and Pasiansi. The study was conducted over a 3-month period from October 2013 to February 2014. All children from the communities served by these health facilities, who were attending at RCH clinics for regular child health care and immunization, were involved. Sample size was 350, estimated using Kish Leslie formula for cross-sectional studies (Kish, 1965). The prevalence of infection of 35% (Moyo *et al.,* 2012) was used. The minimum sample size obtained was 350. Children aged 2-59 months who attended RCH clinics were enrolled into the study serially until the sample size was reached. All children who were sick but qualified for admission were excluded.

Data collection

Demographic and clinical data were collected using standardized data collection tool by a clinician. The data included general examination of the child, respiratory and cardiovascular systems of the child. Other clinical data included immunization status, HIV status and presence of other chronic conditions. Nutritional status was assessed using WHO criteria for the classification of malnutrition (Chen *et al.*, 1980). HIV results were collected from the RCH cards, those who didn't have the HIV results were sent for Provider Initiated Testing and Counselling.

A nasopharyngeal specimen (one from each child) was collected. To obtain the specimen the patient's head was tipped slightly backward and rayon- tipped flexible aluminium-shaft swab (Medical Wire and Equipment Company, Town, UK) passed directly backwards, parallel to the floor of the nasopharynx. Once the swab was in place, it was rotated 180 degrees or left it in place for 3 – 5 seconds to saturate the tip before being slowly removed (O'Brien *et al.*, 2003).

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Swabs were immediately placed in Stuart Transport Media and transported within two hours to the laboratory where microbiological tests were conducted.

Laboratory procedures

Fresh specimens were inoculated directly onto 5 – 10% sheep blood agar and chocolate agar under 5% CO_2. Isolates were identified as *S. pneumoniae* by colony morphology, α-haemolysis, bile solubility and susceptibility to optochin. Confirmed isolates were subjected to antimicrobial susceptibility testing as recommended by Clinical and Laboratory Standard Institute (CLSI) Guidelines 2012 on Muller Hinton Agar (HIMEDIA) supplemented with 5% sheep blood (Wikler, 2007). The following antibiotics discs were used for susceptibility tests:-Penicillin 10IU, ampicillin 10µgm, amoxicillin/clavulanic acid 20/10 µgm, co-trimoxazole 1.25/23.75 µgm, erythromycin 15 µgm ciprofloxacin 5 µgm (Oxoid UK). Briefly colonies of *S. pneumoniae* were picked using a sterile wire loop and emulsified into sterile 5ml of normal saline to obtain turbidity equivalent of 0.5 McFarland standard. Using a sterile non toxic cotton swab the inoculums were plated uniformly on agar plates as previously described (O'Brien *et al.*, 2003). The interpretation of the zone diameters were done based on CLSI guideline (Cockerill *et al*., 2012).

For quality control, *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25923 were used as positive and negative control strains, respectively. Each batch of newly prepared media was tested for sterility and performance tests. Optochin discs were tested with positive and negative controls.

Data analysis

Data were recorded and coded in the questionnaire and entered in the Microsoft Excel, then analysed by using STATA version 13 (Texas, USA). Continuous variables were summarized using mean with standard deviation (SD) or median and interquartile range (IQR) depending on their distribution. Categorical variables were summarized using percentage. Univariate and multivariate logistic regression analysis were used to determine predictors of nasopharyngeal colonization among underfives. Odds ratio (OR) and the respective 95% confidence interval (CI) were computed. Predictors without collinearity and with a p-value of less than 0.05 on univariate analysis were subjected to multivariate analysis. Predictors with a p-value of <0.05 on multivariate analysis were considered statistically significant.

The study variables were dependent variables (nasopharyngeal *S. pneumonia* carriage status, antimicrobial susceptibility pattern) and independent variables (age, recent use of antibiotics, children being exposed to passive smoking in the house, number of family members in the household, children whose caregivers had respiratory symptoms in the last week, children currently attending school, children with a respiratory infection in the past week, signs of malnutrition, HIV serostatus and children who are on anti-retroviral medication (ARV)).

Ethical considerations

The study was approved by Catholic University of Health and Allied Sciences/Bugando Medical Centre Ethics Review Board. The consent was obtained from guardian or parent of respective child.

Results

Study enrolment

A total number of 1,802 children were screened for enrolment in the study. Among these patients, 953 children were excluded, as they were below 2 months of age. A total of 849 patients were eligible but only 350 children were randomly selected. Patients were recruited

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patients from Makongoro (n=101; 28.9%), Nyamagana (n=89; 25.4%), Buzuruga (n=111; 31.7%) and Pasiansi (n=49; 14.0%).

