Streptococcus pneumoniae Colonization among Children in Galle, Sri Lanka: A Cross-Sectional Study

by

Chi Zhang

Duke Global Health Institute
Duke University

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Approved:

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Gayani Tillekeratne, Advisor

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Gaya Wijayaratne

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Chris Woods

_____
Larry Park

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Joy Noel Baumgartner

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

Background: Streptococcus pneumoniae, a Gram-positive bacterium that is found in the human respiratory tract, is the most common cause of bacterial pneumonia globally. Pneumococcal pneumonia can be effectively prevented by administering pneumococcal vaccines but pneumococcal vaccination is not provided through the public healthcare sector in Sri Lanka at present. This study will serve as evidence instructing future decisions regarding the utility of vaccination.

Methods: A cross-sectional survey was conducted in Galle, Sri Lanka from July to September 2019 . Eleven Medical Officer of Health (MOH) clinics, which provide routine vaccinations to infants and children through the public health sector, were selected as the study setting. The parents of consecutive children ≤5 years of age were approached for consent. A nasopharyngeal sample was collected from each enrolled child and socio-demographic and clinical data were obtained by interviewing the parents. Routine microbiological testing was conducted to confirm the presence of S. pneumoniae isolates. Antibiotic susceptibility testing was performed on confirmed isolates using Kirby-Bauer disc diffusion. Sociodemographic and clinical characteristics associated with S. pneumoniae colonization were assessed using bivariable and multivariable logistic regression in R.

Results: Among 123 enrolled patients, 26 (21.1%) were found to be colonized with S. pneumoniae. Higher risk of S. pneumoniae colonization was found to be associated with living with other children <5 years (Unadjusted OR=4.58, 95%CI: 1.69-12.83); Adjusted OR=3.99, 95%CI: 1.19-13.39) in both bivariate and multivariate analysis. With age >2 years was found to be associated with lower risk of being infected (Unadjusted OR=0.19, 95%CI: 0.02-0.84) in bivariate analysis, and drinking boiled water was found protective to the carriage than no treatment (Adjusted OR=0.11, 95%CI: 0.02-0.65) in multivariate analysis. For antibiotic resistance, the non-susceptible prevalence was 94.4% to oxacillin/penicillin, 72.2% to erythromycin, and 44.4% to clindamycin. All isolates were susceptible to levofloxacin.

Discussion: This is the first report of S. pneumoniae colonization prevalence among children in Southern Province, Sri Lanka. One-fifth of children were found to be colonized with S. pneumoniae, and oxacillin non-susceptibility prevalence was high. Further characterization must be performed to identify serotypes of colonizing strains and to correlate with serotypes present in available vaccines. Our results provide evidence regarding burden of pneumococcal colonization in Sri Lanka, and may help guide pneumococcal vaccine decisions in Sri Lanka.

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1. Introduction

Pneumonia is the leading infectious cause of death among children under 5 years of age globally. In 2017, pneumonia caused 808,694 pediatric deaths, accounting for 15% of all deaths in children under 5 years worldwide (WHO, 2019). Streptococcus pneumoniae, a Gram-positive bacterium that is found in the human respiratory tract, is the most common cause of bacterial pneumonia. In 2016, S. pneumoniae was estimated to cause 341,029 deaths among children under five years old worldwide, accounting for 52% of all pediatric deaths (Troeger, 2018).

Pneumococcal pneumonia can be effectively prevented by administering pneumococcal vaccines, which cover between 7 and 13 serotypes associated with invasive disease, to children, There are more than 90 known serotypes of S. pneumoniae, with the distribution of serotypes varying by age, time, and geography (WHO, 2019). The pneumococcal conjugate vaccine covering seven pneumococcal serotypes (PCV7) was introduced in 2000 and the universal use of PCV7 contributed to a 39% decrease in hospitalization for pneumonia among children under 2 years old (CDC, 2008). Now, PCV10 (containing serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) and PCV13 (additionally containing serotypes 3, 6A, and 19A) have been available on the market, replacing PCV7. WHO recommends PCVs to be included into the childhood immunization programs worldwide.

However, In Sri Lanka, a low-or-middle-income country (LMIC), pneumococcal vaccination is not provided through the public healthcare sector. Limited data exist regarding the colonization prevalence with S. pneumoniae in healthy children and the circulating serotypes. In one study of 11 Asian and Middle Eastern countries conducted through the Asian Network for Surveillance of Resistant Pathogens (ANSORP), Sri Lankan children were found to have a S. pneumoniae carriage prevalence of 23.9%, with serotypes 19 and 23 being the most common (Lee, 2001). However, this study was hospital-based and the data were from 2001. In another study at the Lady Ridgeway Hospital in Colombo, the largest children's hospital in Sri Lanka, 23 isolates of S. pneumoniae from clinical cultures were obtained in total. Serotypes 19F, 14, 23F, and 6B were the most common among the isolates (Batuwanthudawe, 2009). However, this study was conducted between 2005 to 2007 and such distribution may have changed throughout years. Moreover, a worrisome rise in antibiotic resistance among S. pneumoniae isolates has also been noted in Sri Lanka. The ANSORP studies have observed an increase in penicillin nonsusceptibility in Sri Lanka from 41.2% in 1998-1999 to 76.5% in 2000-2001 (Song, 1999; Lee, 2001); more recent data are limited .

