

Molecular Assessment of *Streptococcus pneumoniae*: Prevalence and Risk Factors among School-Age Children in Tchibanga, Gabon



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ABSTRACT

Context: *Streptococcus pneumoniae* (pneumococcus) is a major pathogen responsible for respiratory infections, meningitis, and septicemia, particularly among children. Current data on the transmission and risk factors of this infection among school-aged children in Gabon remains scarce. This study aims to address this gap by investigating Molecular Assessment of *Streptococcus pneumoniae*: Prevalence and Risk Factors among School-Age Children in Tchibanga, Gabon.

AOR = 1.18, (95% CI, 0.68–2.02), $p = 0.03^*$

Methods: Using a prospective and cross-sectional design, the study was conducted from June 13 through November 30, 2023, among school-aged children presenting with respiratory symptoms or other suspected infections. Nasopharyngeal swabs were collected using COPAN FLOQSwabs™ and subsequently analyzed by real-time PCR amplification of the *lytA* gene, specific to *S. pneumoniae*, to confirm bacterial colonization. Questionnaire-derived sociodemographic, clinical, and environmental variables were assessed. Risk factor analysis was performed using R v4.4.2, employing logistic regression with significance thresholds of $p \leq 0.05$ and 95% CI.

Results: Of the 430 children studied, *S. pneumoniae* infection prevalence was 32.8% (95% CI, 0.29 – 0.37). Significant risk factors associated with the infection included children aged 3 to 5 years. AOR = 1.18, (95% CI, 0.68–2.02), $p = 0.03^*$, lack of maternal education (AOR = 2.58, 95% CI, 2.82–7.62), exposure to household tobacco smoke (AOR = 2.2, 95% CI; 1.4 – 2.9), lack of adequate sanitation (toilets and wastewater disposal) (AOR = 1.7, 95% CI, 1.6 – 2.9), presence of fever (AOR = 2.21, 95% CI, 1.65 – 2.31), and lack of pneumococcal vaccination (AOR = 2.67, 95% CI, 1.39– 3.81).

Conclusion: This study reveals a high prevalence of *S. pneumoniae* infection among school-aged children in Tchibanga, highlighting the importance of strengthening prevention strategies, including vaccination and recognition of environmental determinants of disease. These results could guide public health policies to reduce the health and economic toll of pneumococcal infections in this area.

Keywords: *Streptococcus pneumoniae*, prevalence, risk factors, school-aged children, Tchibanga, Gabon.

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I. INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is a major pathogen responsible for invasive infections (pneumonia, meningitis, septicemia) and non-invasive infections. Every year, this kills a child under five every minute - with nearly all deaths happening in developing countries [1]. The nasopharyngeal carriage rate of this bacterium ranges from 20% to 60% in children, with higher rates in sub-Saharan Africa (30% to 70%) due to overcrowding, limited access to healthcare, and insufficient vaccine coverage [2]; [3]. In Gabon, data are limited, but a study in Libreville revealed a carriage rate of 40% to 50% among children younger than five years [4]. In rural areas like Tchibanga, unfavorable socio-economic conditions and limited access to healthcare may exacerbate this situation. Although the pneumococcal conjugate vaccine (PCV) has been introduced, its coverage remains insufficient in some regions.

School-aged children, particularly exposed in school and community settings, facilitate bacterial transmission [5]. Factors such as overcrowding, significant exposures included environmental tobacco smoke, low socioeconomic status (SES), and absence of vaccination increase the risk of infection [3]. This hospital-based study assesses *S. pneumoniae* prevalence and risk factors among schoolchildren in Tchibanga, with potential implications for vaccination policy and environmental health.

II. MATERIALS AND METHODS

II.1. Study Design

This study is a descriptive and analytical cross-sectional survey conducted from June 13 to November 30, 2023, among school-aged children receiving care at the Marcel Ngoubou Regional Hospital Center in Tchibanga, southwest Gabon.

The study aims to determine the prevalence of *Streptococcus pneumoniae* infection and identify associated risk factors.

