

Original Research Article

Impact Assessment of Pneumococcal Conjugate Vaccination on Nasopharyngeal Pneumococcal Carriage: A case study of vaccinated and unvaccinated Children under-Five Years attending Mbale Municipality Health Centers, Eastern Uganda.

Running Title: Pneumococcal Carriage in Mbale district

ABSTRACT

Aims: To determine the nasopharyngeal carriage of *Streptococcus pneumoniae* among children under-5 years of age who have been vaccinated with Pneumococcal Conjugate Vaccine (PCV) and those unvaccinated in Mbale Municipality Health Centres.

Study design: Cross sectional study

Place and Duration of Study: Mbale Municipality Health Centres, between September 2015 and June, 2016.

Methodology: We enrolled 280 participants (140 vaccinated with pneumococcal conjugate vaccine and 140 unvaccinated). Demographic data and vaccination status were obtained from the care takers, evidenced by the vaccination card. We collected nasopharyngeal swabs that were bacteriologically investigated to isolate and identify *Streptococcus pneumoniae*. Pneumococcal carriage was defined by a positive culture, with an identification

of *Streptococcus pneumoniae*.

Results: The overall prevalence of *Streptococcus pneumoniae* was 123 out of 280 (43.93%; 95% Confidence interval: 39.88-47.69). The prevalence of pneumococcal carriage among the pneumococcal conjugate vaccinated children was 14.29% (95% CI: 11.33-16.73), compared to the 85% (95% CI: 81.34-88.61) among unvaccinated children. The odds ratio of pneumococcal carriage was 7.4 among the unvaccinated children compared to the PCV immunized children.

Conclusion: Vaccination with PCV offers protection to the risk of pneumococcal carriage. Grounded on this, vaccination of children is key to prevention against pneumonia infection and the associated mortality.

Keywords: Children under-five years, nasopharyngeal carriage, Pneumococcal Conjugate Vaccine, *Streptococcus pneumoniae*.

1. INTRODUCTION

Pneumonia is the leading cause of mortality among children under-five years of age, and is an independent predictor of childhood mortality. It kills more children under-five years than human immune deficiency virus (HIV), malaria, and measles combined [1-3]. Worldwide, pneumonia accounts for 16% of the under-five mortalities [4].

There are estimated 35-93% risks of pneumococcal colonization among children under-five years of age, mainly from low income countries [5-7]. The high risk of infection with pneumonia is ascribed to the potent causative agents; the most common being bacteria and viruses. It is caused by *Streptococcus pneumoniae*, *Haemophilus influenzae type b* (Hib), and respiratory syncytial virus (RSV). *S. pneumoniae* is the most common cause of bacterial pneumonia, the second being Hib, followed by RSV [7-11].

Streptococcus pneumoniae accounts for a third of all childhood pneumonia-associated deaths [12]. It is found in the child's nose or throat; from where it is then aspirated into the respiratory secretions to colonize the nasopharynx and ultimately cause blood-borne disease [8, 9].

Worldwide, interventions to reduce childhood pneumonia have been recognized. These vary from exclusive breastfeeding for the first six months, apt antibiotics use in case management, and pragmatic vaccinations against *Haemophilus influenzae* type b, pneumococci and measles [10, 11]. In line with this, Uganda initiated a 10-valent Pneumococcal Conjugate Vaccine (PCV-10) to its National Immunization Program In 2014 [2] as a public health intervention to prevent pneumococcal infection. This has however not brought up the much desired health impact as the burden of pneumonia among children under-five remains high. At the same time, there remains paucity of data regarding the PCV-10 protective effect as a measure of vaccine efficacy as roll out programs intensify. To this, we report on pneumococcal infection carriage burden among children vaccinated with PCV-10 and the unvaccinated. This is hoped to serve as a basis for enacting prevention and sensitization strategies in this region.

2. MATERIAL AND METHODS

Study design, population: This was a cross sectional laboratory based study that involved bacteriologic examination of nasopharyngeal swab samples to investigate the effect of pneumonia among children attending Namakwekwe, Namatala, Maluku, Busamaga and Wanale health centers. Reported pneumonia symptoms were defined as guided by World Health Organization. In this context, cough and/or difficult breathing, and fast breathing (with or without fever). Upper respiratory tract infections were considered whenever there was cough or runny nose without fever, difficult breathing, or fast breathing.

