RESEARCH ARTICLE

Pneumococcal carriage and antibiotic susceptibility patterns in mother-baby pairs in a rural community in Eastern Uganda: a cross-sectional study [version 1; peer review: 1 approved, 1 not approved]

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Abstract

Background: Pneumonia poses a significant threat to the lives of children below five years old worldwide, contributing to a high number of hospitalizations and death. Morbidity and morbidity are especially common in children under five and the elderly, although any age group can be affected. This study aimed to estimate pneumococcal carriage and determine antibiotic susceptibility patterns of the pneumococci isolated from mother-baby pairs in Ngora district after the rollout of the pneumococcal vaccine. We hypothesized that high carriage of *Streptococcus pneumoniae* in mothers leads to carriage in their babies and hence a greater chance of contracting pneumonia.
Methods: Consecutive sampling was used to select 152 mother-baby pairs from community visits and those seeking care at the health facility. We collected nasal swabs from both baby and mother for culture and sensitivity testing using the Kirby-Bauer's agar disc diffusion method.
Results: This study found that there was a low prevalence of pneumococcal carriage in the mother-baby pair in Ngora district. We also observed high rates of microbial resistance to penicillin, which is the first-line drug for the management of pneumonia in Uganda.
Conclusions: The relationship between pneumococcal carriage and immunization status suggests that the pneumococcal vaccine is protective against pneumococcal carriage. Resistance of *S. pneumoniae* to commonly used antibiotics was high.

Open Peer Review

Reviewer Status ✓ ✗

	Invited Reviewers	
	1	**2**
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Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Pneumococcal carriage, mother-baby pair, antibiotic susceptibility pattern, immunization with PCV 10, Eastern Uganda.

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Competing interests: No competing interests were disclosed.

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Introduction

Pneumonia poses a significant threat to the lives of children below five years worldwide, contributing to a high number of hospitalizations and death[1–3]. Morbidity and morbidity is especially common in children under five and the elderly, although any age group can be affected[4,5]. One third of the overall global childhood mortality due to pneumonia has been attributed to *Streptococcus pneumonia*[4]. Developing countries are now faced with the double burden of community-acquired and nosocomial pneumonia, with both the under-funded health care systems and individual caretakers of the affected patients bearing negative economic consequences[6]. *Streptococcus pneumoniae* has been reported to be the most commonly isolated bacteria in both community-acquired and nosocomial pneumonia[5]. A high proportion of mortality due to community-acquired pneumonia has been documented, with up to 81% of these deaths occurring outside of hospital due to challenges related to access to health care in low- and middle-income countries[7]. The evolution of antimicrobial resistance among organisms causing pneumonia has made the consequences of childhood pneumonia even worse.

Over two million children die annually from pneumonia each year, accounting for almost 1 in 5 under five deaths in low- and middle-income countries on average, as compared to 1 in 66 children in high-income countries[8].

Pneumonia, together with diarrhea and malaria, was responsible for the deaths of 2.2 million children under five in 2012 in Sub-Saharan Africa, accounting for a third of all deaths in under-fives in this region[9]. In Uganda, pneumonia kills around 24,000 children below the age of five every year[2]. The overall pneumococcal carriage among children under five was estimated to be 56% when the PCV-10 and PCV-13 coverage in Uganda was 42% and 54%, respectively[4]. With improved PCV-10 and PCV-13 vaccine coverage, most of the invasive serotypes should be covered[4]. Pneumococcal carriage among mothers may lead to pneumococcal infections in children by droplet infection. This study aimed to estimate pneumococcal carriage and determine antibiotic susceptibility patterns of the pneumococci isolated from mother-baby pairs in Ngora district, Eastern Uganda.