In order to add to the body of evidence regarding pneumococcal colonization burden and help inform future decisions regarding the utility of vaccination, we explored the proportion of healthy children colonized by S. pneumoniae in a community setting in Galle, Sri Lanka.

2. Methods

2.1 Study area and clinics

Galle, a major city in southwestern Sri Lanka that lies 119 km from the capital Colombo, is the administrative capital of both Galle District and the Southern Province. Galle is further subdivided into administrative units including 19 Assistant Government Agents (AGA) divisions with 2423 villages in total. With a population of over 1 million, nearly 90% of Galle's citizens live in rural areas.

Among the 19 AGA divisions, one urban division (Galle Four Gravets) and one rural division (Bope-Poddala) were selected as the study sites due to proximity to the study laboratory. Galle Four Gravets, with a population over 100,000, has both urban and rural sectors but more than 85% of people live in urban sectors. Bope-Poddala, which has half the population of Galle Four Gravets, is a purely rural division and is also the location of the study laboratory.

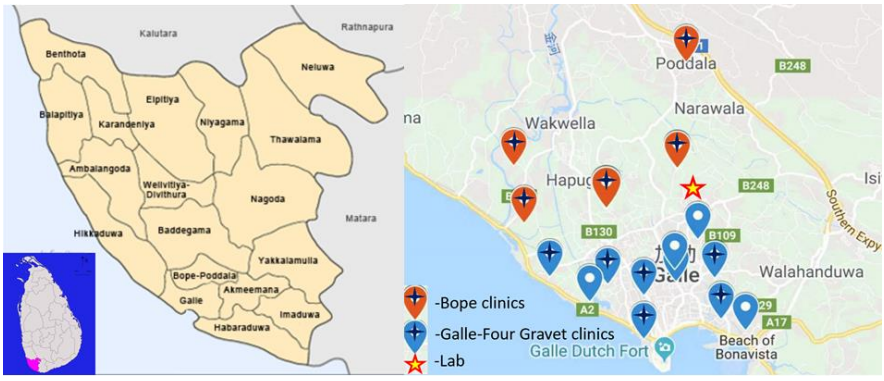

Figure 1. Location of Galle District (left) and locations of clinics (right)

Medical Officer of Health (MOH) clinics are facilities that provide free public health services including standard immunizations to children and pregnant women in Sri Lanka. There were eleven MOH clinics in the selected urban division and five MOH clinics in the selected rural division during the study period.

Through the MOH clinics, infants receive the five doses of oral poliovirus vaccines & Pentavalent (diphtheria and tetanus toxoid with pertussis containing vaccine- hepatitis B- Hemophilus influenzae type b) vaccines at the age of 2 months, 4 months 6 months, 1.5 years, and 5 years. In addition, children receive two doses of measles, mumps, and rubella vaccine at the age of 9 months and 3 years (MOH, 2017). Vaccination for nine diseases are provided free of charge and it is estimated by WHO and UNICEF that at least 96% of children in Sri Lanka have been covered by the national immunization program in 2018 regarding each of these nine vaccines (WHO %UNICEF, 2018). Vaccinations are administered through each MOH Clinic one to two days each month. All the clinics within the same AGA division are operated by the same group of doctors and midwives and they provide immunization services in different clinics as scheduled.

2.2 Data collection

A cross-sectional survey was conducted from July to September 2019. All five clinics in the rural area and six of eleven clinics in the urban area were selected according to clinic schedules (Figure 1). Clinics were visited by a trained study doctor

and study research assistant during the clinic hours (generally 10am to 1pm on scheduled days). All children five years of age or less were eligible to participate unless they had ongoing or recent (within 14 days) pneumococcal diseases including pneumonia, bacteremia, and meningitis; hospitalization or antibiotic treatment within the past 14 days; or their parents refused participation. On average, the team was able to enroll ten children during a 3-hour window during each visit.

A questionnaire was administered to the parents of enrolled children to obtain information regarding the family background, environmental risk factors and the medical history of the child. One nasopharyngeal (NP) swab specimen was obtained from each child following the questionnaire. Each NP specimen was collected using a sterile nylon flexible minitip flocked swab and was immediately placed into a 2-ml cryovial containing 1 ml Skim-milk tryptone glucose glycerol (STGG) transport media. The NP samples were transported to the research laboratory at room temperature on the same day as collected and immediately stored at -80°C (Lee, 2001).

2.3 Culture and antimicrobial susceptibility testing

The frozen NP-STGG was thawed at room temperature and vortexed for 20 seconds, and then 200 μl of the specimen was transferred to an enrichment broth composed of 5-ml Todd Hewitt broth containing 0.5% yeast extract (THY) with 1 ml rabbit serum. After 6 hours incubation at 37°C in a candle jar, one loop (approximately 10 μl) of the THY enriched culture was plated on a blood agar plate (BAP) with 5%

sheep blood (Remel, Lenexa, US). One milliliter of the THY enriched culture was transferred into a screw-cap 2ml cryovial and stored at -80°C for pneumococcal serotyping at a later date. The BAPs with 5% sheep blood were incubated for 24 hours at 37°C in a candle jar.

After 24 hours incubation, colonies suspected to be S. pneumoniae colonies based on color and morphology were plated onto a new BAP with human blood and incubated at 37°C in a candle jar for 24 hours. An optochin disk was put in the center of the plating area and S. pneumoniae was identified isolates with a halo diameter >14 mm after incubation, as per the disk diffusion method.