Sample: We analyzed nasopharyngeal swabs. This was collected after the caretakers signed an informed consent. The swabs were taken by trained technicians using a pre-packed sterile calcium alginate flexible swab (*Copan, Brescia, Italy*). The first author, who oversaw the sample

collection process and conducted training before the commencement of the study is well conversant with the technique, having led three teams that were involved earlier surveys. Swabbing was done at the back of the nasopharynx as guided by World Health Organization (2013). Following collection, swabs were placed in a millilitre of skim-milk tryptone glucose glycerol (STGG) transport media, and transported to the Microbiology laboratory at Mbale Regional Referral Hospital at -20°C . All swabs were processed within 8 hours of collection for primary culture, isolation and identification.

Sample analysis: Nasopharyngeal swabs were processed as earlier described by Rutebemberwa et al [13]. A swab was mixed thoroughly by vortexing and streaked on tryptone soya agar base plates (*Oxoid Ltd., Basingstoke, Hampshire, UK*) supplemented with 5% whole sheep blood and chocolate agar. A 6mm diameter containing $5\mu\text{g}$ of ethylhydrocupreine optochin disk (*Himedia Laboratories, Mumbai, India*) were inserted aseptically in the first streak area and plates were incubated at 37°C in 5% carbon dioxide using a candle jar for 24 to 48 hours. Pneumococci were identified using a zone of inhibition around the optochin disk, and bacteriologic characteristics which included: alpha hemolysis, flat and irregularly shaped colonies. Instances where two or more morphologically different colonies were identified, these were separately isolated.

Identification of *S. pneumoniae*: Gram-positive cocci alpha hemolytic colonies on the plates were picked up with sterile wire loop, re-suspended to make a 0.5MCFaland's standard using densitometry technique and sub-cultured onto tryptone soya agar base supplemented with 5% sheep blood. Optochin-susceptible strains with ≥ 14 mm in diameter of the zone of inhibition were identified as *S. pneumoniae*; strains with zones of inhibition < 14 mm were subjected to bile solubility test (tube method) using 2% sodium deoxycholate or bile salt (*Oxoid Ltd.*). The overall flow chart explaining identification algorithm for *S. pneumoniae* isolates was adopted from the standard operating procedure of Mbale Regional Referral Hospital as given below;

