RESEARCH ARTICLE

REVISED Pneumococcal nasopharyngeal carriage and antimicrobial susceptibility profile in children under five in southern Ethiopia [version 3; peer review: 2 approved]

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Abstract

Background: *Streptococcus pneumonia* causes high morbidity and mortality, particularly in children under five. Nasopharyngeal (NP) carriage predisposes individuals to pneumococcal infection and horizontal spread within the community. Overuse of antibiotics has been linked to increased risk of antimicrobial resistance to *S. pneumonia*. We investigated NP carriage rate and resistance to commonly prescribed antibiotics in under-five children visiting a public referral center in southern Ethiopia.
Methods: In total, 413 under 5 children who visited the outpatient department for a health check-up, immunization or acute mild illnesses underwent NP sampling. Parent/caregiver surveys were administered at the clinic. Sterile plastic applicator rayon tipped swabs were used for NP sampling. Antimicrobial susceptibility testing was performed using modified the disk diffusion method.
Results: *S. pneumonia* NP carriage was observed in 39% [95% confidence interval (CI): 34.4–43.8]. Living with one or more sibling (AOR (adjusted odds ratio) 1.95: 95% CI: 1.01, 3.76), age group of 3-23 months (AOR 2.31: 95% CI: 1.07, 4.98), co-sleeping with family (AOR 2.09, 95% CI: 1.16, 3.79), attendance at kindergarten/day-care (AOR 1.84: 95% CI: 1.09, 3.11) and malnutrition independently increased *S. pneumonia* carriage at the individual level. *S. pneumonia* was highly resistant to Oxacillin (38.5%), Tetracycline (37.3%), and Trimethoprim-sulfamethoxazole (34.2%). Multi-drug resistance was observed in 42.2% of isolates.
Conclusions: A high streptococcal NP carriage rate was observed in under-five children. The high level of resistance to commonly used antibiotics calls for enhancing national surveillance of resistance

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patterns and enforce antibiotic stewardship efforts.

Keywords
Nasopharyngeal carriage, Streptococcus pneumonia, antimicrobial susceptibility, under-five children, Ethiopia

This article is included in the Antimicrobial Resistance collection.

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REVISED **Amendments from Version 2**

A sentence reflecting our limitation to evaluate locally available antibiotics for susceptibility was added.

Any further responses from the reviewers can be found at the end of the article

Abbreviations

EDHS = Central Statistical Agency of Ethiopia, PCV = pneumococcal conjugate vaccine, NP = nasopharyngeal, STGG = skim milk tritons glucose glycerol, and *S. pneumonia* = *Streptococcus pneumonia.*

Introduction

Streptococcus pneumonia (pneumococcus) is a Gram-positive extracellular pathogen associated with high morbidity and mortality in children all over the world, particularly in developing countries like Ethiopia[1]. *S. pneumonia* is the most important cause of bacterial pneumonia and meningitis worldwide. For instance, in 2010, it accounted for 33% of the deaths in children under 4 years old[2]. In Africa, pneumococcal disease is estimated to cause nearly half a million deaths among children under-five years annually[3]. The World Health Organization (WHO) in 2010 estimated 541,000 global child deaths due to pneumococcal infections in under-five years children[3]. Ethiopia is among the countries with the highest burden of pneumonia, especially in children under-five[4]. In 2010, 312,857 cases community acquired pneumonia and 12,284 deaths caused by *S. pneumonia* were reported in children under-five[5].

Pneumococcal disease often follows nasopharyngeal (NP) colonization with homologous strains. The mucosal epithelium of the nasopharynx is the primary site of pneumococcal colonization[6]. *S. pneumonia* NP carriage, a necessity for the development of the disease, is considered to be an important source of horizontal spread of this pathogen within the community[7]. Vaccination is one of the key interventions to prevent pneumococcal disease and colonization. In Ethiopia, vaccination with a streptococcal conjugate vaccine (PCV 10) for S. pneumoniae started in 2011 through the vaccine alliance, Gavi support[8]. The coverage of PCV-10 has since increased to reach more than 60% in 2019[9].