Methods

Ethical statement

We obtained ethical approval for this study from Mbale Regional Referral Hospital Research and Ethics Committee (MRRHREC060418). A letter of introduction to the study was obtained following approval from the Busitema University Faculty of Health Sciences community education program office and used by the researchers to seek permission from the Medical Superintendent of Ngora District Health Centre IV. Written informed consent for participation was obtained from participants preceding to the actual data collection exercise. Access to collected data was restricted to persons directly involved in the study only. Participants were free to withdraw their consent to participate in the study at any stage of the study. Such a decision did not affect the medical care they received or possible participation in future research studies in any way. The procedures were verbally explained to the research participants by the researchers and those who agreed to continue with the study could consent and participate.

Research design and setting

A cross-sectional study was carried out in Ngora district Health Centre IV and Ngora Health Center IV catchment area from 7th April to 5th May 2018. It which serves a population of approximately 142,487. Ngora district is one of the districts in Teso sub-region and was part of Kumi district until 2010, when it was established as a separate district by a Uganda parliamentary act. Ngora district covers an area of approximately 715.9 square kilometers and is predominantly inhabited by the Iteso and Kumam ethnicities. According to the Ugandan healthcare hierarchy of organizations, a Health Center IV is expected to serve a population of up to 100, 000 people, meaning at its current capacity, this health facility operates above the level of a Health Center IV[10].

Study population and sampling

Children under five years old and their mothers were selected in a 'mother-baby pair' to determine the relationship between carriage of pneumococci in the baby, prevalence of pneumococci in mothers and the child's immunization status. The inclusion criteria were mother –baby pairs from selected villages in the community, and those who visited Ngora Health Center IV for routine immunization with babies under the age of five years. For this study, we defined a baby as any person under the age of five years and a mother was considered as either biological or any other female in direct care of the baby for at least four weeks. This was aimed at comparing samples from the baby, and the person in closest contact with the baby for the longest duration of time in the most recent past to allow presumed cross transmission/cross-infection. Exclusion criteria were babies above five years, and those presented by male care takers as those were presumed to spend less time with the babies. Mothers who did not consent or opted out at any stage of the research were excluded as well. The purpose of the research was explained to the mothers and they were asked to voluntarily participate in the research.

Consecutive sampling was used. Every participant meeting the criteria of inclusion was selected at the young child clinic and in randomly selected villages in the community until the required sample size was achieved. This method is relatively easy to employ and reduces the chances for intentional and unintentional manipulation by staff, or errors due to confusion. A total of 152 mother-baby pairs fulfilling the inclusion criteria were sampled. Sample size was estimated using the Kish and Leslie formula developed in 1965.

$$N=\frac{z^2(pq)}{e^2}$$

Where N = sample size, z = confidence level at 95 % (standard value of 1.96), p = estimated prevalence of pneumonia in Uganda (27.2%)[11], q = 1-p (72.8%) and e = margin of error at 5%. Therefore, N = 304.

Data collection and variables

Demographic data was captured using a pre-tested questionnaire. The questionnaire was pre-tested with 10 mother-baby

pairs at Mbale regional referral hospital Young Child Clinic (YCC) because the mother-baby pair in Mbale hospital had homogenous characteristics with our target population in Ngora district. After pre-testing of the questionnaires, some questions were rephrased for clarity. The Uganda national immunization guidelines require all children to receive their first PCV dose at six weeks (1.5 months), second dose at 10 weeks (2.5 months) and third dose at 14 weeks (3.5 months). Therefore, in the questionnaire, full immunization was defined as any child 14 weeks (3.5 months) of age and above, who had received all the three PCV doses as stipulated in the national immunized schedule. Partial immunization included children above six weeks (1.5 months) of age who had received less than three doses of PCV, whether off schedule, or still on schedule as per the national immunization guidelines. All children below five years of age who had not received any single dose of PCV including those at below six weeks (still on schedule) were categorized as not immunized.