One loop of the identified S. pneumoniae isolate was plated on a new BAP with human blood for antimicrobial susceptibility testing. Susceptibility to oxacillin (substituted for penicillin), erythromycin, clindamycin, levofloxacin, tetracycline and vancomycin was identified using the disk diffusion method. Isolates were categorized as susceptible, intermediate, or resistant to antimicrobials according to standard Clinical & Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013).

2.4 Statistical analysis

At the time of study design, data regarding community prevalence of S. pneumoniae carriage among healthy children in Sri Lanka were not available from sample size calculation; estimates from developed countries may not be representative. To guarantee an adequate sample size, an estimated prevalence of 50% was used for

sample size calculation. With 95% confidence level and 0.1 margin of error, the sample size was calculated as 97. Descriptive analyses were performed to identify the prevalence of colonization and prevalence of antibiotic resistance. Fisher's exact test for univariate analysis and logistic regression models for multivariate analysis were used to explore the association between risk factors and S. pneumoniae carriage using R. Fisher's exact test was used for the univariable analysis (unadjusted). Risk factors were selected into a logistic regression model (adjusted) using the "backward selection" method to better estimate the risk of colonization by controlling the potential confounding effects by different variables.

2.5 Ethical approval

Ethical approval were obtained from the Ruhuna University Ethical Review Committee (Sri Lanka) and the Duke University Duke Health Institutional Review Board (US).

3. Results

Study cohort and sociodemographic characteristics

In total, 126 children were enrolled in the study. The parents of two children refused to continue sample collection after finishing the questionnaire and the NP sample from one child could not be collected; the data for these three children were excluded from further analyses. Among the 123 children, median age was 12 months (IQR 6-34) and 42.3% were male (Table 1). Of enrolled children, 85 (69.1%) were enrolled from urban areas and 31 (25.2%) were enrolled from rural areas. Of note, for the purpose of these analyses, 26 children from two clinics in the rural district were re-categorized as being from urban regions because they geographically locate in urban areas. Most families had a monthly income of 30,001-45,000 Rs (157-235 USD; average monthly income 16377 Rs in Sri Lanka 2016). The minority of families reported smokers at home (35, 28.5%) or the use of indoor stoves (35, 28.5%).

Table 1. Sociodemographic characteristics of healthy children ≤5 years of age enrolled in southern Sri Lanka, 2019

Sociodemographic characteristic	N (total=123)	Median (IQR) or %
Age (months)	123	12 (6, 34)
Sex		
Male	71	57.7%
Female	52	42.3%
Height (cm)	99	71 (64,82)
Weight (kg)	113	8.4 (6.6,10.3)
Residence		
Urban	85	69.1%
Rural	31	25.2%
Unspecified	7	5.7%
Child's education		
Montessori	10	8.1%
No	113	91.9%
Mother's age (years)	123	32 (28,36)
Father's age (years)	121	36 (31,40)
Mother's education		
<O/L (Original level)	18	14.6%
O/L	38	30.9%
A/L (Advanced level)	55	44.7%
>A/L (diploma, graduate)	12	9.8%
Father's education		
<O/L	20	16.3%
O/L	55	44.7%
A/L	35	28.5%
>A/L (diploma, graduate)	11	8.9%
Missing	2	1.6%
Family monthly Income		
<15,000 Rs	1	0.8%
15,001- 30,000 Rs	18	14.9%
30,001- 45,000 Rs	47	38.8%
>45,000 Rs	57	47.1%
Breastfeeding status		
Ongoing	24	19.5%
Weaned	99	80.5%
Smokers at home		
Yes	35	28.5%
No	88	71.5%
Bathing frequency/week		
0-3	45	36.6%
4-7	77	62.6%
Missing	1	0.8%
Toilet		
Private household toilet	118	95.9%
Public toilet	2	1.6%
Missing	3	2.4%
Exposure to pets		
Yes	34	27.6%
No	89	72.4%
Use of indoor stove		
Yes	35	28.5%
No	83	70.3%
Missing	5	4.2%
Colonized		
Yes	26	21.1%
No	97	78.9%

Prevalence of S. pneumoniae colonization and risk factors for colonization

Among 123 children, 26 (21.1%) were found to be colonized by S. pneumoniae. Details are presented in Table 2.

Table 2. Colonization and risk factors among healthy children ≤5 years of age enrolled in southern Sri Lanka, 2019