Several socio-demographic and clinical characteristics including young age, family size, low income, number of siblings, and malnutrition predicted NP pneumococcal colonization[10]. Household and environmental factors such as overcrowding, exposure to tobacco smoke[11] and exposure to indoor air pollution also increased the risk of NP colonization[10]. The transmission of *S. pneumonia* occurs through respiratory droplets or more commonly from individuals who are asymptomatic carriers[6]. Pneumococcus susceptible individuals may become colonized upon exposure and can remain so for weeks to months. Acquisition of invasive serotypes could lead to pneumococcal disease, commonly after a 1-to-3-day incubation period[12].

The increasing frequency and rapid spread of antimicrobial resistant pneumococcal strains is a global health threat. Antimicrobial resistance has made the choice of antimicrobial agents for treatment of pneumococcal infections more complicated and costly[11,13]. Nasopharyngeal colonization by antimicrobial resistant *S. pneumonia* had been increasing in different parts of the world including Ethiopia[14,15]. Minimizing NP carriage rate is an important step for prevention and control of pneumococcal disease. The variable risk factors in different populations and the risk factor differences necessitate generating evidence in various settings to better understand the factors that predispose to increased risk of exposure to *S pneumoniae*. We aimed to investigate the prevalence and predictors of NP pneumococcal colonization as well as antimicrobial susceptibility pattern of isolates in a setting where there is a high prevalence of undernutrition and low socioeconomic status.

Methods

Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Review Board (IRB) of College of Medicine and Health Sciences, Hawassa University (IRB reference number: IRB/006/11). The purpose and importance of the study was explained to each study participants. To ensure confidentiality of participants, data collection tools were anonymous with no participant identifiers. Participants were interviewed alone to maintain privacy. All participants were not paid for the test. Informed written consent was obtained from a parent or guardian for children to participate in the study. The study incurs no cost to the study participants and were interviewed free of charge.

Study location and sampling technique

The study was conducted between November 2018 and March 2019 at outpatient departments (OPD) of two public Hospitals – Adare and Hawassa University comprehensive specialized Hospitals (HUCSH) in Hawassa City, which were purposively selected to represent primary healthcare and referral facilities in the region; Adare General Hospital is a primary care facility while HUCSH is the main referral hospital in southern Ethiopia. In the study district the coverage of three doses of pneumococcal conjugate vaccine (PCV) coverage was at 61% in 2019[16], which showed significant improvement from the 2016 reported coverage of 53%[17].

Sample size (n) was calculated using single proportion formula (Equation 1) assuming a prevalence (p) of 43% based on data reported in North West Ethiopia (43.8%)[18] with 95% confidence interval (z=1.96) and 5% precession (d), and 10% non-response rate which resulted in a sample size of 417. A systematic random sampling method was used to select participants – every k^{th} child was selected from a total of OPD attendees every day. The list of all children who presented to the OPDs everyday was used as a sampling frame to decide the value of *k*. Parents or legal authorized representatives were invited to participate in the study. The informed consent

process was administered to those who agreed to participate in the study.

$$Equation\ 1:\ n = \frac{z^2 * p(1-p)}{d^2}$$

Inclusion and exclusion criteria

Inclusion were all under-five children who visit OPDs of the two hospitals during the study period and who consent to participate in the study.

Exclusion criteria included subjects who had an illness that made nasal swabbing difficult, and those with severe respiratory problems (for example acute attack of bronchial asthma), had anatomical abnormalities of the nose (e.g. cleft palate) and who were on antibiotics in the two weeks prior to the start of the study.