Specimen collection and transport

Nasal pharyngeal specimens were collected from the posterior nasopharynx of the mother and the baby using sterile cotton swab sticks moistened with 0.9% physiological saline. Sample collection was carried out at the young child clinic in Ngora Health Centre IV and at the selected villages in the community by qualified hospital laboratory technicians. Separate swabs were used to collect samples from a mother and a baby in a mother-baby pair. To prevent sample contamination, the swab was placed in a casing containing Amies transport medium and immediately placed into a cool box containing ice packs for transportation to Busitema University Microbiology laboratory for culture and susceptibility testing within 12 hours.

Laboratory procedures

Samples were cultured on sheep blood agar and chocolate agar, followed by 24 hours of incubation at 37°C anaerobically. The isolates were identified morphologically by colony appearance and Gram staining. Optochin sensitivity and bile solubility testing were conducted on colonies that were potentially identifiable as *S. pneumoniae* by alpha-haemolytic appearance on the culture media and lancet shaped Gram-positive cocci appearing in pairs.

A 0.5 McFarland standard of *S. pneumoniae* was made from a 24-hour subculture by suspending colonies in sterile normal saline and inoculated by swabbing onto a plate of Mueller-Hinton agar supplemented with 7% sheep blood for susceptibility testing. Antibiotic susceptibility to penicillin G (1U), chloramphenicol (30µg), tetracycline (10µg), clindamycin (2µg), erythromycin (30µg) and ceftriaxone (30µg) was determined using modified Kirby-Bauer agar disc diffusion methods and the disc zone diameters were interpreted using the Clinical and Laboratory Standards Institute Guidelines[12].

Data management

Collected data were entered in Microsoft Excel 2010, cleaned, coded and imported to SPSS Version 16.0 statistical package for analysis. Data were analyzed using descriptive statistics, frequencies and bivariate analyses (cross-tabulations). The primary outcome was pneumococcal carriage and the secondary outcome was antibiotic resistance. Statistical frequency distribution tables and graphs were used for data presentation in terms of proportions, absolute values, percentages and confidence intervals for point approximations at 95% level of confidence, with $P<0.05$ considered as statistically important.

Data quality control

Laboratory procedures were performed by laboratory scientists under close supervision of a clinical microbiologist to ensure quality results were obtained. Data was double entered into Microsoft Excel for accuracy and reliability. ATCC 49619 *S. pneumoniae* was used as a control strain for quality assurance during isolation and drug susceptibility testing.

Results

Demographic characteristics of the study participants

At the young child clinic, a total of ninety-three pairs were sampled, two of whom were excluded because the babies were presented by male care takers, and one mother opted out due to fear of discomfort associated with the procedure for sample collection. Ninety pairs were recruited. In the community, a total of sixty-nine pairs were sampled, five were excluded because they presented children above five years of age, while two were presented by male caretakers. Sixty-two were recruited, making a total of 152 mother-baby pairs.

The study participants comprised of 152 mothers and 152 babies. Of the 152 babies, 74 were male and 78 were female with the age range of 0–59 months. The youngest mother was 16 years, whereas the oldest was 44 years. None of the mothers who participated in the study reported having formal employment[13].

Prevalence of pneumococci in mother-baby pairs

During the study, 304 samples were collected; 152 from the mothers and 152 from the children, making 152 mother-baby pairs. All samples were cultured, and antibiotic susceptibility was carried out on the isolated pneumococci. Out of 152 samples from the mothers, only five (5/152) isolates of pneumococci were obtained whereas seven (7/152) were isolated from the babies. Only one mother-baby pair (1/152) was found to be colonized with pneumococci in both mother and baby and the rest of *S. pneumoniae* colonized either the mother or baby.

Immunization coverage

During data collection, immunization status of the baby was categorized as fully immunized, not immunized and partially immunized among different age groups (Figure 1). There was high immunization coverage among the children above 12 months old but lower in the 3.5-<12 age group.

Antibiogram

The antibiotic susceptibility testing was done on positive isolates for both mother and baby.