Baseline characteristics of patients enrolled in the study

Among 350 children enrolled in the study, 172 (49.1) were female. Of 350 children; 309 (88.3%) were below 2 years of age. Out of 350 children 11 (3.1%) children were attending school and 13 (3.7%) children were found to have chronic disease, including 6 children with sickle cell disease (SCD), 1 with chronic heart disease(CHD), 3 with adenoids, 2 with epilepsy and one with tuberculosis. Of 350 children; 11 (3.14%) children were born from mothers with positive HIV status (HIV exposed). However, overall 6 (1.7%) of children were HIV positive (Table 1). A total of 253 (72.3%) children had received at least one dose of pneumococcal vaccine (Prevanar 13) whereas 83 (23.7%) had used antibiotics at median duration of 5 days in the past 14 days (Table 1).

Table 1: Baseline characteristics of the 350 underfives attending 4 RCH clinics in Mwanza city

Characteristic	Category	Frequency	%
Sex	Female	172	49.1
Age	Below 2 yrs	309	88.3
	2 yrs and above	41	11.7
Health facility	Makongoro	111	31.7
	Buzuruga	101	28.9
	Pasiansi	49	14.0
	Nyamagana	89	25.4
School enrolment	In school	11	3.1
	Not in school	339	96.9
Caretaker URTI symptoms *	Present	109	31.1
	Not present	241	68.9
Immunization status	Complete	324	92.6
	Incomplete	26	7.4
Pneumococcal vaccination status	Not vaccinated	97	27.7
	Vaccinated	253	72.3
HIV status	Positive	6	1.7
	Negative	342	98.3
Chronic disease	Not present	337	96.3
	Present	13	3.7
Mother's education level	No school	6	1.7
	Primary	255	72.9
	Secondary	87	24.9
	Post secondary	2	0.6

*URTI: Upper respiratory tract infections

Clinical characteristics

Of 350 children; 175(50%) had cough and only 9 (2.6%) presented with difficulty in breathing. A total of 83 (23.7%) of children used antibiotics with median duration of 5 days and 253 (72.3%) had received one dose or more of pneumococcal vaccine. (Table 2)

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Table 2: Clinical characteristics of the 350 underfives attending 4 RCH clinics in Mwanza city

Patient's characteristic	Frequency	Percentage
Cough	175	50.0
Sneezing	68	19.4
Running nose	147	42.0
Difficulty in breathing	9	2.6
Respiratory infection		
None	106	30.3
Pneumonia	9	2.6
URTI	235	67.2
Antibiotics	83	23.7
Days of antibiotics	5	[4-7]*
Co-trimoxazole prophylaxis	10	2.9
Streptococcal l vaccine	253	72.3
No. of vaccine		
No vaccine	97	28.0
One	42	12.0
Two	44	12.6
Three	166	47.4
No. of children to take care	2	(1-3)*

*Represent Range not percentage

Nasopharyngeal carriage of S. pneumoniae and susceptibility pattern

Out of 350 underfives; 43 (12.3%) were found to carry *S. pneumoniae* in their nasopharynx. Among 43 children whom were colonized 32 (74%) received pneumococcal vaccine; where 8 children received one dose, 6 children received two doses and 18 children received three doses of PCV13 respectively. The resistance levels of *S. pneumoniae* to penicillin, trimethoprim-sulphamethaxazole, erythromycin and ciprofloxacin were 40%, 88.2%, 41.7% and 0.0% respectively (Table 3).

Table 3: Susceptibility pattern of 43 *S. pneumoniae* isolates from 350 underfives

Antibiotics	Sensitivity	Number	Percentage (%)
Penicillin	Sensitive	26	60.00
	Resistance	17	40.00
Erythromycin	Sensitive	25	58.33
	Resistant	18	41.67
Ciprofloxacin	Sensitive	43	100
	Resistant	-	-
Co-trimoxazole	Sensitive	5	11.76
	Resistant	38	88.37
Amoxicillin/Clavulanic acid	Sensitive	40	93.02
	Resistant	3	6.8
Gentamicin	Sensitive	35	81.4
	Resistant	8	19.60

***Risk factors for nasopharyngeal carriage of* S. pneumoniae**

Children with chronic diseases and those at school were 3.4 and 4.4 times more at risk to be carriers of *S. pneumoniae* than their counterpart group (OR; 3.4; (95% CI[1.0-11.6] p value-0.05) and (OR; 4.4; (95% CI [1.2-15.7] pvalue-0.023), respectively. Number of children at home, positive HIV status and someone smoking showed an association with *S. pneumoniae* carriage but the differences were not statistically significant (Table 4). In multivariate analysis, clinical features of the patients like sneezing, increased body temperature and increased respiratory rate according to age showed statistically association with nasopharyngeal colonization (Table 4).