II.2. Study Population

II.2.1. Inclusion and Exclusion Criteria

Only children aged 3 to 15 years, presenting with respiratory symptoms (cough, fever, difficulty breathing) or other suspected infections, and with parental/guardian consent, were included in this study. Children who had received antibiotic treatment within the 7 days before sampling, and those whose parents or guardians refused participation, were excluded.

II.3. Sampling

II.3.1. Sample Size

As elsewhere, the sample size was calculated using Cochran's formula for cross-sectional studies, assuming an expected prevalence of 30% (based on similar studies in sub-Saharan Africa) ([6], a margin of error of 5%, and a 95% confidence interval.

$$n = [(Z\alpha / 2)^2 \times (P \times (1 - P))] / d^2$$

Where Z-score of 1.96 (representing the 95% confidence interval threshold, $p = 0.30$ (estimated prevalence), and $d = 0.05$ (margin of error). To account for potential dropouts, the initial sample size of 323 was increased by 10% following the recommendations of Mba [7]. Therefore, this study ultimately included 430 participants.

II.3.2. Recruitment

Children were recruited consecutively from those visiting the pediatric department or pediatric emergency unit of the hospital.

II.4. Data Collection

II.4.1. Questionnaire

Parent/guardian-completed structured questionnaires were used for data collection on sociodemographic characteristics (age, sex, parental education level, occupation, housing conditions), medical history (pneumococcal vaccination, recurrent respiratory infections), and environmental factors (exposure to tobacco smoke, overcrowding).

II.4.2. Nasopharyngeal Swab Protocol

After informing the child (or their guardian) of the procedure, a swab from the COPAN FLOQSwabs™ kit (nylon swabs with universal transport medium, UTM) was gently inserted into the participant's nostril to the nasopharynx, where it was rotated for 5 to 10 seconds to collect the sample. Once removed, the swab was labeled with the required information, placed in Stuart transport medium, and transported to the laboratory for molecular analysis.

II.4.3. Molecular Diagnosis of *S. pneumoniae*

Molecular diagnosis to confirm the presence of the bacterium involved DNA extraction from nasopharyngeal swabs, followed

using *lytA* gene-targeted real-time PCR (a ~200-300 base pair fragment specific to *Streptococcus pneumoniae*), which encodes an autolysin involved in cell wall lysis. This molecular diagnosis was performed using Forward primer: 5'-ACGCAATCTAGCAGATGAAGCA-3', and Reverse primer: 5'-T C G T G C G T T T T A A T T C C A G C T - 3'. The annealing temperature was set between 60 and 62 °C, adapted to the melting temperature (T_m) of the primers provided by Sigma-Aldrich (Merck) France.

II.5. Data Analysis

After being recorded on standardized forms and exported to Excel 2016 spreadsheet for cleaning, the data were analyzed by R software version 4.4.2. First, a descriptive analysis was conducted, summarizing the data using frequencies and percentages for categorical variables, and means or medians for continuous variables. Next, in the bivariate analysis, statistical tests (chi-square test or Fisher's exact test) were used to assess associations between *S. pneumoniae* carriage and potential risk factors. A logistic regression model was ultimately used to pinpoint independent risk factors linked to the infection. Variables with a p -value ≤ 0.05 in the bivariate analysis were included in the multivariate model.

II.6. Ethical Considerations

Before commencing, the study was approved by the Research Ethics Committee of the Gabonese Ministry of Health through the Regional Health Director of the South, via letter No. 0436/PHO/SG/DRSS/SGP/D. Written informed consent was obtained from parents or guardians before including children. Data confidentiality was strictly maintained.

III. RESULTS

III.1. Overall Prevalence of *Streptococcus pneumoniae* Infection Among Study Participants (N=430)

A total of 430 (95%) nasopharyngeal swabs from school-aged children (3 to 15 years) were collected and examined during this study. Of these, 47.21% were from male children ($n = 203$). The overall prevalence was 32.8% (95% CI, 0.29–0.37) indicated that 141/430 participants were infected with *Streptococcus pneumoniae*.