Generally, a high trend of anti-microbial resistance was observed among the *S. pneumoniae* isolated (Table 1). The highest resistance patterns were recorded with chloramphenicol (50%)

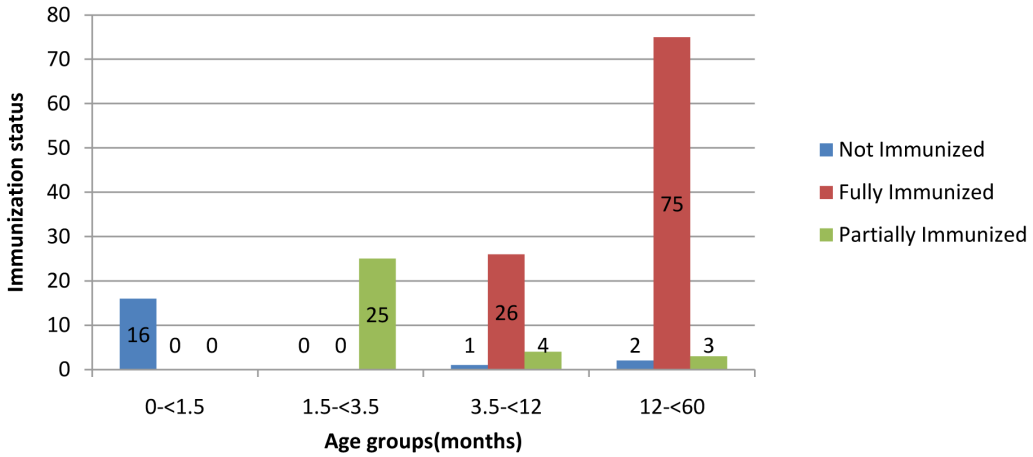

Figure 1. PCV-10 immunization coverage among children under five years old.

Table 1. Antibiotic susceptibility patterns of *S. pneumoniae* to the commonly used antibiotics.

Drugs	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Penicillin G	6 (50)	3 (25)	3 (25)
Chloramphenicol	6 (50)	0 (0)	6 (50)
Tetracycline	4 (33)	2 (17)	6 (50)
Clindamycin	9 (75)	1 (8)	2 (17)
Erythromycin	8 (67)	0 (0)	4 (33)
Ceftriaxone	8 (67)	1 (8)	3 (25)

and tetracycline (50%), whereas the lowest resistance was recorded in clindamycin (17%).

Babies that were fully immunized had a lower likelihood of being colonized by *S. pneumoniae* than their non-immunized counterparts $P<0.05$. Other factors examined by this study were not significantly associated with colonization with *S. pneumoniae* among the babies.

Discussion

We determined the prevalence of pneumococcal carriage and factors associated with colonization of pneumococci in a mother-baby pair in our study. Out of the 304 nasal swabs cultured, only 12 (3.95%) were positive for pneumococci, seven (4.61%) in children under five and five (3.29%) in mothers. We report a low carriage of pneumococci among mothers and babies that were included in this study. In contrast, a previous study in Iganga/Mayuge reported high carriage rates of over 50% in children aged under five years[4]. In the Iganga/Mayuge study, participants were selected on the basis of presentation with pneumonia symptoms as defined by WHO guidelines, as opposed to our study, which included all children that fulfilled our selection criteria and did not take into account signs and symptoms of pneumonia. In a similar study carried out in Kenya, 90.0% of children were colonized with pneumococci. Both the Iganga/Mayuge and Kenyan studies were carried out prior to the introduction of PCV-10, accounting for the difference in carriage observed in our study. Different studies have shown varied carriage rates of pneumococci among children under five in Uganda and elsewhere[4,14,15], with most of them reporting a higher carriage rate than reported in our study. A systematic review reported the carriage rate in Africa to range between 21–94%[16], with more studies done among children than in adult population. The high immunization coverage for PCV-10 in Ngora district could further explain the low carriage rate of pneumococci in our study as opposed to the Iganga/Mayuge study, which indicated a high carriage rate of 56% at a lower immunization coverage of 42% for PCV-10 and 54% for PCV-13. There was a statistically significant relationship between the pneumococcal carriage and immunization status of the babies in our study (Table 2). Pneumococcal carriage is a prerequisite for disease; therefore, our findings suggest that full immunization with PCV-10 is protective against pneumococcal carriage and hence pneumonia caused by *S. pneumoniae*. Several studies have reported a decrease in the burden of invasive pneumococcal disease and serotype distribution since the introduction of the PCV vaccines[17–20]. The immunization coverage for the first dose of PCV-10 (PCV1) in Ngora district in the current study was 97.78% (133/136), and 2.22% (3/136) of children above six weeks had not received PCV1. Of the 152 participants, 10.53% [16] were children below six weeks and were therefore not eligible for immunization with PCV10. The immunization coverage for PCV3 was 90.99% (101/111). Children below fourteen weeks who had not received PCV3 were excluded from the denominator because they were not eligible.