84

85

86

87

88

89

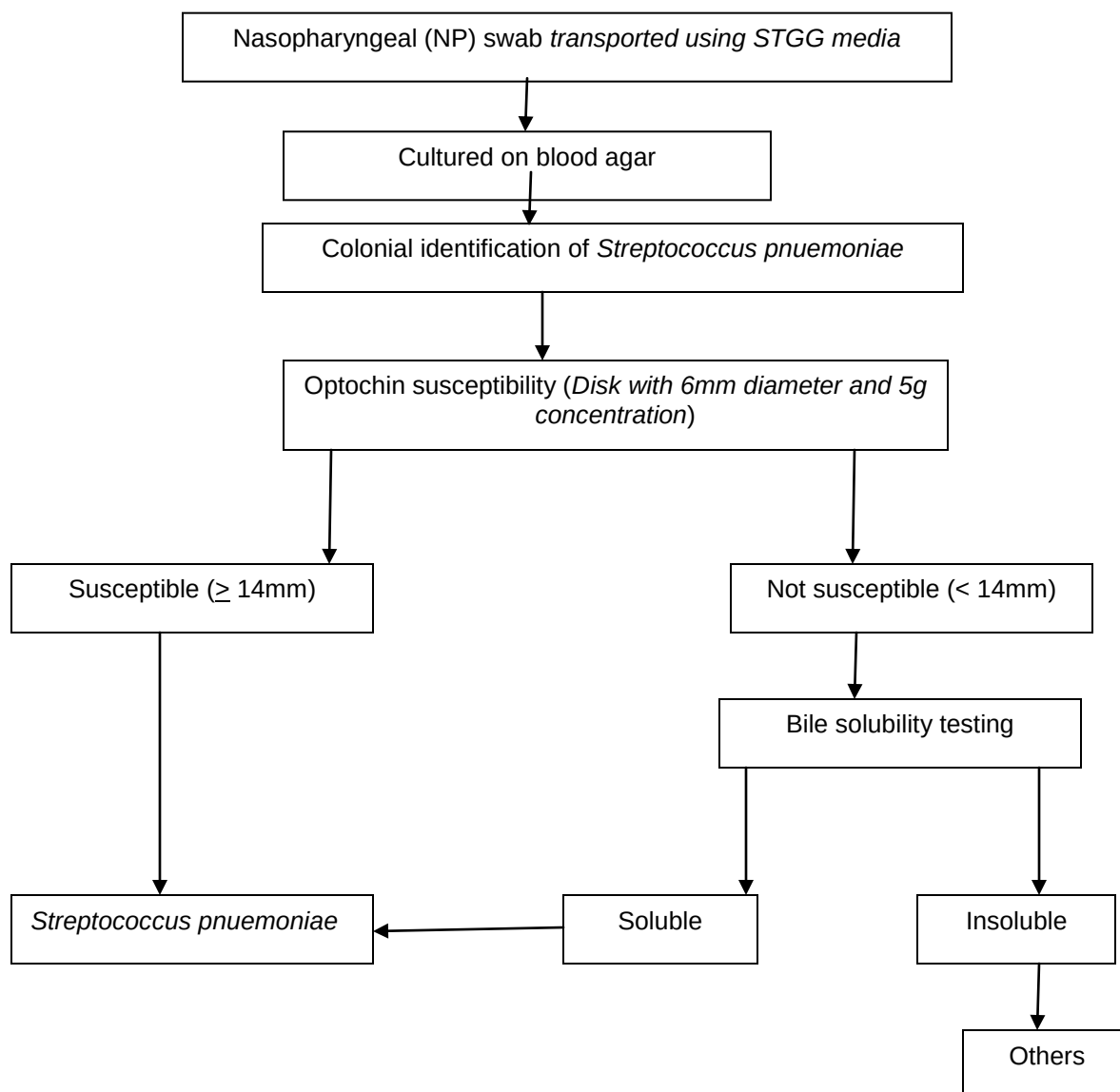
90

91

92

93

94



95

Data analysis: Data was analyzed by SPSS, and presented in a bar graph and a table.

96

Pearson's square and p-value was used to interpret the statistical association. A p-value less

97

than 0.05 was considered as statistically of significant association.

98

Quality control measures: Known strain of *Streptococcus pneumonia* (ATCC 49619) was

99

used. Quality control of media was done by performing sterility and fertility testing. Standard

100

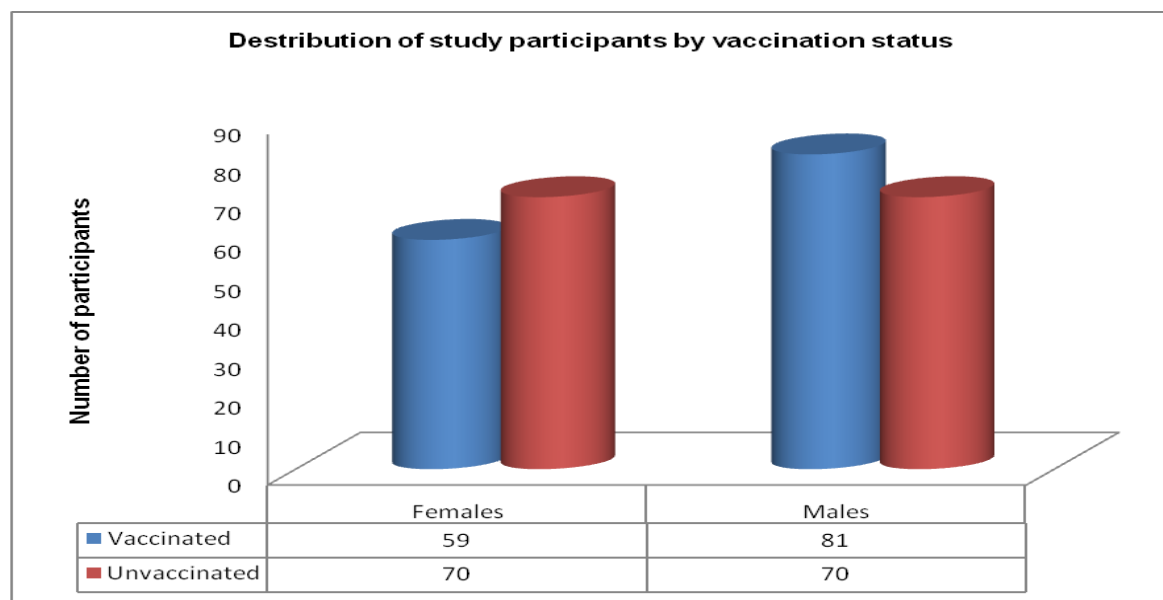
operating procedures (SOPs) were strictly followed.