Data collection

Structured questionnaires developed for the purpose of the study, pilot tested on 5% of the sample before implementation, were used to collect information on socio-demographics, clinical data, and associated factors. Based on the results of the pilot testing of whether questions were correctly understood by the interviewers and respondents or not, the questionnaires were revised to improve clarity. The pilot testing did not reveal significant errors in the questionnaires. The tools were first developed in English (see extended data[19]), translated to local language (see extended data[20]), and back translated to English by an independent translator to ensure internal validity. In addition to interviews of parents/guardians, medical records of participants were reviewed to abstract past medical history. PCV vaccination status was also assessed through interviews with parents/guardians and vaccination card. Trained data collector healthcare professionals administered the questionnaires to the parents or LAR in a quiet room; data collectors also measured child's weight to the nearest 0.1kg and height/length to the nearest 1cm using electronic weighing scale and length/height board. Anthropometrics were then interpreted using WHO Z scores, where a score of <-2 is considered to indicate undernutrition.

Sample collection and processing

Nasopharyngeal specimens were collected using sterile swabs in two replicates. One NP specimen was collected per child by gentle insertion of sterile flexible plastic applicator rayon tipped swab (Copan, Brescia, Italy, catalogue number: 26061), which was done by tilting slightly backwards and immobilize child's head while gently restraining the child's body. Once in place, the swab was rotated and left in place for five seconds to saturate the tip before slowly removing it. After collection, the sample was immediately placed in 1ml skimmed milk tritons glucose glycerol (STGG) transport media in tubes. Any excess samples were cut off before inoculating in the transport medium in tubes, after which the caps were tightened securely. The NP specimen was processed within 8 hours of collection and in cases where delay was encountered, it was stored at -20°C. Culturing the NP swab-STGG specimens was done on tryptone soy agar base (Oxoid, Basingstoke, Hampshire, England; Catalogue number: 105459). Briefly, the NP swab-STGG specimens were mixed thoroughly by vortexing for 30 seconds, 10 µl of the sample was then used to inoculate the plates and streaked using a sterile wire loop. The streaked plates were incubated into 5% CO2 incubator at 37°C for 24 hours. Plates were fully examined for any growth and the plates displaying no growth were re-incubated before being reported as negative. Identification of positive culture results was performed based on the appearance of colonies and the hemolytic pattern – small and watery growth surrounded by a greenish zone of alpha-hemolysis on the media.

Optochin susceptibility and a bile solubility biochemical test

We employed similar microbiological methods to those reported by Gebre *et al.*[18]. Briefly, to isolate pneumococci, suggestive colonies were sub-cultured and tested for optochin susceptibility and bile solubility. Optochin susceptible strains with ≥14mm in diameter zone of inhibition were identified as *S. pneumonia*. Next, alpha hemolytic strains with zone of inhibition <14 mm underwent bile solubility test using 2% sodium deoxycholate or bile salt base (Oxoid, Basingstoke, Hampshire, England; Catalogue number: 89904).

Bacterial cell suspension samples were prepared from freshly streaked presumed positive colonies of *S. pneumonia* in sterile normal saline. An adjusted 1ml of suspension was divided into two equal amounts of 0.5 ml in each tube. Then 0.5 ml of normal saline was added to one tube and 0.5 ml of 2% bile salt to the other tube as a test followed by incubation in 5% CO2 incubator at 37°C for up to 2 hours. A loss of turbidity in the bile tube but not in the saline control tube was considered as a positive test.

Antimicrobial susceptibility test

Disk diffusion (modified Kirbye-Bauer) method on Mueller Hinton agar (Oxoid, Basingstoke, Hampshire, England, Catalogue number: 105437) supplemented with 5% sheep blood was employed for AST[21]. Standard disks of commonly used antibiotics including Tetracycline – 30µg, Trimethoprim/sulfamethoxazole – 1.25+23.75µg, Oxacillin – 1µg, Chloramphenicol – 30µg, and Erythromycin – 15µg (Oxoid, Basingstoke, Hants RG24 8PW, UK) were used antimicrobial susceptibility testing of all the isolates.