We also report a low carriage rate of pneumococci among the mothers. A similar study in coastal Kenya indicates that pneumococcal carriage was associated more with children under five than adults[15]. The low carriage rate of pneumococci among adults has been attributed by other studies to the development

Table 2. Factors associated with pneumococcal carriage.

Factors	Odds ratio	P-value	(95% CI)
Age group (months) 0-<1.5 1.5-<3.5 3.5-<12 12-<60	1.3603	0.478	0.5818-3.1804
Immunization status	7.1617	0.010	1.5939-32.1791
Sex of the babies	2.4658	0.290	0.4633-13.1224
Age of the mother	0.9025	0.194	0.7731-1.0536

of natural immunity[21]. The upper respiratory tract apparently appears a disadvantageous niche for *S. pneumococci* due to the development of mucosal host defenses such as sIgA[22,23]. Also vaccination with PCV has resulted in the development of herd immunity in the adult population against *S. pneumoniae*[15]. Cases of pneumococcal colonization are, however, reported to rise in the elderly population due to immune senescence[24], with many countries not considering the importance of immunization to this group of people.

In our analysis, there was no statistically significant association between carriage of pneumococci and sex/gender of the child. This finding is similar to the results of a systematic review in Africa, which noted that there was no association between pneumococcal carriage and gender[16], although one study associated pneumococcal carriage with males and the other that reported association of carriage with females.

A trend of antimicrobial resistance was observed in chloramphenicol, tetracycline and erythromycin. Other studies have similarly reported resistance of pneumococci to commonly used antibiotics[25,26]. In a study of erythromycin-resistant *S. pneumoniae*, 81% of the isolates were resistant to tetracycline and 76% were multi-drug resistant, whereas 12% were resistant to clindamycin, tetracycline, chloramphenicol and kanamycin combined[25]. As opposed to our study, low resistance rates to tetracycline, erythromycin, chloramphenicol and ceftriaxone were reported in Tanzania[26]. Again, contrary to our findings, an earlier study in Uganda reported no resistance to erythromycin and ceftriaxone[27], indicating the emergence of antimicrobial resistance against those drugs, which may be attributed to the irrational use of antibiotics in Uganda and also due to the fact that such drugs are given empirically since there is no laboratory capacity to carry out culture and sensitivity studies.

Limitations

We were not able to serotype the pneumococci isolated to determine the circulating serotypes and to link pneumococcal carriage to development of the disease.

Conclusions and recommendations

We report low pneumococcal carriage in the mother-baby pair in Ngora district. There was no significant relationship between pneumococcal carriage in the mother and prevalence in the baby. The relationship between pneumococcal carriage and immunization status suggest that PCV-10 is protective against pneumococcal carriage. Resistance of *S. pneumoniae* to commonly used antibiotics was high.

