Nasopharyngeal carriage of *Streptococcus pneumoniae* and antimicrobial susceptibility pattern among school children in South Ethiopia: post-vaccination era

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Abstract

**Objective:** The aim of this study was to investigate nasopharyngeal carriage rate and antibiotic susceptibility patterns of *Streptococcus pneumoniae* among school children.

**Results:** Three hundred eleven (43.8%) became culture positive for *S. pneumoniae*. The carriage rate among children, 3–5 years old was 62.5%, which was higher than the carriage rate of 38.6% among 6–13 years old children. Age ≤ 5 years and co-sleeping with siblings remained significantly associated with *S. pneumoniae* carriage. 155 (49.8%) of the isolates were resistant to co-trimoxazole, 152 (48.9%) of the isolates were resistant to tetracycline, and 88 (28.3%) of isolates were resistant to oxacillin. Multi drug resistant *S. pneumoniae* was observed in 90 (28.9%) of isolates. There is high prevalence of *S. pneumoniae* in primary school children in our study area. Relatively high carriage rate of resistance to oxacillin, tetracycline and co-trimoxazole were observed. These findings provide baseline data for future studies to further compare pneumococcal carriage rates and antibiotic resistance patterns.

**Keywords:** Nasopharyngeal, Carriage, *S. pneumoniae*, Sodo Zuria Woreda, Antibiotic, School children

Introduction

World health organization (WHO) estimates that ~ 1.6 million people, including up to 1 million children aged < 5 years, die of invasive pneumococcal disease every year, with developing countries bearing the greatest burden [1–4]. *Streptococcus pneumoniae* is a leading cause of childhood disease worldwide [5] which is responsible for 30% of pneumonia associated deaths in such settings [6].

*Streptococcus pneumoniae* is the leading cause of pneumonia in children and the most common cause of morbidity among infectious agents in Ethiopia [7]. Each year, 104,000 under five deaths are due to pneumococcal infections who are not vaccinated [8, 9]. The prevalence of nasopharyngeal carriage of *S. pneumoniae* varies according to age, geographic area, crowding, concomitant respiratory tract illness and antibiotic consumption. Prospective studies have revealed considerable intra-individual changes in nasopharyngeal carriage rates of *S. pneumoniae* over time [10–12]. The percentage of carriers also increases significantly in schools, orphanages, military camps and persons with chronic respiratory diseases [13].

Multidrug-resistant (MDR) *S. pneumoniae* clones have already spread throughout the world due to nasopharyngeal colonization over the last few years [10]. Antibiotic-resistant *S. pneumoniae* infections may require higher doses of antibiotic, longer duration of treatment and hospitalization, the use of more expensive medications, or use of medications with greater side-effect potential [14]. Because of the highest frequency of pneumococcal colonization and the highest crowding index are found in young children, this group is the most important vector for horizontal dissemination of pneumococcal strains within the community. Part of the strategy to prevent pneumococcal disease focuses on prevention of
nasopharyngeal colonization [10], accurate diagnosis and effective antimicrobial chemotherapy [15].

Early nasopharyngeal acquisition of *S. pneumoniae* is associated with both high carriage in young infants and a high rate of pneumococcal disease [16]. Since nasopharyngeal carriage is the necessary first step in the pathogenesis of pneumococcal disease [17], knowledge about the carriage rate is the necessary first step for effective prevention and control of pneumococcal disease.

Even after the introduction of Pneumococcal conjugate vaccine (PCV-10) in Ethiopia, *S. pneumoniae* mortality among under fives is still increasing [18]. PCV effectively induces protection against nasopharyngeal carriage of vaccine type pneumococci in young children as well as in older age groups, it is necessary to know the current carriage status of *S. pneumoniae* in order to allow evaluation on the role of vaccination, to determine risk factors for *S. pneumoniae* carriage and to assess the burden of antibiotic resistance on these strains.

### Main text

**Methods**

Institution based cross-sectional study was conducted in selected schools of Sodo Zuria Woreda of Wolaita Zone from Sept 5 to Dec 25, 2016. Sodo Zuria Woreda is one of the 12 districts in Wolaita zone. It has 31 kebeles (the smallest administrative structure in Ethiopia) and 7 governmental health centers. The total population is about 200,866. Sodo Zuria District consists of 43 primary schools. Within the town and the district, six primary schools namely, Shola Kuto, Waja Kero, Ofa, Gandeba, Delbo wogene and Waraza Gerera from Sodo zuria district were selected as the study schools.