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Table 4: Factors associated with nasopharyngeal colonization

Patient's characteristic	Response	*S. pneumoniae*		Univariate		Multivariate	
		Yes N (%)	No N (%)	OR [95% CI]	p-value	OR [95% CI]	p-value
Age	≥ 2 years	5(12.20)	36(87.8)	1		-	-
	< 2 years	38 (12.3)	271(87.7)	1.0 [0.4 - 2.7]	0.985		
Sex	Male	21 (11.8)	157 (88.2)	1			
	Female	22 (12.8)	150 (87.2)	1.1 [0.6 - 2.1]	0.777	1.2 [0.6-2.3]	0.635
Chronic disease	No	39 (11.6)	298 (88.4)	1			
	Yes	4 (30.8)	9 (69.2)	3.4 [1.0-11.6]	0.050	-	
HIV Status	Negative	42 (12.2)	302 (87.8)	1			
	Positive	1 (16.7)	5 (83.3)	1.4 [0.2-12.6]	0.743	-	-
No. of people in house	≤3 people	10 (11.00)	81 (89.0)	1			-
	>3 peoples	33 (12.7)	226 (87.3)	1.2 [0.6-2.5]	0.662	-	-
Schooling	No	39 (11.5)	300 (88.5)	1		-	-
	Yes	4 (36.4)	7 (63.6)	4.4 [1.2-15.7]	0.023	5.6[1.4-22.0]	0.014
Smoking in the house	No	34 (11.3)	266 (88.7)	1			
	Yes	9 (18.0)	41 (82.0)	1.7 [0.8-3.8]	0.188	-	-
Pneumococcal vaccine	Yes	32 (12.7)	221 (87.4)	1			
	No	11 (11.3)	86 (88.7)	0.9 [0.5-2.3]	0.739	-	-
Body temperature	< 37.5°C	33 (9.8)	304 (90.2)	1		-	-
	≥ 37.5°C	10 (76.9)	3 (23.08)	30.7[8.0-17.2]	<0.001	2.1 [1.1-3.5]	0.024
DIB	No	37 (10.85)	304 (89.2)	1			
	Yes	6 (66.7)	3 (33.3)	16.4[3.9-8.3]	<0.001	-	-
Sneezing	No	29 (10.28)	253 (89.7)	1			
	Yes	14 (20.60)	54 (79.4)	2.3 [1.1-4.6]	0.023	2.3 [1.1-5.0]	0.029
Respiratory rate		42 [38-48]	36 [28-42]	1.06[1.03-1.10]	<0.001	1.1 [1.03-1.1]	0.003

In performing multivariate analysis, a risk factor of chronic diseases and difficulty in breathing were not subjected into multivariate analysis despite having a p-value of < 0.05 in the univariate analysis because of collinearity with respiratory rate.

Discussion

In this study *S. pneumoniae* colonization prevalence was found to be 12.3% , which is almost similar to the one reported in northern part of Tanzania (Batt *et al*., 2003). However lower than 35% reported in Dar es Salaam (Moyo *et al.*, 2012). This difference may be due to geographical variation (Chiu *et al.,* 2001) and low population density in Mwanza City compared to Dar es Salaam City. Other reasons are likely to include timing of the study and the fact that our study was conducted in the vaccination era. Studies have shown that overcrowding increases the rate of *S. pneumoniae* colonization (Mercat *et al*., 1991; Obando *et al*., 2011) and this is due to high rate of transmission as a result of close contact (Musher, 2003). Higher prevalence rates of *S. pneumoniae* have been reported in neighbouring countries of Uganda (62%) (Joloba *et al*., 2001a) and Kenya (65%) (Abdullahi *et al.*, 2012). The observed difference of the carrier state in different countries could be due to wide geographical and climate variation (Appelbaum, 1992).

In our study, nasopharyngeal colonization did not differ significantly among age groups which is similar to another study in the USA (Guillemot *et al.*, 1998). This observation could be explained by the fact that most of the children in our study had been recently covered with pneumococcal vaccine. The prevalence of colonization tends to decrease with age (Abdullahi *et al*., 2012) because of formation of antibodies against various serotypes as age increases. It has been considered that the immune response to pneumococci in naturally exposed and non-vaccinated children depends on antibody against the polysaccharide capsule. This may verify that immunization with polysaccharide conjugate vaccines generates serotype specific antibody response. In the current study there was no age influence because majority of study participants

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were below 2 years, of whom 80.6% had received pneumococcal vaccine. In most studies age range of children were wide, and there was no vaccination influence (Talarico, 2009).