III.2. Sociodemographic Determinants of *Streptococcus pneumoniae* Prevalence in Children: Bivariate and Multivariable Logistic Regression Analysis (N=430).

In bivariate and multivariate logistic regression analyses of the overall prevalence of *Streptococcus pneumoniae* infection based on the sociodemographic characteristics of the children included in the study, a significant association was observed with two factors: young age (children aged 3 to 5 years, AOR = 1.18, (95% CI, 0.68–2.02), $p = 0.03^*$) and lack of maternal education (AOR = 2.58, 95% CI: [2.82–7.62], $p \leq 0.001$). In contrast, gender, number of household members, place of residence, and type of housing showed no significant association with *S. pneumoniae* infection (see Table 1).

Table 1: Bivariate and Multivariable Analysis of Sociodemographic Factors Associated with *S. pneumoniae* Carriage in a Pediatric Cohort (N=430)

Variables	Total Number of Participants Tested (%)	Overall Prevalence of <i>Streptococcus pneumoniae</i> , % (n/N)	Bivariate Analysis		Multivariate Analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Gender						
Female	227 (52.79)	29.96 (68/227)	Reference	-	1	-
Male	203 (47.21)	36 (73/203)	1.31 [0.88 – 2]	0.22	-	-
Age groups (years)						
3 - 5	168 (39.07)	53.33 (98/168)	3.86 [2.37 – 6.34]	≤0.001	1.18 [0.68 – 2.02]	0,03*
6 - 10	107 (24.9)	25.23 (27/107)	0.23 [0.19 – ; 0.55]	≤0.001	-	-
11 et 15	59 (36.03)	11.51 (16/139)	Reference	-	1	-
Mother's Education Level						
None	105 (24.42)	41.90 (44/105)	1.69 [1.05 – 2.73]	0.024	2.58 [1.55 – 4.27]	≤0.001*
Primary	152 (35.35)	41.45 (63/152)	1.84 [1.17 – 2.80]	0.005	1.46 [0.86 – 2.49]	0.23
Secondary	142 (33.02)	16.90 (24/142)	Reference	-	1	-
Higher Education	31(7.21)	32.26 (10/31)	0.97 [0.38 – ; 2.24]	1	-	-
Number of Household Members						
2 - 4	110 (25.58)	22.73 (25/110)	Reference	-	1	-
5 - 7	145 (33.72)	56.55 (82/145)	4.96 [3.15 – 7.89]	≤ 0.001	0.71 [0.31 – 1.60]	0.40
8 and above	175 (40.7)	24.11 (34/141)	0.34 [0.21 ; 0.53]	≤ 0.001	1.5 [1.1 ; 2.13]	0.27
Residence						
Tchibanga (Urban)	259 (60.23)	38.6 (100/259)	Reference	-	1	-
Other (rural)	171 (39.77)	23.98 (41/171)	0.5 [0.32 – 0.79]	0.002	0.24 [0.49 – 1.01]	0.12
Type of Housing						
Permanent House	188 (43.72)	19.68 (37/188)	Reference	-	1	
Semi- Permanent House	191 (44.42)	35.60 (68/191)	1.26 [0.82 – 1.92]	0,30	-	-
Traditional Housing	51(11.86)	70.59 (36/51)	0.5 [0.32 – 0.79]	≤ 0.001	1.6 [0.62 – 1.80]	0.84

Key: 1 : Reference group ; OR (odds ratio with 95% CI); * significant at p<0.05. A : ajusted

III.3. Prevalence of *Streptococcus pneumoniae* Infection Stratified by Environmental Exposures in the Pediatric Study Cohort (N=430).

Bivariate and multivariate logistic regression analyses examining the overall prevalence of *Streptococcus pneumoniae* infection in relation to the environmental factors to which the children in the study were exposed revealed two factors with significant associations: exposure to household tobacco smoke (AOR = 2.2, 95% CI: [1.4–2.9], p = 0.02*) and the lack of an adequate sanitation system (toilets and wastewater disposal) (AOR = 1.7, 95% CI: [1.6– 2.9], p = 0.01*). In contrast, the presence of domestic animals in the household, bed-sharing with others, and the absence of a potable water source at home showed no significant association with *S. pneumoniae* infection (see Table 2).