Variable/risk factor	Category	Colonized	Free	Colonized percentage	Unadjusted OR (95% CI) FISHER's	P value	Adjusted OR (95% CI)	p valu
Gender	Boy	13	58	*18.3%*	ref		ref	
	Girl	13	39	*25.0%*	1.48 (0.57, 3.89)	0.381	1.30 (0.23, 7.33)	0.39
Age	<=2 year	24	67	*26.4%*	ref		ref	
	> 2 years	2	30	*6.3%*	**0.19 (0.02 , 0.84)**	**0.022**	0.13 (0.01, 1.36)	0.08
Residence	Urban	21	64	*24.7%*	ref		ref	
	Rural	4	27	*12.9%*	0.45 (0.10 , 1.53)		0.65 (0.13, 3.22)	0.59
	Unspecified	1	6	*14.3%*	0.51 (0.01 , 4.60)	0.439	1.89 (0.10, 37.18)	0.67
Education	Montessori	1	9	*10.0%*	ref			
	Daycare		0	*NA*			NA	0.93
	Kindergarten		0	*NA*				
	No	25	88	*22.1%*	2.54 (0.32, 116.4)	0.687		
Household income	<=45,000 Rs	15	51	*22.7%*	ref		ref	
	>45,000 Rs	11	46	*19.3%*	0.81 (0.30, 2.12)	0.665	1.20 (0.36, 3.97)	0.76
Breastfeeding status	Ongoing	24	75	*24.2%*	ref		ref	
	Weaned	2	22	*8.3%*	0.29 (0.03, 1.31)	0.101	0.50 (0.05, 5.17)	0.56
Living with children <5 years of age	No	11	75	*12.8%*	ref		ref	
	Yes	15	22	*40.5%*	**4.58 (1.69, 12.83)**	**0.001**	**3.99 (1.19, 13.39)**	**0.02**
Toilet	Private household toilet	25	93	*21.2%*	ref		NA	0.99
	Public toilet	1	1	*50.0%*	3.66 (0.05, 294.32)	0.388		
Exposure to smoking at home	Yes	7	26	*21.2%*	1.01 (0.32, 2.88)		1.46 (0.36, 5.91)	
	No	19	71	*21.1%*	ref	1.000	ref	0.59
Drinking water source	None/ breastfeeding only	4	20	*16.7%*	0.70 (0.15, 2.49)			0.07
	Tap	18	63	*22.2%*	ref		NA	
	Well	4	13	*23.5%*	1.08 (0.23, 4.08)			0.99
	Bottled	0	1	*0.0%*	0.00 (0.00, 138.37)	0.879		0.99
Drinking water Treatment	None/ breastfeeding only	4	20	*16.7%*	0.44 (0.08, 2.29)		0.08 (0.01, 0.70)	**0.02**
	No	6	13	*31.6%*	ref		ref	
	Boiled	14	57	*19.7%*	0.54 (0.15, 2.03)		0.11 (0.02, 0.65)	**0.01**
	Filtered	2	7	*22.2%*	0.63 (0.05, 4.93)	0.637	0.39 (0.03, 4.48)	0.44
Bathing water source	Tap	21	79	*21.0%*	ref		NA	0.99
	Well	5	18	*21.7%*	1.04 (0.27, 3.39)	1.000		
Bathing frequency (per week)	0-3	14	31	*31.1%*	ref		ref	
	4-7	12	65	*15.6%*	0.41 (0.15, 1.09)	0.065	0.84 (0.26, 2.64)	0.75
Exposure to pets	Yes	5	29	*14.7%*	0.56 (0.15, 1.73)		0.47 (0.11, 2.06)	
	No	21	68	*23.6%*	ref	0.332	ref	0.31
Use of indoor wood stove	Yes	5	30	*14.3%*	0.53 (0.14, 1.64)		0.33 (0.07, 1.50)	
	No	20	63	*24.1%*	ref	0.325	ref	0.15
Chronic medical condition present	Yes	6	13	*31.6%*	1.93 (0.53, 6.30)		2.89 (0.61, 13.79)	
	No	20	84	*19.2%*	ref	0.232	ref	0.18

Use of daily medications	Yes	0	6	*0.0%*	0.00 (0.00, 3.182)		NA	NA
	No	26	91	*22.2%*	ref	0.341		
PCV vaccinated	Yes	0	0	*NA*	NA		NA	NA
	No	25	94	*21.0%*		1.000		
Hospitalization (in past 6 months)	Yes	1	7	*12.5%*	0.52 (0.01, 4.34)			
	No	25	90	*21.7%*	ref		NA	NA
	Unsure	0	1	*0.0%*	0.00 (0.00, 141.65)	1.000		
History of Infection within past 6 months	Yes	7	22	*24.1%*	1.24 (0.39, 3.60)		1.84 (0.47, 7.11)	
	No	19	74	*20.4%*	ref	0.795	ref	0.38
History of pneumococcal infection within past 6 months	Yes	0	1	*0.0%*	0.00 (0.00, 143.68)		NA	NA
	No	26	95	*21.5%*	ref	1.000		
Antibiotic intake within past 6 months	Yes	0	2	*0.0%*	0 (0.00, 21.55)			
	No	24	94	*20.3%*	ref		NA	NA
	Unsure	2	1	*66.7%*	7.65 (0.38, 465.35)	0.152		

Sociodemographic features associated with S. pneumoniae colonization on bivariable and multivariable analysis are listed in Table 2. On bivariable analysis, younger age (<2 years) and living with other children under 5 years old were found to be significantly associated with a higher risk of S. pneumoniae colonization. Children ≤2 two years had more than five times the odds of being colonized as children >2 years of age. Living with other children (one or more) under five years old was associated with more than 4 fold greater odds of being colonized. However, living with children between 5-8 years old was not found significantly associated with a higher risk of colonization with an odds ratio of 1.50 (95% CI: 0.61-3.71, p value = 0.37).

After adjusting for all selected predictor variables in the regression model, there was still a statistically significant association between living with other children under 5 years of age and S. pneumoniae colonization (OR=3.99, 95%CI: 1.19-13.39, p=0.025), consistent with the univariate analysis (OR=4.58, 95%CI: 1.69-12.83, p=0.001). Adjusted

for the coeffects from other predictor variables, younger age (<2 years) was no longer associated with colonization but there was a trend towards significance (OR 0.089, 95%CI: 0.01-1.36).