Following inoculation of the bacteria suspension on the Mueller-Hinton agar plate, which is supplemented with 5% sheep blood agar, and then air drying, the antibiotic disks were dispensed aseptically using an automatic disk dispenser. Next, the plates were incubated in a 5% CO2 incubator at 37°C for 24 hours. Finally, zone diameters of growth inhibition were measured to the nearest millimeters using a ruler and were interpreted using cut-off points for each antibiotic disk, which range from 0.5µg/mL for vancomycin to 8µg/mL for gentamicin and tetracycline, in the Clinical and Laboratory Standard Institute (CLSI) result interpretive standards[21]. Categorically, results were interpreted as susceptible, intermediate, or resistant[22]. *S. pneumonia* ATCC 49619 provided by the

Ethiopian National Quality Assurance Directorate (Catalogue number: 0947L) was used as a positive quality control strain for all procedures.

Data analysis

Bivariate and multivariate binary logistic regression models containing sociodemographic and clinical variables to assess independent predictors of pneumococcal NP carriage were produced. Variables with p-value <0.2 in the bivariate model were included in the multivariate model. Odds ratios and 95% confidence intervals (CI) were used to measure the association between potential risk factors and occurrence of NP carriage at the individual level. Level of significance for the multivariate models was set at p-value < 0.005. Anthropometrics were assessed following standard procedures[23], Z-score of <-2.0 was used as a cut off to define wasting, stunting, and underweight for weight-for-height, height/length-for-age and weight-for-age assessments respectively[24]. All the statistical tests were performed using Stata version 14.0 (StataCorp, Texas, USA).

Results

Socio-demographic characteristics

A total of 413 children participated in the study with 99.04% response rate; 226 (54.7%) were female. Age of the children ranged from 3–59 months with mean age (standard deviation – SD) of 36.63 (18.85) months. The majority, 308 (74.6%) of the children were from an urban setting; 157 (38.0%) of the parents/guardians attended primary education; and 230 (55.7%) of the parents/guardians were housewives. More than half, 215 (52.1%), of participants were from a family who had an average monthly income of USD 30 to 60 (Table 1[25]).

Prevalence of pneumococcal nasopharyngeal carriage

The overall prevalence of pneumococcal NP carriage rate was 39% [95% confidence interval (CI): 34.4–43.8]. The highest

Table 1. Pneumococcal nasopharyngeal carriage rate and predictors in children under-five.

Variable	NP carriage rate		COR (95% Confidence Interval)	p-value	AOR (95% Confidence Interval)	p-value
	Number (%) Total tested	Number (%) Positive				
Sex						
Male	226(54.7)	95(42.0)	1.33(0.89, 1.98)	0.162	1.22(0.78, 1.93)	0.385
Female	187(45.3)	66(35.3)	ref	ref	ref	ref
Age (months)						
3–23	201(48.7)	104(51.7)	4.66 (2.66, 8.16)	<0.001*	2.31(1.07,4.98)	0.032*
24–41	105(25.4)	37(35.2)	2.37(1.26,4.44)	0.007	1.45(0.66,3.18)	0.349
42–59	107(25.9)	20(18.7)	ref	ref	ref	ref
Place of residence						
Rural	308(74.6)	115(37.3)	ref	ref	ref	ref
Urban	105(25.4)	46(43.8)	1.31(0.84, 2.05)	0.241	1.10(0.65,1.85)	0.732
Mother/guardian education						
No formal education	113(27.4)	50(44.2)	1.53(0.73, 3.22)	0 .263		
Primary education	157(38.0)	60(38.2)	1.19(0.58, 2.45	0 .632		
Secondary & above	102(24.7)	37(36.3)	1.09(0.51, 2.35)	0 .810		
College and above	41(9.9)	14(34.1)	ref	ref		
Mother/guardian occupation						
Employed	67(16.2)	23(34.3)	ref	0.710		
Merchant	116(28.1)	43(37.1)	1.13(.60, 2.12)	0.305		
Housewife	230(55.7)	95(41.3)	1.35(.76, 2.38)	ref		
Average family income						
≤ 35 USD	62(15.0)	25 (40.3)	1.16(.63, 2.15)	0 .632		
36-65 USD	215(52.1)	86(40.0)	1.15(.74, 1.79)	0.545		
> 65 USD	136(32.9)	50(36.8)	Ref	ref		
Number of rooms in the house						
Single room	127(30.8)	52(40.9)	1.13(0.74,1.73)	0.586		
More than one room	286(69.2)	109(38.1)	ref	Ref		
Family size						
Fewer than five	280(67.8)	99(35.4)	ref	ref	ref	Ref
Five or more	133(32.2)	62(46.6)	1.597(1.052.43)	0.029	1.31(0.80, 2.13)	0.286