Table 2: Environmental Risk Factors and *Streptococcus pneumoniae* Prevalence in Children: Bivariate and Multivariate Logistic Regression Analysis (N=430)

Variables	Total Number of Participants	Overall Prevalence of <i>Streptococcus pneumoniae</i> , % (n/N)	Bivariate Analysis		Multivariate Analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Exposure to Household Tobacco Smoke						
Yes	126 (29.3)	29.92 (48/126)	1.4 [0.88–2.2]	0.14	2.2 [1.4–2.9]	0.002 [‡]
No	304 (70.7)	44.08 (93/211)	Reference	-	1	-
Presence of Domestic Animals in the Household						
Yes	107 (24.88)	16.82 (18/107)	0.33 [0.17 – 0.58]	≤ 0.001	--	-
No	323 (75.32)	38.08 (123/323)	Reference	-	1	-
Does the Child Share a Bed with Others?						
Yesi	170 (39.53)	18.82 (32/170)	0.32 [0.20 – 0.51]	≤ 0.001	-	-
No	260 (60.53)	41.92 (109/260)	Reference	-	1	-
Presence of a Potable Water Source in the Home						
Yes	376 (66.59)	19.78 (121/376)	1	-	1	-
No	54 (33.41)	7.3 (20/54)	1.24 [0.65 – 2.32]	0.53	1.08 [1.3–1.7]	0.19
Existence of an Adequate Sanitation System (Toilets, Wastewater Disposal)						
Yes	353 (82.09)	27.80 (84/353)	1	-	1	-
No	77 (17.91)	74.03 (57/77)	7.8 [4.35–14.5]	≤ 0.001	1.7 [1.61–2.9]	0.01*

Key: 1: Reference group; OR (odds ratio with 95% CI); * significant at $p < 0.05$. A: adjusted

III.4. Clinical Symptom Profile and *S. pneumoniae* Infection Prevalence Among Children (N=430)

Table 3 shows that, following bivariate and multivariate logistic regression analyses of the overall prevalence of *Streptococcus pneumoniae* infection in relation to the symptoms presented by the children at the time of the study, only the presence of fever was significantly associated with the disease (AOR = 2.21, 95% CI: [1.65–2.31], $p \leq 0.001^*$)

Table 3: Bivariate and Multivariate Logistic Regression of the Overall Prevalence of *Streptococcus pneumoniae* Based on Children's Symptoms at the Time of the Study (N=430)

Variables	Total Number of Participants Tested (%)	Overall Prevalence of <i>Streptococcus pneumoniae</i> , % (n/N)	Bivariate Analysis		Multivariate Analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Presence of Respiratory Symptoms						
Yes	308 (71.63)	31.82 (98/308)	1		1	-
No	122 (28.37)	35.25 (43/122)	1.07 [0.67–1.69]	0.82	-	-
Presence of Fever						
Yes	153 (35.58)	41.83 (64/153)	1.87 [1.21 – 2.89]	0.003	2.21 [1.65 – 2.31]	≤ 0.001
No	277 (64.42)	27.8 (77/277)	1	-	1	-
Presence of Cough						
Yes	108(25.12)	62.96 (68/108)	1.29 [0.79 – 2.08]	0.21	-	-
No	322(74.88)	31.37 (101/322)	1	-	1	-
Presence of Breathing Difficulties						
Yes	163 (37.91)	33.13 (54/163)	1.02 [0.66 – 1.58]	0.91	-	-
No	267 (62.09)	32.58 (87/267)	1		1	-

Key: 1: Reference group; OR (odds ratio with 95% CI); * significant at $p < 0.05$. A: adjusted

III.5. Distribution of *Streptococcus pneumoniae* Infection Prevalence by Medical History in the Pediatric Study Cohort (N=430)

Table 4 indicates that, Logistic Regression (Bivariate and Multivariate) analyses examining the overall prevalence of *Streptococcus pneumoniae* infection concerning the medical history of the children in the study, only the lack of pneumococcal vaccination was significantly associated with this infection (AOR = 2.67, 95% CI: [1.39–3.81], $p \leq 0.001^*$).