Moreover, drinking water treatment method appeared to be significantly associated with S. pneumoniae colonization in the multivariate analysis, Compared with children who were breastfed only, children who started drinking water had more than ten times greater odds of being colonized (95%CI: 0.01-0.70, p=0.022) if the water was untreated. No difference was observed between water source (from tap or well) and colonization (p>0.9). Boiling the water showed a strong protective effect towards colonization with a ten-fold lower odds (p=0.014) on adjusted analysis.

Prevalence of antibiotic resistance

Of the 26 S. pneumoniae isolates, antibiotic susceptibility testing was performed on 18 . Among all tested isolates, only one isolate was susceptible to oxacillin, leading to a non-susceptible rate to oxacillin/penicillin of 94.4% (Figure 2). Only one isolate was non-susceptible to vancomycin (this requires further test for confirmation) and all isolates were susceptible to levofloxacin. Twelve isolates (66.7%) were resistant and one isolate was intermediate to erythromycin (72.2% non-susceptible), and 13 isolates (72.2%) were resistant and one isolate intermediate to tetracycline. The prevalence of resistance was lower for clindamycin (8 isolates, 44.4%).

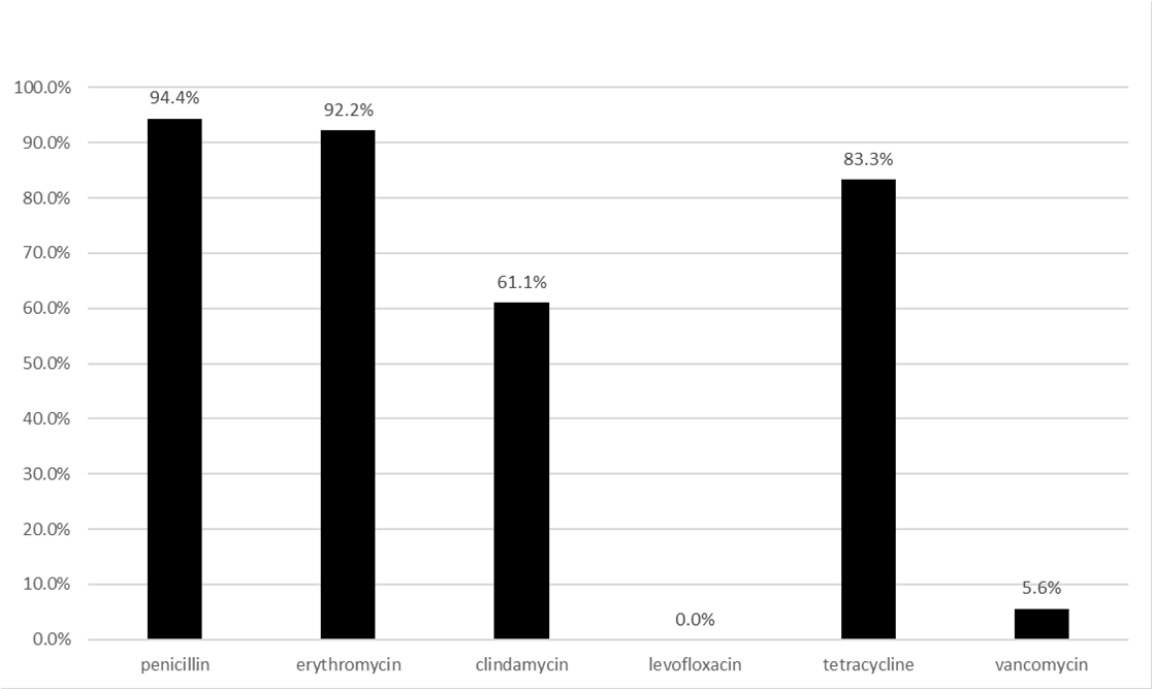

Figure 2. Prevalence of antibiotic resistance of S. pneumoniae isolates identified among healthy children ≤5 years of age enrolled in southern Sri Lanka, 2019

4. Discussion

There have been many studies exploring the colonization prevalence of S. pneumoniae in healthy children in South Asia and the rest of the world, but data in Sri Lanka have been limited to date. This study is one of the first to explore the carriage rate of S. pneumoniae among children under five years old in Sri Lanka. Lack of data regarding colonization and invasive disease is an obstacle in decision making regarding the introduction of PCV in Sri Lanka.

In our study, we found 26 children were carriers and identified a carriage rate of 21.1%. In both univariate and multivariate analyses, the risk of being colonized was strongly associated with living with other children under 5 years old. This finding is consistent with other studies showing a strong association between S. pneumoniae carriage and having siblings at home (Principi, 1999; Koliou, 2018), which increases the risk of household transmission of the bacterium if siblings are carriers. Moreover, we found that living with other children more than 5 years of age did not significantly increase the risk of being colonized with S. pneumoniae. The prior studies did not specify the age range of siblings. One potential explanation for our finding is that children over five years of age are not at high risk of being colonized with S. pneumoniae, thus reducing the risk of household transmission. Crowding, presented as day care center attendance, is another key factor has been found strongly associated

with S. pneumoniae colonization in other studies (Bogaert, 2004; Raymond, 2000; Koliou, 2018).