101

3. RESULTS

Characteristics of study participants: In total, we enrolled 280 children under-five years of age. These were constituted an equal number (n=140) of PCV vaccinated and unvaccinated children. The mean age was 23 months (range: 4-59 months. There were 151 males and 129 females. Additionally, participants 81 (53.64%) of the male participants were vaccinated, while 59 (45.74%) were unvaccinated as indicated in figure 1.

Figure 1: showing the vaccination status of study participants



Isolation of *Streptococcus pneumoniae* among study participants: The overall prevalence of *Streptococcus pneumoniae* was 123 of the 280, which represents 43.93% (95% Confidence interval: 39.88-47.69). *Streptococcus pneumoniae* was isolated in only 13 (9.29%) of the vaccinated participants, while it was isolated among 110(78.57%) children who had not been vaccinated with PCV as indicated in table 1.

Table 1: showing *Streptococcus pneumonia* isolated

Immunization Status	Streptococcus pneumonia
---------------------	-------------------------

	No	Yes	Total	Odds ratio (P-value)
Immunized	127 (90.71)	13 (9.29)	140 (100)	7.4259 (0.000)
Not Immunized	30 (21.43)	110 (78.57)	140 (100)	
Total	157 (56.07)	123 (43.93)	280 (100)	

4. DISCUSSION

This study has established high nasopharyngeal carriage risk of *Streptococcus pneumoniae*. This was found at 78.57% and 9.29% among the unvaccinated and the vaccinated respectively. Among Our findings are in agreement with a systematic review on pneumococcal carriage in Sub-Saharan Africa which indicated a carriage risk of 21-94% [6]. The prevalence is however lower than the 72% that has been reported in Gambian communities [15]. Additionally, *Streptococcus pneumoniae* was isolated in only 13 (9.29%) of the vaccinated participants compared to the 110 (78.57%) children who had not been vaccinated with PCV. From this, it may be deduced that the high burden of pneumococcal carriage in Mbale district is high, and therefore risks more children under-five to suffer from pneumococcal disease. This is similar to what has been reported elsewhere in Uganda [16, 17]. As this was a study among vulnerable study group, this raises great concerns as the management of pneumococcal disease is inadequate in reference to access and quality of care in most set up. This phenomenon has hitherto been reported [16, 18], and is considered critical to the preventive approach (wide implementation of the PCV vaccination) to subdue related mortalities. We found 7.4 odds of acquiring pneumococcal infection among the unvaccinated children, this is similar to the earlier study report [19], thus it is really evident that childhood vaccination offers a protective advantage in our set up.

Although our study has established high carriage and therefore eventual risk of pneumococcal infections among children under-five years of age, some of the study limitation are: 1) we did not

study seasonal variability of pneumococcal carriage, and relate it to prevention aspects. 2) we did not perform molecular assays and sero-typing to clearly report the existing serotypes in our set up. 3) our study design did not explore the associated factors of pneumococcal infection, which one would regard critical to disease control.

5. CONCLUSION

Based on our study findings, we report a high prevalence of pneumococcal carriage among children under-five years in Mbale district. The carriage is more among the unvaccinated group; thus PCV remains key to the prevention of pneumonia among children under-five years. Similarly, pneumonia remains high in children who were not vaccinated with PCV hence conforming the case mortality of pneumonia in children below five years. Thus, concerted efforts to boost vaccination uptake would lessen childhood mortality risk due to pneumococcal infections

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethical approval was obtained from research and ethics committee of International Health Science University. We ensured strict confidentiality and high ethical standards. We obtained written informed consent from each participant. Results were only availed to the attending doctor for proper management. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

157

158 **REFERENCES**

159 1. Wardlaw TM, Johansson EW, Hodge M. World Health Organization; UNICEF. Pneumonia: the
160 forgotten killer of children. 2006; Page 40; ISBN: 9789280640489 / 9280640488

161 2. The Global Burden of Disease. Mortality and Causes of Death Collaborators. Global, regional,
162 and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of
163 death, 1980-2015: a systematic analysis for the Global Burden of Disease Study. *Lancet*.
164 2015; 388: 1459-1544

165 3. Fischer CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA et al. Global burden of
166 childhood pneumonia and diarrhoea. *Lancet*. 2013; 381:1405–16.