Variable	NP carriage rate		COR (95% Confidence Interval)	p-value	AOR (95% Confidence Interval)	p-value
	Number (%) Total tested	Number (%) Positive				
Number of siblings						
One or more	281(68.0)	135(48.0)	3.77(2.31, 6.15)	<0.001*	1.95(1.01,3.76)	0.047*
None	132(32.0)	26(19.7)	ref	ref	ref	Ref
Co-sleeping with family						
Yes	322(78.0)	138(42.9)	2.22(1.32, 3.74)	0.003	2.09(1.16,3.79)	0.031*
No	91(22.0)	23(25.3)	ref	ref	ref	Ref
Child attending Kinder/Day care						
Yes	134(32.4)	76 (56.7)	2.99(1.95, 4.58)	<0.001*	1.84(1.09,3.11)	0.023*
No	279(67.6)	85 (30.5)	ref	ref	ref	ref
Weight-for-Height						
Wasted	52(12.6)	29(55.8)	2.19(1.22,3.94)	0.009*	2.17(1.07,4.34)	0.031*
Normal	361(87.4)	132(36.6)	ref		ref	ref
Height -for-age						
Stunted	100(24.2)	57(57.0)	2.66(1.68,4.22)	<0.001*	2.68(1.58,4.55)	<0.001*
Normal	313(75.8)	104(33.2)	ref	ref	ref	
Weight-for-age						
Underweight	61(14.8)	36(59.0)	2.62(1.50,4.56)	0.001*	1.70(0.87,3.33)	0.123
Normal	352(85.2)	135(35.5)	1		ref	
Immunization status						
Three doses	250(60.5)	94(37.6)	1.00(.35, 2.85)	0.994		
Two doses	64(15.5)	25(39.1)	1.07(0.35, 3.31)	0.909		
One dose	16(3.9)	6(37.5)	ref	ref		
Zero dose	83(20.1)	36(43.4)	1.28(.42, 3.84)	0.664		

USD – United states dollar; COR – Crude Odds Ratio; AOR – adjusted odds ratio; NP – nasopharyngeal; Ref – reference

prevalence of NP carriage was observed in those aged 3 – 23 months (49.8%). More boys than girls had NP colonization (42.0% in girls versus 35.3% in boys). Of the study participants who lived in urban settings, 46 (43.8%) were carrier for *S. Pneumonia* (Table 1[25]).

Predictors of nasopharyngeal carriage

In bivariate analysis, sociodemographic variables including sex, place of residence, and age of the child had statistically significant association with pneumococcal NP carriage. Similarly, family factors including larger family size, presence of other siblings, and co-sleeping with other family members were predictors of NP pneumococcal colonization. Attendance at day care centers and presence of acute and chronic malnutrition were associated with an increased probability of NP Streptococcal colonization. Interestingly, level of PCV vaccination and lack of any vaccination were not associated with probability of NP pneumococcal colonization.