All children in primary school who are ≤ 13 years of age were considered as the source population; and the study population was randomly selected primary school students below grade five. Students with wounded nose (nasal trauma and injuries) and previous history of infection who take antibiotics were excluded from the study.

The sample size was determined based on single population proportion formula by assuming a nasopharyngeal carriage of 88% according to a study in Gambia [19]. The expected margin of error (d) was 0.05 and the confidence interval (Zα/2) was 95%. With the above sampling parameter and a 10% contingency for possible non-respondents we came up with a sample size of 357. To improve the power of the test the sample size was further doubled and the final sample size was 714 primary school children.

A two-stage sampling technique was employed to draw a representative sample of 714 school aged and preschool children. Then lists of all primary schools having any of under 5th grades were identified by the help of the district education officials. Overall 25 schools, all children under the 5th grade were identified. A total of six schools were selected using probability proportional to size of enrolment. Children were allocated based on probability allocation to the size of each selected school, and participants were selected from the sampling frame.

The selection of primary schools was based on probability proportion to size (PPS); the number of schools required from the district six to derive the sampling interval (SI) divided the total population of attendants in each school. The first cluster was selected by multiplying the sampling interval by a random computer generated three-digit decimal number between 0 and 1. The resulting number was traced in the cumulative population column, and the first cluster was taken from the corresponding school. The following clusters were identified by adding the sampling interval to the previous number.

Questionnaires were completed by interviewing the parents (guardians). Clinical data, the medical history of the children and associated risk factors were collected by trained nurses in collaboration with kindergartens.

Nasopharyngeal specimens were collected by laboratory technicians using rotating cotton-tipped flexible swabs; during the smooth insertion through the nostrils of each individual, whose head was tipped backward; the swabs were left in the posterior nasopharynx for 5 s to saturate the tip. Once a swab specimen was collected, it was placed in a tube of skim milk–tryptone–glucose–glycerin (STGG) transport medium and transported to the WSU microbiology laboratory within 1 h of collection for processing.

A loopful nasopharyngeal specimen from STGG tube was inoculated onto colombia agar (Oxoid, England) on plates containing 5% sheep blood and 5 µg/ml gentamicin. The inoculated plates were incubated for 24 h at 35–37 °C in an atmosphere of 5–10% CO2 using a candle jar. *S. pneumoniae* was identified by its colony morphology, haemolytic activity, optochin susceptibility and bile solubility [20]. Zones of inhibition > 14 mm indicate susceptibility, 7 to 13 mm are indeterminate and a zone < 7 mm is resistant to optochin when a 6 mm size disc is used and the culture is incubated in 5% CO2 [21].

The Kirby–Bauer disc-diffusion test was used to determine antibiotic susceptibility testing. A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used for evenly inoculating onto Muller–Hinton agar (Oxoid, England) plates containing 5% sheep blood and allowed to dry. The plates were incubated at 37°C for 24 h, and the diameters were measured [22]. Isolates were classified as susceptible, intermediate resistant or resistant to the antibiotics based on the recommendations of Clinical Laboratory Standard Institute (CLSI) 2014.
Multidrug resistant defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [23]. The quality of transport media, culture media and antimicrobial susceptibility was checked using a standardized reference strain of S. pneumoniae ATCC 49619. The data was edited, cleaned, and restructured in Epi-data analysis v2.2 then exported to SPSS version 21, for analysis. Bivariate analysis and multiple logistic regressions were used. P value less than 0.05 was used to declare statistical significant association.

Result

Socio-demographics information

710 out of 714 children were involved in the study. The age of these children ranges from 3 to 13 years giving the mean age of 8.06 (95% CI (7.88, 8.24)), 50.3% of the children were females. (Additional file 1: Table S1). Table 1 Nasopharyngeal carriage rate of S. pneumoniae in relation to age and sex, Sodo Zuria Woreda, South Ethiopia, 2014

| Age       | Carriage status (n = 311) | Male | Female | Total (%) |
|-----------|--------------------------|------|--------|-----------|
|           |                          | Positive (%) | Negative (%) | Female Positive (%) | Negative (%) | Total (%) |
| 3–5 years | 28 (9)                   | 18 (9) | 45 (28.3) | 29 (14.6) | 120 (16.9) |
| 6–13      | 124 (39.9)               | 183 (91) | 114 (71.7) | 169 (85.4) | 590 (83.1) |
| Total     | 152 (21.4)               | 201 (28.3) | 159 (22.4) | 198 (27.9) | 710 (100) |

Risk factors associated with S. Pneumonia carriage

Age ≤ 5 years and slept with siblings remained significantly associated with S. pneumoniae carriage. The odds of carrying pneumococci were approximately twice as high among those children (Table 2).