Data availability

Underlying data

OSF: Pneumococcal carriage and antibiotic susceptibility patterns in mother-baby pairs in a rural community in Eastern Uganda: a cross-sectional study. https://doi.org/10.17605/OSF.IO/H9X7R[13]

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Current Peer Review Status: ✔ ✘

Version 1

Reviewer Report 11 January 2021

https://doi.org/10.5256/f1000research.24529.r76579

✘ **Adnan Al-Lahham**
School of Applied Medical Sciences, German Jordanian University, Amman, Jordan

The article "Pneumococcal carriage and antibiotic susceptibility patterns in mother-baby pairs in a rural community in Eastern Uganda: a cross-sectional study" should be a good one since it contains results of the pneumococcal carriage in the rural area of eastern Uganda.
comments to the article:

1. Regarding the abstract: It should contain at least the summary of the results obtained but has no results at all, and it has some mistakes like morbidity and morbidity. One more comment is that the background starting to talk about pneumonia which has nothing to do with the research results. A third comment on the abstract: it contains only text and the results are only two lines.

2. The Introduction contains repeated data as in the abstract background, and it talks mainly about the pneumonia not carriage.

3. In the methodology: There are mistakes like Nasal Pharyngeal, not Nosopharyngeal. Secondly, in the laboratory procedures, it is stated that samples were cultured anaerobically which is wrong and the CLSI reference from 2014 is not the recent one.

4. In the results:
   a. 152 samples were taken and cultured from mothers and children as pairs. As a result, only 12 isolates were obtained. This result is not accepted.
   b. Why immunization status, since there is no data showing these cases are vaccinated or not.
   c. The table with the sensitivity shows only the 12 strains which is not ok since the method is not valid for the isolation.
   d. There are no data available about the serotypes or the macrolide resistant phenotypes or genotypes

As a result: This paper is not eligible for indexing and cannot be accepted from my side.

Is the work clearly and accurately presented and does it cite the current literature?
No

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

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

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Streptococcus pneumoniae resistance, epidemiology and typing

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 28 Sep 2021

Jacob Stanley Iramiot, Busitema University, Mbale, Uganda

The abstract has been improved for a better read. A thorough review of grammar and other language issues has been done. There have been a couple of mentions of pneumococcal carriage as a precursor to pneumonia in children which should better inform the audience. The authors mentioned the CLSI 2014 because that was the version available and used by the team then. The authors acknowledged that they were not able to do serotyping in the limitation section of the manuscript. About the validity of the methods, it is not clear to the authors what the reviewer really means by this because standard methods and procedures were followed and as available in our setting.

Competing Interests: No competing interests were disclosed.

Reviewer Report 01 December 2020

https://doi.org/10.5256/f1000research.24529.r73502

Wilber Sabiti
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The authors sought to decipher the relationship between pneumococcal carriage and PCV-10 vaccine. They asked whether the vaccine had an impact on the carriage of pneumococcal bacteria among the under-five children. In a cross-sectional study involving participants from Ngora district health centre IV and its catchment area, they showed a significant reduction of carriage following vaccination. Although the sample size was small in relation to the population covered by Ngora health centre IV, having a baseline carriage prior to vaccination and prevalence in the area helped give credence to the findings and conclusions. Nevertheless, it is not clear whether the authors used purposive or random sampling. Random sampling would have been better for such a sample in a large population. The article is well written, easy to read and follow and the conclusions are reflective of the findings. The discussion is well balanced putting the findings into context of other studies done before. The study adds evidence to the value of pneumococcal vaccine and I strongly recommend a follow on study covering the whole country. The addition of an immunological analysis arm to the study would serve as evidence that the observed associations are indeed due to vaccination.

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?
Yes

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?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I have expertise in Microbiology, Molecular biology and Immunology. I understand all aspects of the paper. It will be great for authors to explain their sampling method and why they chose it.

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.

Author Response 28 Sep 2021

Jacob Stanley Iramiot, Busitema University, Mbale, Uganda

Thank you very much for that very useful feedback

Competing Interests: I declare no conflict of interest