Next, we constructed a multivariable regression model including variables with a p-value < 0.2 in the bivariate analysis. Being under two years of age (AOR 2.31: 95% CI: 1.07, 4.98), those living with one or more siblings (AOR 1.95: 95% CI: 1.01, 3.76), history of co-sleeping with family members (AOR 2.09, 95% CI: 1.16, 3.79) and attendance at kindergarten/day care (AOR 1.84: 95% CI: 1.09, 3.11) were found to result in an increased probability of pneumococcal NP colonization (Table 1[25]). Children who were stunted, 2.17(1.07,4.34); and wasting, 2.68(1.58,4.55) had a higher probability of pneumococcal colonization.

Antimicrobial susceptibility bacterial isolates

Antimicrobial susceptibility was determined for all 161 isolates of *S. pneumonia* to six commonly prescribed antimicrobial agents: Oxacillin, tetracycline, Erythromycin, TMP-SMX, and chloramphenicol. Among tested antimicrobial agents, higher rates of *S. pneumonia* resistance was reported in Oxacillin, 62 (38.5%); Tetracycline, 60 (37.3%) and Trimethoprim-sulfamethoxazole, 55 (34.2%). Comparatively, the lowest resistance rate was exhibited by Erythromycin, 11 (6.8%); chloramphenicol, 117(10.6%); and Vancomycin, 13(8.1). multi-drug resistance to two or more antimicrobials was identified in 68 (42.2%) isolates (Table 2[23]).

Discussions

In the current study, we showed that streptococcal colonization is a common condition. The findings highlight the importance

Table 2. Antimicrobial susceptibility patterns of *S. pneumoniae* isolated among children visiting pediatrics OPD in governmental Hospitals Hawassa City, Southern Ethiopia in 2019.

Anti-microbial Agents	Susceptibility Pattern					
	Resistant		Intermediate		Sensitive	
	No.	(%)	No.	(%)	No.	(%)
Oxacillin	62	(38.5)	13	(8.1)	86	(53.4)
Tetracycline	60	(37.3)	17	(10.6)	84	(52.2)
Erythromycin	11	(6.8)	16	(9.9)	134	(83.2)
Trimethoprim-sulfamethoxazole	55	(34.2)	14	(8.7)	92	(57.1)
Chloramphenicol	17	(10.6)	7	(4.3)	137	(85.1)
Vancomycin	13	(8.1)	0	(0.0)	148	(91.9)

No. – Number; OPD – Outpatient Department

of pneumococcus as a potential cause of bloodstream infections and cause septicemia, meningitis, and pneumonia[26]. Ethiopia introduced the PCV vaccine in the expanded program of immunization (EPI) schedule for under-five children since 2011 and the coverage has since increased. However, carriage rate of *S. pneumonia* and pneumococcal invasive disease remains a public health problem. The prevalence of pneumococcal nasopharyngeal carriage among children < 5 years in Hawassa City was 39% [95% confidence interval (CI): 34.4–43.8], a finding similar to reports from Jimma (43.8%)[18] and Gondar (41.03 %)[27]. However, higher prevalence (64.8%) was reported in the Wolayita Zone of southern Ethiopia[28], which is similar to carriage rates reported in other African countries, for example 64.8%[29] and 65.8% of NP colonization in Kenya[30]. The factors affecting the variabilities in the burden of streptococcal NP colonization have yet to be explored but could include differences in socioeconomic and population characteristics[31].

Even though the findings in this study represent health facility data in under-five children who visited the recruitment centers for mild health conditions, routine health check-up or vaccination, a community-based NP sample collection would have enabled inference into the general population of under-five children. Furthermore, both recruitment facilities are urban hospitals which could limit representation of rural communities. However, the study included a significant proportion of rural residents since both hospitals have both urban and rural catchments. Lastly, even though we tested resistance of isolates to a range of antibiotics, commonly used antibiotics including penicillin and cephalosporins were not evaluated due to the lack of specific antibiotic discs.

In this study, NP carriage of *S. pneumonia* was significantly lower among children whose aged 24–41 months old, a finding similar to other studies in Arsi zone, South East Ethiopia[32] and Gondar, North West Ethiopia[27]. The decline in *S. pneumonia* colonization rate with increasing age maybe due to the progressive acquisition of mucosal immunity as a result of repeated colonization by several serotypes and the potential reduction of exposure[33]. Supporting this argument, our study showed that co-sleeping with other family and living with one or more siblings is associated with increased odds of NP colonization. Similar findings were reported in elsewhere[28,32].