Age younger than 2 years has been widely recognized as a high risk factor for S. pneumoniae carriage in studies in both developed countries and developing countries (WHO, 2013; Bogaert, 2004; Kumar, 2014; Hadinegoro, 2016). Our result on univariate analysis result was consistent with this finding from prior studies. However, the significant association disappeared after adjusting for other variables. The adjustment for breastfeeding status ($p<0.001$) and history of infection ($p=0.003$), which are significantly associated with age, may explain the difference between the two analyses. Most children <2 years were breastfed (96.3%) while only 48% children over 2 years were breastfed. For age group <2 years, only 17.3% had a history of infection while 48% of children over 2 years of age had a history of infection. After inclusion into the regression model, age itself was not individually associated with the outcome while in univariate analysis it integrated effects from these two variables which are also recognized as risk factors for colonization (Levine, 1999). The significance level also changed for breastfeeding status and history of infection after inclusion into the model, and age remained a risk factor.

Additionally, the source of drinking water and treatment of drinking water appeared to be significantly associated with colonization. Boiled drinking water, either from a tap or a well, was associated with a ten-fold lower risk of colonization compared

to drinking untreated water. Prior studies do not note an association between drinking water treatment and S. pneumoniae colonization. Further studies are needed to confirm these findings and whether treatment of drinking water may be a correlate for another factor associated with colonization..

The ANSORP studies have observed an increase in penicillin nonsusceptibility in Sri Lanka from 41.2% in 1998-1999 to 76.5% in 2000-2001 (Song, 1999; Lee, 2001). It increased to more than 90% in 2007 (Batuwanthudawe, 2009) and now we observed a higher non-susceptible rate of 94.4% to oxacillin/ penicillin. All 996 isolates across the 11 countries tested were susceptible to vancomycin in the 1999 ANSORP study (Song, 1999) and one isolate appeared to be non-susceptible to vancomycin in this study. All isolates are susceptible to levofloxacin. Resistance to erythromycin, which was not addressed in previous ANSORP studies, was found to be prevalent (72.7%) in Asian countries (Kim, 2012) and we also find a similar high prevalence of resistance to erythromycin (66.7%). Globally, the prevalence of resistance to clindamycin was approximately 22% and we found a higher prevalence of 44.4%.

Overuse and misuse of antibiotics is one of the key factors contributing to the rise of antibiotic resistance. The "National Strategic Plan for Combating Antimicrobial Resistance in Sri Lanka 2017–2022" reports nearly 13% (US$ 220 million) of the total annual health budget is spent on antimicrobials in Sri Lanka (Zawahir, 2019). One source of the overuse and misuse of antibiotics has been self-medication of antibiotics

(SMA) in community settings (Senadheera, 2017). Antibiotics are used frequently, especially in developing countries, and antibiotic dispensing without prescription has been noticed as a major contributor to antimicrobial resistance in resource-limited settings (Zawahir, 2019). In Zawahir's study of 242 community pharmacies located across all provinces in Sri Lanka, in visits by simulated clients asking to purchase four kinds of antibiotics for which prescriptions were required, 47% of pharmacies asked for prescriptions and 61% of all pharmacies dispensed antibiotics without a prescription. Moreover, only 7% of the staff in the pharmacies suggested that the clients see a doctor. Such consumption of antibiotics without instruction from professionals may increase the risk of overuse and misuse of antibiotics. Moreover, such practice is not rare in Sri Lanka. In Senadheera's study, more than 25% of people who used antibiotics within three months were self-diagnosed without prescription in Colombo District. Convenience is the most popular reason for SMA and more than 85% of patients purchase their antibiotics from pharmacies. The self-medication and selection of antibiotics are largely based on patients' own experiences but only half of them are able to name an antibiotic. Moreover, more than 85% of patients who self medicated with antibiotics stopped their medications at the disappearance of symptoms rather than finishing the whole course or consulting a doctor, which might worsen antibiotic resistance.

We were not able to complete S. pneumoniae serotyping in this study. However, others have shown that the most common S. pneumoniae serotypes were 19F, 14, 23F, and 6B in Sri Lanka in 2007 and that 60% were covered by PCV7 (Batuwanthudawe, 2009). Two years later in 2009, the coverage of PCV7 dropped to 52.5% and serotype 19A (8.2%) emerged and became the most prevalent non-PCV7 serotype in Asia, with 86% resistant to erythromycin (Kim, 2012). Although PCV7 has not been administered through the public sector in Sri Lanka, such changes in serotype are likely due to PCV7 distribution in other Asian countries. These effects associated with PCV7 were also identified in another study showing the changes of serotype distribution in Asian regions (Tai, 2016). Compared with the pre-PCV7 period, a drop of the PCV7 serotype coverage was observed in many regions: Hong Kong from 89.2% to 55.6%, Taiwan from 87.1% to 54.1%, South Korea from 64.5% to 34.6%, Japan from 68.6% to 53.1%, and Thailand from 74.1% to 68.3%. A slight increase was found in mainland China from 57.6% to 60.5%. The study was a pooled analysis combining studies from different regions, thus the variance between regions might be the explanation for the slight increase in mainland China (Tai, 2016). The serotype distribution varies by age, region and time thus requires prompt surveillance. Such information can further inform the decision making of the introduction of PCV into the national immunization program in Sri Lanka. Also, surveillance is also necessary after successful introduction to

understand the effects of PCV towards the profile of serotype distribution. This study will finish serotyping in the short future to better inform the local health system.