Antimicrobial susceptibility pattern

Resistance rate of 48.9% to tetracycline, 45.3% to co-trimoxazole and 28.3% to oxacillin were isolated. The lowest resistance rate was exhibited by chloramphenicol which was 12.5% (Additional file 2: Figure S1).

Antibiogram pattern

Out of 311 strains, 90 (28.9%) were multidrug resistant, 23 (10.9%) strains were resistant to 3 antimicrobials, nine isolates were resistant to 4 antimicrobials and 4 (2.1%) of the isolates were pan resistant (Table 3).

Table 2 Risk factors associated with nasopharyngeal carriage of S. pneumonia among school children, Sodo Zuria Woreda, South Ethiopia, 2014

| Associated factors | Carriage rate | No (399) | Yes (311) | P-value | COR (95% CI) | P-value | AOR (95% CI) |
|--------------------|---------------|----------|-----------|---------|--------------|---------|--------------|
| Male               |               | 201      | 152       | 0.85    | 0.96 (0.62–1.48) |         |              |
| Age ≤ 5 years      |               | 47       | 73        | 0.00    | 2.64 (1.46–4.78) | 0.02    | 2.09 (1.13–3.87) |
| Having at least one sibling |   | 356      | 284       | 0.50    | 1.22 (0.50–2.53) |         |              |
| Living with one or more siblings < 6 years old | | 217 | 182 | 0.04 | 1.61 (1.03–2.51) |         |              |
| Co-sleeping with siblings | | 229 | 233 | 0.00 | 2.25 (1.41–3.60) | 0.01 | 1.89 (1.15–3.09) |
| Passive smoking    |               | 42       | 63        | 0.01    | 2.39 (1.30–4.39) |         |              |
| Income < 500 birr/month |       | 127      | 114       | 0.21    | 1.33 (0.85–2.08) |         |              |
| Have one room in the house | | 89       | 78        | 0.30    | 1.30 (0.79–2.15) |         |              |

COR crude odds ratio, AOR adjusted odds ratio

Discussion

Streptococcus pneumoniae carriage rate in healthy children under 5 years of age ranged from 20 to 93.4% in low income countries which was generally higher than...
reported in lower-middle income countries (range 6.5–69.8%) [24]. The carriage rate of *S. pneumoniae* among children ≤ 5 years, 62.5%, in this study was corroborated with the cumulative prevalence in African countries 64.8% [24] and Kenya 56.7% [25] but higher than the reported carriage rates elsewhere [24–31]. A 38.6% carriage rate in children aged 5–15 years in the current study is in harmony with 41% reported in Kenya [32] but much higher findings, 53.6% for 5–10 years old children in northern India [33] and, > 80% for children aged 5–14 years in Gambia were also documented [3]. On the contrary, much lower prevalence, 8.2% for 10–19 years old children in Brazil [34] as compared with this study were noticed. Possible reasons for the difference in the carriage rate might be due to variation in specimen, age, geographic area, socio-demographic factors, the availability of PCV vaccine, and the study time.

In the literature, colonization rates were independently related to risk factors such as young age [30, 34–37], day care center attendance [27, 35] having young siblings [30, 35, 36], the number of bedroom occupants <5 years of age [36] and exposure to passive smoke in the household [38]. In our study, nasopharyngeal carriage of *S. pneumoniae* was high on children ≤ 5 years old and on those who co-sleeping with his/her siblings. The decline of carriage rate as age increases could be as a result of decreased close contact among adults as compared with young children. However, it could also reflect acquisition of local mucosal immunity as a result of repeated colonization by many different serotypes [32].

Penicillin resistant in various studies has been reported to be as low as 2.2% [27] to as high as 83.5% [39]. Penicillin resistance rate in Ghana was 37.3% [40], which is nearly comparable with the current 28.3% result. High resistance to co-trimoxazole, 45.3% was noted in this study which was also evidenced across literature from different parts of the world [2, 33, 38, 41, 42]. In the contrary to this, much lower finding, 12.7% were documented from Zambia [15]. Seventeen percent resistance to erythromycin in the current study differed with no resistances elsewhere [2, 3, 40] to 96%, 77% and 33.5% resistance in Taiwan [38], Hong Kong [41], and Greece [4] respectively. In this study, we found 48.9% resistance to tetracycline, which is nearly comparable with 42.9% resistance from Spain [13]. A higher prevalence of tetracycline resistant *S. pneumoniae* isolates, 63.4% and 60.8% were reported from Ghana [40] and India [33] respectively. But in some other countries, lower tetracycline resistance rate of 32.3% [3], 26.4% [4], 23% [15] and 1% [43], were also reported. Low resistance prevalence to chloramphenicol were reported from African countries: 13.3% from Ghana [39], 6.3% from Gambia [3], 3.9% from Zambia [15] and 4% from Kenya [2], which is comparable with our 12.5% finding. But in contrast to our study, 33.7% was also reported from Hong Kong [41]. A wide variation in antibiotic resistance rate of *S. pneumonia* emphasizes the need for periodic local surveillance, in order to prevent and manage pneumococcal disease.