The high resistance to Tetracycline (37.8%) was consistent with other studies which reported in Gondar (33.2%) and Hawassa (42.6%)[18,33], but was lower than prevalence of tetracycline resistant of *S. pneumonia* isolates from Wolayta Sodo (48.9%) and Jima (53.2%)[18,34]. The high resistance to tetracycline maybe due to widespread inappropriate prescription, which exerts selection pressure for the presence of increased tetracycline-resistant bacterial isolates. *S. pneumonia* isolates were also resistant to Trimethoprim-sulfamethoxazole and Oxacillin, which is in agreement with other reports[18,33], reflecting local and regional antimicrobial use practices. Oxacillin is often used for soft tissue infections and injuries, including for road traffic accidents.

A worrying finding in our study was the high prevalence (42.2%) of multi-drug resistant *S. pneumonia* isolates (i.e. resistant to two or more drugs), which could be linked to mobile genetic units (including plasmids, gene cassettes in integrons and transposons)[34], lack of effective medicines, inappropriate dispensing, medication sharing, counterfeit drugs, bacterial evolution, climate changes, lack of medical practitioner with proper training, poor-quality and unhygienic sanitary conditions[35].

Conclusion and Recommendation

The prevalence of pneumococcal Nasopharyngeal Carriage in the study area was high. The proportion of drug resistance to Tetracycline, Trimethoprim-sulfamethoxazole and Oxacillin was very high. Younger age, co-sleeping with family and living with one or more sibling independently predicted the probability of pneumococcal Nasopharyngeal carriage. The results of the study will have critical input to enforce antimicrobial stewardship efforts in the study area and beyond. Furthermore, surveillance of carriage and antimicrobial resistance in different populations will help to formulate targeted interventions.

Data availability

Underlying data

Figshare: Last Pneum Referal2.sav siraj Dem F.sav. https://doi.org/10.6084/m9.figshare.13297724.v1[25]

This project contains the following underlying data:

- Last Pneum Referal2.sav siraj Dem F.sav (Deidentified nasopharyngeal colonization data)

Extended data

Figshare: Questionnaire (English Version).docx. https://doi.org/10.6084/m9.figshare.13297781.v1[19]

This project contains the following extended data:

- Questionnaire (English Version).docx

Figshare: Amharic version of the questionnaire. https://doi.org/10.6084/m9.figshare.13356641.v1[20]

This project contains the following extended data:

- Questionnaire_Translated version (Amharic).docx

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 3

Reviewer Report 13 July 2021

https://doi.org/10.5256/f1000research.58336.r89045

✔ **Abate Yeshidinber Weldetsadik**
Department of Pediatrics and Child Health, St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

I approve the revision.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric, paediatric pulmonary and critical care, quality of health care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 30 June 2021

https://doi.org/10.5256/f1000research.56639.r85118

✔ **Ritah F. Mutagonda**
Department of Clinical Pharmacy and Pharmacology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

I have understood the response to the comments raised, and I approve the manuscript to be considered for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease particularly malaria and HIV; pharmacokinetics and pharmacogenomics studies; pharmacodynamics research.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 19 May 2021

https://doi.org/10.5256/f1000research.56639.r85117

?

Abate Yeshidinber Weldetsadik
Department of Pediatrics and Child Health, St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

I have noticed most of my concerns are addressed in the revision but it didn't mention the limitations including the antibiotics tested are not the ones mostly used for streptococcal infections (penicillin and cephalosporin).

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric, paediatric pulmonary and critical care, quality of health care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 09 March 2021

https://doi.org/10.5256/f1000research.30484.r79335

?