4.1 Implications for policy and practice

Adding a vaccine to a national program includes three steps: "Deciding on the introduction of a vaccine," "Planning and managing the vaccine introduction" and "Monitoring and evaluation" (WHO, 2014). Regarding vaccine implementation decision-making, the disease, available vaccines and strength of the health system are three key factors. The main barrier so far towards PCV introduction in Sri Lanka is the lack of evidence regarding the disease burden (Kularatna, 2015). Kularatna's study, lead by the Ministry of Health in Sri Lanka, reported the estimate of adjusted invasive pneumococcal disease (IPD) incidence as 206.3 per 100,000 children under five years of age. Incidence of pneumococcal pneumonia, meningitis and sepsis were for 147.9, 13.2 and 45.2 per 100,000 children, respectively. Kularatna's study was valuable for estimating the burden of IPD and more importantly, for revealing the awareness and attention from the health system in Sri Lanka on introducing PCV into its national immunization program. The burden of IPD is important and the risk of being colonized by S. pneumoniae among healthy children is also instructive regarding the preventive role of vaccines. Other regions in South Asia have conducted studies exploring the carriage rate of S. pneumoniae among healthy children under five years old. Indonesia (46.2%) (Hadinegoro, 2016), India (27.9%) (Kumar, 2014), Thailand (25.4%)

(Thummeepak, 2014) and Taiwan (14.8%) (Chen, 2007) have implemented such studies as efforts and may have potentially inspired the decision making for the policy makers to integrate PCV into its immunization program. Taiwan implemented PCV13 into its regional immunization program in 2015 (Chi, 2018). The president of India announced that PCV would be available to 2.1 million children under its universal immunization program in 2017 (Anupam, 2017). Indonesia's government is now planning to include PCV in its national immunization program under the support from the Global Alliance for Vaccines and Immunization (GAVI)..

4.2 Implications for further research

For future research directions, a wider national level study is expected to better instruct the introduction of PCV13. Moreover, a guideline with list of antibiotic to be tested in such studies is suggested incase failing to track particular antibiotics in case of random selection of antibiotics studied. In a longer term, surveillance studies on the prevalence of S. pneumoniae colonization and antibiotic resistance and serotype distribution are of same importance after the introduction of PCV13.

4.3 Study strengths and limitations

Some limitations in this study must be noted. The prevalence of S. pneumoniae colonization (21.1%) is an underestimate according to standard microbiological procedures (da Gloria Carvalho, 2010). We defined S. pneumoniae isolates as those with a halo diameter>14mm to optochin disk after 24 hours incubation. However, there are

rare S. pneumoniae isolates that are resistant to optochin disk, requiring confirmation with the bile solubility test for those suspected isolates with a halo diameter ≤14mm (da Gloria Carvalho, 2010). Others have noticed the existence of optochin-resistant variants of pneumococcal strains (Pikis, 2001). For example, two serotype 6B isolates of strain HV109 were tested with reverse results in optochin test, one susceptible and one resistant (MIC=32mg/mL, MIC=2mg/mL) but they were both bile soluble. Moreover, such optochin-resistant S. pneumoniae isolate case has been found in a 4-year old boy in Sri Lanka. Resources for bile solubility test were lacking during the study period so we may have failed to identify a small proportion of S. pneumoniae isolates. These isolates were stored and can be retested in the future. Other limitations include that we have not finished serotyping to evaluate the coverage of PCV13. In addition, participants of this study may not be representative to children outside the study regions, Galle Four Gravet and Bope-Poddala.

This study fills a gap in knowledge regarding the prevalence of S. pneumoniae colonization and prevalence of antibiotic resistance; we will identify serotype distribution in future to further evaluate the coverage of PCV13. Moreover, in the event that PCV is introduced in Sri Lanka in the future, these data will serve as a baseline to evaluate the impact of PCV13.

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5. Conclusion

This is the first report of S. pneumoniae colonization prevalence among children in Southern Province, Sri Lanka. One-fifth of children were found to be colonized with S. pneumoniae, and oxacillin non-susceptibility prevalence was high. Further characterization must be performed to identify serotypes of colonizing strains and to correlate with serotypes present in available vaccines. Our results provide evidence regarding burden of pneumococcal colonization in Sri Lanka, and may help guide pneumococcal vaccine decisions in Sri Lanka.

Appendix A-Questionnaire

DGHI S.PNEUMONIAE STUDY (SRI LANKA)

STUDY ID _____ DATE ___ / ___ / ___ DIVISION: _____ SEEN BY: _____ REVIEWED BY: _____ DATA ENTRY BY: _____

Part A. Child Basic information

1. Date of Birth: ____/____/_____(DD/MM/YY)

2. Age: ____ years ____ months

3. Sex: ❑Male ❑Female

4. Height/Length: _____ cm

5. Weight: _____kg

6. Education: ❑Montessori ❑Daycare ❑Kindergarten ❑Other_____ ❑No (Go to Question 8)

7.Start date : ____/_____(MM/YY)

Weekly attendance days:_____(1-7)

Class size: ❑0-5 ❑6-10 ❑11-15 ❑16-20 ❑>20

8.Relationship to the child of interviewee: ❑Mother ❑Father ❑Grandparent ❑Other guardian_____

9.Marital status of parents: ❑ Married ❑Divorced

Part B. Parental information

1. Mother's information

1.Age: _____ years

2.Education: ❑ <O/L ❑ O/L ❑ A/L ❑> A/L (diploma, graduate)

3.Occupation:

❑Healthcare professional ❑Military/police/security ❑Teacher

❑Merchant/ shop/office worker ❑Manual/ unskilled laborer ❑Skilled laborer

❑Housewife/ unemployed ❑Retired ❑Other_____

2. Father's information

1.Age: _____ years

2.Education: ❑ <O/L ❑ O/L ❑ A/L ❑> A/L (diploma, graduate)