| Frequency of resistance | Antibiotics pattern | No | % |
|-------------------------|---------------------|----|---|
| R₀                      | Susceptible to all drugs | 97 | 31.2 |
| R₁                      | Oxacillin            | 12 | 3.9 |
|                         | Tetracycline         | 24 | 7.7 |
|                         | Co-trimoxazole       | 27 | 8.7 |
|                         | Oxacillin/tetracycline | 12 | 3.9 |
| R₂                      | Oxacillin/co-trimoxazole | 17 | 5.4 |
|                         | Tetracycline/co-trimoxazole | 16 | 5.1 |
|                         | Tetracycline/chloramphenicol | 9 | 2.9 |
|                         | Tetracycline/erythromycin | 7 | 2.3 |
|                         | Oxacillin/tetracycline/co-trimoxazole | 36 | 11.6 |
| R₃                      | Tetracycline/co-trimoxazole/erythromycin | 14 | 4.5 |
|                         | Tetracycline/co-trimoxazole/chloramphenicol | 8 | 2.6 |
|                         | Co-trimoxazole/chloramphenicol/erythromycin | 9 | 2.9 |
|                         | Oxacillin/tetracycline/co-trimoxazole/erythromycin | 10 | 3.2 |
| R₄                      | Oxacillin/tetracycline/chloramphenicol/erythromycin | 9 | 2.9 |
| R₅                      | Oxacillin/tetracycline/co-trimoxazole/erythromycin/chloramphenicol | 4 | 1.3 |
Conclusion
Nasopharyngeal carriage of *S. pneumoniae* among school children can be a potentially risk for children. Children ≤ 5 years age and co-sleeping with siblings play a role in pneumococcal carriage. Higher prevalence of resistance to oxacillin, tetracycline and co-trimoxazole was observed. Hence, nationwide surveillance for resistance pattern and serotype distribution in Ethiopia is necessary to guide the rational choice of antimicrobial agents.

Limitation
Serotyping assessment was not conducted.

Additional files

**Additional file 1:** Table S1. Socio-demographic characteristics of school children in Sodo Zuria Woreda, South Ethiopia, 2014.

**Additional file 2:** Figure S1. Antibiotic susceptibility pattern of *S. pneumoniae* strains, Sodo Zuria Woreda, South Ethiopia, 2014.

Abbreviations
AOR: adjusted odds ratio; ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Science Institute; COR: crude odds ratio; IPD: invasive pneumococcal disease; MDR: multi drug resistant; PCV: pneumococcal conjugate vaccine; RTI: respiratory tract infection; SPSS: Statistical Package For Social Science; STGG: skim milk–tryptone–glucose–glycerin; WHO: World Health Organization.

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Authors’ contributions
FWW, FBS: conceived the study, FWW, FBS, EGT, and TMB: participated in the design of the study and performed the statistical analysis, FBS, FWW, EGT, TMB: interpreted the data; FBS, FWW, TMB, EGT: supervised data collectors: FBS: drafting the article or revisiting it critically for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available. Anyone interested can get upon reasonable online request by writing to fitha2007@yahoo.com.

Ethical approval and consent to participate
Ethical clearance was obtained from Ethical Review Committee at College of Health Sciences and medicine in Wolaita Sodo University. Letter of cooperation was taken from zonal education department and informed verbal consent from the heads of the schools was obtained. Permission was obtained from the parents or legal guardian and verbal assent from the selected child was obtained after informing about the purpose of the study, their right to proceed or withdraw from study anytime they want anonymity and confidentiality of the study, which was maintained strictly throughout. The objectives as well as the nature of the study were explained to the parents and study participants. The study participants were informed about risks concerning specimen collection which is a little discomfort and sneezing during inserting of the swab. The final result wasn’t linked to individual students.

Consent to publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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