167 4. Rudan I, O'Brien KL, Nair H, Liu L, Theodoratou E, et al. Epidemiology and etiology of
168 childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying
169 risk factors and causative pathogens for 192 countries. *Journal of Global Health*. 2013; 3.

170 5. Adegbola RA, DeAntonio R, Hill PC, Roca A, Usuf E, et al. Carriage of *Streptococcus*
171 *pneumoniae* and Other Respiratory Bacterial Pathogens in Low and Lower-Middle Income
172 Countries: A Systematic Review and Meta-Analysis. *PLoS ONE*. 2014; 9

173 6. Usuf E, Bottomley C, Adegbola RA, Hall A. Pneumococcal Carriage in Sub-Saharan Africa—
174 A Systematic Review. *PLoS ONE*. 2014; 9.

175

176 7. Kinney K, Johnson C, Papasian RT, Hall CG, Kurth MA, Jackson. Early onset *Haemophilus*
177 *influenzae* sepsis in the newborn infant *Pediatr Infect Dis J*. 1993; 12 (9) 739-743

8. Stensballe LG, Hjuler T, Andersen A, Kaltoft M, Ravn H, Aaby P, Simoes EA. Hospitalization for respiratory syncytial virus infection and invasive pneumococcal disease in Danish children aged <2 years: a population-based cohort study. *Clin Infect Dis*. 2008; 46:1165–1171.
9. Madhi SA, Klugman KP Vaccine Trialist Group. A role for *Streptococcus pneumoniae* in virus-associated pneumonia. *Nat Med*. 2004; 10:811–813.
10. Ampofo K, Bender J, Sheng X, Korgenski K, Daly J, Pavia AT, Byington CL. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics*. 2008; 122:229–237.
11. Bogaert D, de Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect Dis*. 2004; 4:144–54.
12. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *The Lancet*. 2009; 374: 893–902.
13. Rutebemberwa E, Mpeka B, Pariyo G, Peterson S, Mworzi E, et al. High prevalence of antibiotic resistance in nasopharyngeal bacterial isolates from healthy children in rural Uganda: A cross-sectional study. *Ups J Med Sci*. 2015; 120: 249–256.
14. Simell B, Auranen K, Kayhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. *Expert Rev Vaccines*. 2012; 11:841–55.
15. Philip C, Hill A, Akisanya K, Yin-Bun C, Mark SG, Lahai BM, Greenwood Richard A, Adegbola. Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Gambian Villagers. *Clinical Infectious Disease*. 2006; 43, 6
16. Kalyango JN, Lindstrand A, Rutebemberwa E, Ssali S, Kadobera D, et al. Increased Use of Community Medicine Distributors and Rational Use of Drugs in Children Less than Five

Years of Age in Uganda Caused by Integrated Community Case Management of Fever. *The American Journal of Tropical Medicine and Hygiene* 2012; 87: 36–45.

17. Nantanda R, Hildenwall H, Peterson S, Kaddu-Mulindwa D, Kalyesubula I, et al. Bacterial aetiology and outcome in children with severe pneumonia in Uganda. *Ann Trop Paediatr*. 2008; 28: 253–260.

18. Kallander K, Burgess DH, Qazi SA. Early identification and treatment of pneumonia: a call to action. *Lancet Glob Health*. 2016; 4: e12–13.

19. Tate JE, Kisakye A, Mugenyi P, Kizza D, Odiit A, et al. Projected health benefits and costs of pneumococcal and rotavirus vaccination in Uganda. *Vaccine*. 2011; 29: 3329–3334.

ACRONYMS AND ABBREVIATIONS

PCV: Pneumococcal Conjugate Vaccine; PPSV: Pneumococcal Polysaccharide Vaccine; CDC: Centers for Disease Control and Prevention; WHO: World Health Organization; MOH: Ministry of Health; B.A: Blood agar; BHI: Brain Heart Infusion; CHO: Chocolate