Ritah F. Mutagonda

Department of Clinical Pharmacy and Pharmacology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

This work addresses a very pertinent issue which is the prevalence of pneumococcal nasopharyngeal carriage and antimicrobial susceptibility profile in children under five in southern Ethiopia. With the increase in antimicrobial resistance which is currently a global health concern the information obtained from antimicrobial surveillance studies give us the clue of the current trends in pathogen antimicrobial resistance in different populations which will enable the development of targeted approaches to help control antimicrobial resistance. There are recent similar studies conducted in Ethiopia that are not featured in this manuscript eg. Negash *et al.* 2019[1].

There are minor comments which need to be addressed to improve the quality of the manuscript as described below:

Abstract:

- The summarized methodology sub-section needs to be improved a bit by adding information with regards to the study design, the study site, study duration, sampling technique and how data was analysed so that the reader can easy grasp this information when reading the abstract.

- Be consistent with the way you present information. Example in the methodology it is written 'under 5' while in the conclusion it is written 'under-five'.

Introduction:

- Inconsistency of how you write under-five' is also observed here.

- I do understand there is pneumococcal vaccine (PCV) which is given to children. This information is presented in the results section so I think it will be very informative to describe a bit in the introduction section as one of pneumococcal preventive measures. Could also describe the coverage which might have an impact on the pneumococcal carriage rate in children.

Methodology:

- I suggest that the Ethics approval and consent to participate sub-section should come after the data analysis part.

- Add subsection describing the study design --- analytical cross-sectional study.

- In data analysis, describe how you were able to analyse antimicrobial susceptibility test results. State the dependent and independent variables. Please remove this sentence *"PCV vaccination status was assessed through interviews with parents/guardians and vaccination card."* It does not fit under data analysis part.

Results:

- Remove this sentence *"Next, we constructed a multivariable regression model including variables with a p-value < 0.2 in the bivariate analysis."* Under the predictors of nasopharyngeal carriage part.

References

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease particularly malaria and HIV; pharmacokinetics and pharmacogenomics studies; pharmacodynamics research.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 May 2021

Birkneh Tilahun Tadesse, Hawassa University, Hawassa, Ethiopia

We thank the reviewer very much for taking the time to review our paper and for the important suggestions. We agree with the importance of keeping consistency using such terms as “under-five” and we have revised throughout the paper to conform to that. We also appreciate the comment on including more information regarding the introduction of PCV in Ethiopia and we have included a paragraph in the introduction to address that. We have also made the requested formatting changes while trying to conform to the Journal's formatting requirements. Please note that the final formatting was based on the requirement of the Journal. The sentence regarding the regression models was included to ensure that there is a good flow.

Competing Interests: No competing interests were disclosed.

Reviewer Report 21 January 2021

https://doi.org/10.5256/f1000research.30484.r76205

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Abate Yeshidinber Weldetsadik
Department of Pediatrics and Child Health, St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Hussein and colleagues reported a study on "Pneumococcal nasopharyngeal carriage and antimicrobial susceptibility profile in children under five in southern Ethiopia". They underwent nasopharyngal swab culture in 413 children and found out that streptococcal carriage is common and especially increased in selected group of children with risk factors. They also reported high level of bacterial resistance.

While it is similar to previous studies from similar setting, the study adds to the current knowledge in the region. However, their culture was determined for a group of antibiotics which are not primarily used to treat strept infection including penicillin and cephalosporins. The conclusion of strept infection as a common cause of sepsis and other infection, while a possibility and a clear risk, is also over-calling of the result in this context. These two limitations should be mentioned and rephrasing would make the paper more acceptable.

Language edit is also strongly recommended.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric, paediatric pulmonary and critical care, quality of health care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 May 2021

Birkneh Tilahun Tadesse, Hawassa University, Hawassa, Ethiopia

We thank the reviewer for taking the time to review our paper. As suggested, copyediting was done by a native speaker. We have reworded the first sentence of the discussion to indicate the possibility of severe infections following Streptococcal colonization.

Competing Interests: None!