3.Occupation:

❑Healthcare professional ❑Military/police/security ❑Teacher

❑Merchant/ shop/office worker ❑Manual/ unskilled laborer ❑Skilled laborer

❑Housewife/ unemployed ❑Retired ❑Other_____

3.Other guardian's information (if the interviewee is not parent)

1.Age: _____ years

2.Education: ❑ <O/L ❑ O/L ❑ A/L ❑> A/L (diploma, graduate)

3.Occupation:

❑Healthcare professional ❑Military/police/security ❑Teacher

❑Merchant/ shop/office worker ❑Manual/ unskilled laborer ❑Skilled laborer

❑Housewife/ unemployed ❑Retired ❑Other_____

4. Average monthly household income (total):

❑ <15,000 Rs ❑ 15,001- 30,000 Rs ❑ 30,001- 45,000 Rs ❑>45,000 Rs

Part C. Community-related risk factors

1.1 Breastfeeding status: ❑Ongoing ❑Weaned

1.2 Total lengths for breastfeeding: _____months (0 if none)

2.1 Number of people currently live at home with the child?

_____Elder(≥ 65 yrs) ____ Adults _____ Children (5-18 yrs) ____ Children (<5 yrs)

2.2 Number of people currently share bed with the child?

_____Elder(≥ 65 yrs) ____ Adults _____ Children (5-18 yrs) ____ Children (<5 yrs)

3. Toilet: ❑private household toilet ❑public toilet ❑open defecate ❑other_____

4.1 Number of smokers in the family: ❑0 ❑1 ❑2 ❑3 ❑4 ❑5 or more

4.2 Daily number of cigarettes taken in the family: ❑1-5 ❑6-10 ❑11-15 ❑16-20 ❑>20

4.3 Child's time of exposure to the smoke: _____months

***The following questions refer to THE CHILD**

5.1 Drinking water source: ❑ Breastfeeding only ❑Tap(water board) ❑Tap(community water project)

❑Public well ❑Private well ❑Bottled ❑Other_____

5.2 Drinking water treatment methods: ❑None ❑Boiled ❑Filtered

❑Chlorinated (tap from water board) ❑Other_____

6.1 Bathing water source: ❑Tap ❑Well ❑Lake/river ❑Other_____

6.2 Bathing frequency (per week): ❑0-1 ❑2-3 ❑4-5 ❑6-7

6.3 Use of a community pool: ❑Yes ❑No ❑Unsure

→If yes, average times used per month_____

7. Child's exposure to pets or pets kept at home (feeds at least 4d per week): please indicate the number of pets.

❑Pig___ ❑Sheep___ ❑Cat___ ❑Poultry___ ❑Other _____,_____

❑Cow___ ❑Goat____ ❑Dog___ ❑Horse___ ❑No pets

8. Use of indoor wood stove: ❑Yes ❑No

Part D. Child Medical information

1. Chronic medical conditions: ❑Asthma ❑Diabetes mellitus ❑Eczema/ allergies

❑Congenital disease: _____ ❑Other: _____ ❑ No

2. Daily medications: ❑Yes ❑No

→ If yes, type_____

3. Streptococcus pneumoniae vaccination (CPV): ❑Yes ❑No

→If yes, date of administration: ___/___/___(dd/mm/yy)

4. **During the past 6 months:**

4.1 Hospitalization: ❑Yes ❑No ❑Unsure

→If yes, number of days:_____; Last date of admission: ___/___/___(dd/mm/yy)

4.2 History of Infection: ❑Yes ❑No ❑Unsure

→If yes, specify ❑Pneumonia ❑Dengue ❑Flu ❑Cough ❑others_____

4.3 History of Pneumococcal diseases:

❑Pneumonia ❑Ear infections (❑Acute otitis externa ❑Otitis Media with Effusion ❑Acute Otitis Media)

❑Sinusitis ❑Meningitis ❑Bacteremia ❑Unsure ❑No, none of above

4.4 Antibiotic intake: ❑Yes ❑No ❑Unsure

→If yes, Obtained for: _____

Obtained from: ❑Impatient admission ❑Private doctor ❑Outpatient department

❑Pharmacist ❑Friend/ relative ❑Other _____

4.5 If yes, names of antibiotics known: ❑ Yes ❑No

If names known:

Antibiotic	**# days**	**Last date (dd/mm/yy)**
Name unknown		___/___/___
Amoxicillin/ ampicillin		___/___/___
1st gen ceph (ie. cephalexin)		___/___/___
2nd gen ceph (ie. cefuroxime)		___/___/___
3rd gen ceph (ie. ceftriaxone)		___/___/___
Amoxicillin & clavulanic acid		___/___/___
Erythromycin/ azithromycin		___/___/___
Clarithromycin		___/___/___
Anti-TB therapy		___/___/___
Aminogylcoside (ie. gentamicin)		___/___/___
Vancomycin		___/___/___
Metronidazole		___/___/___
Clindamycin		___/___/___
Fluoroquinolone (ie. ciprofloxacin)		___/___/___
Nitrofurantoin		___/___/___
Tetracycline/doxycycline		___/___/___
Trimethoprim & sulfamethoxazole		___/___/___
Carbapenem (ie. meropenem)		___/___/___
Other: _____		___/___/___

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