In this study, those children attending school were 5.6 times more likely to be colonized than those who did not attend school by multivariate analysis. This finding is similar to findings of a recent study in Japan (Otsuka *et al*., 2013). This can be explained by the fact that, children who are attending day care centres or school are having contact with other children of different age and of different risk factors and hence easy to acquire *S. pneumoniae* colonization comparing to children who are staying at home (Doyle *et al.*, 1992; Givon-Lavi *et al.*, 2002). As observed in previous studies (Faden *et al.*, 1997; Pettigrew *et al.*, 2008; Abdullahi *et al.*, 2012) presence of the symptoms of upper respiratory tract infection (URTI) including sneezing, raised temperature and increased respiratory rate showed statistical association with *S. pneumoniae* in the present study. URTI symptoms may be enhancing adherence and growth of *S. pneumoniae*, or the increased secretion may augment the swabbing procedures(Tigoi *et al.*, 2012). More than 70% URTI of the children are caused by viral infection and 19% are due to other organisms (Plotkowski *et al.*, 1986; D'Acremont *et al.*, 2014). The presence of viral infection in respiratory system has been described to enhance *S. pneumoniae* colonization and augment invasiveness (Plotkowski *et al*., 1986)

In the current study, chronic diseases showed statistical association with *S. pneumoniae* carriage in univariate analysis. There is a risk of invasive infection due to *S. pneumoniae* among children with chronic diseases because apart from colonization they have decreased immunity due to chronic diseases. We observed that vaccination has no influence on the carriage, similar to another study in Mexico (Espinosa-de los Monteros *et al*., 2010). This observation could be due to the possibility of selection of serotypes which are not covered by the vaccine (Dagan, 2009). Serotyping could not be done in this study and hence difficult to conclude that high percentage of carriage in the vaccinated children could be due to replacement of the serotypes not covered with vaccine.

As observed in Uganda (Joloba *et al.*, 2001b) high carriage of penicillin resistant pneumococci strains was observed. However, the resistance rate of 40% is significantly lower than the recent findings in Dar es Salaam whereby 67.8% of *S. pneumoniae* strains were resistant to penicillin (Moyo *et al*., 2012). Co-trimoxazole resistance has been reported in different part of the world. In the current study we observed resistance of 88.2%, which is similar to findings reported elsewhere in Tanzania (Moyo *et al.*, 2012), and in the neighbouring Uganda (Joloba *et al.*, 2001a). This can be due to high usage of this drug worldwide and especially to the HIV patients as prophylaxis against opportunistic infections. In Tanzania co-trimoxazole is recommended antimicrobial for urinary tract infections and URTI among children; likely to contribute to its overuse. There is a need to review antimicrobial policy in the country so as to reduce morbidity and mortality associated with the use of non-susceptible antimicrobials.

Wide range of macrolides resistance has been observed in different part of the world, and this is due to increasing rate of their usage in management of respiratory tract infections (Čižman et *al*., 2001). In the current study we observed resistance of 41. 7% to macrolides, which is high but similar to the study in Hungary (Dobay *et al.*, 2003). Elsewhere in Tanzania lower resistant rates have been reported (Batt *et al*., 2003; Moyo *et al.*, 2012). Pneumococcal resistance due to ribosomal mutations has been discovered recently contributing much to the rising macrolides resistance. Quinolones resistance level is low in different parts of the world (Dobay *et al.*, 2003) and this was confirmed also in the current study. This shows that treating *S. pneumoniae* infections using quinolones may be associated with favourable outcome. However, the usage of quinolones remains controversial because of lacking clinical studies, reports of clinical failures and increase of side effects.

Failure to serotyping these isolates was major limitation of the current study; however the magnitude and susceptibility pattern of *S. pneumoniae* isolates were established.

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In conclusion, 12% of underfives are colonized with *S. pneumoniae*, despite good pneumococcal vaccine coverage. The carriage is independently predicted by schooling, chronic diseases and increased respiratory rate, difficulty in breathing and sneezing. In addition a high nasopharyngeal carriage of penicillin resistant *S. pneumoniae* is observed in Mwanza City. Culture and sensitivity are important investigations to every child suspected to have *S. pneumoniae* infection so as to identify the appropriate antibiotic to the individual child.

Competing interest

The authors declare that they have no competing interests

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