Table 4: Logistic Regression (Bivariate and Multivariate) Assessing the Association Between Medical History and *Streptococcus pneumoniae* Prevalence in Children (N=430)

Variables	Total Number of Participants Tested (%)	Overall Prevalence of <i>Streptococcus pneumoniae</i> % (n/N)	Bivariate Analysis		Multivariate Analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Has the child been vaccinated against pneumococcus?						
Yes	405(53.7)	30.62 (124/405)	1	-	1	-
No	25 (46.3)	68 (17/25)	4.8 [1.90–13.2]	0.000	2.67 [1.39–3.81]	≤0.001*
Does the child have a history of recurrent respiratory infections?						
Yes	187(43.49)	19.79 (37/187)	0.33 [0.20–0.52]	≤0.001	-	-
No	243 (56.51)	42.8 (104/243)	1	-	1	-
Does the child have a history of chronic diseases (asthma, sickle cell disease, e tc.)?						
Yes	278 (64.65)	37.8 (105/278)	1.95 [1.23–3.15]	0.036	-	-
No	152 (35.35)	23.7 (36/152)	1	-	1	-

Key: 1: Reference group; OR (odds ratio with 95% CI); * significant at $p < 0.05$. A: adjusted

V. DISCUSSION

Streptococcus pneumoniae infections pose a significant global health burden, especially in low- and middle-income regions where pediatric morbidity and mortality rates are elevated. In Tchibanga, Gabon, this study identified a nasopharyngeal carriage prevalence of 32.8% among school-aged children, aligning with rates reported across sub-Saharan Africa (20–40%) [11] and mirroring findings from Kenya (32.8%) [12] and Gambia (32.8%) [13]. However, this prevalence surpasses figures from Jordan (16.45%) [14], Cameroon (20.9%) [15], and Côte d'Ivoire (25.4%) [16]. Such disparities may stem from regional variations in living conditions (e.g., overcrowding, inadequate ventilation) [9], socioeconomic disparities [17], differential vaccine coverage [10, 13, 15], antibiotic misuse [18, 19], and age-specific social behaviors [20, 21]. Methodological differences in sampling and detection protocols further contribute to these discrepancies [22, 23]. These findings underscore the necessity of region-specific surveillance and interventions to address pneumococcal transmission dynamics. Bivariate and multivariate logistic regression analyses identified children aged 3–5 years as having a significantly elevated risk of *S. pneumoniae* carriage (AOR = 1.18). This aligns with multiple studies highlighting heightened vulnerability in this age group like, a 2022 Kenyan survey noted higher carriage rates in 3–5-year-olds compared to younger children or adolescents [12]. In Addis Ababa, where semi-urban children aged 3–5 years exhibited a 40.2% prevalence vs. 25.8% in those under 2 years [10]. Post-PCV introduction in Gambia, unvaccinated 3–5-year-olds retained a 38.4% prevalence [13]. Senegalese studies linked preschool attendance to a 42.9% prevalence in this group [24]. However, other contexts reveal divergent risk patterns. In Abidjan, infants (6 months–2 years) showed higher carriage than older children [16], Malian infants under 1 year had a 51.3% prevalence [26], Post-PCV Gambian adolescents (6–10 years) reached 45.2% prevalence [13], Rural Mozambique identified elevated carriage (36.9%) in young adults (18–25 years) cohabiting with infected children [27], Senegalese neonates (0–6 months) exhibited 47.5% prevalence, likely due to early pathogen exposure [24]. Focusing on socio-economic, environmental and clinical risk factors for *S. pneumoniae* infection, the present study showed that lack of maternal education correlated strongly with infection (AOR = 2.58), reflecting barriers to healthcare access and health literacy, as observed in sub-Saharan Africa [35, 36].

Also, domestic exposure to tobacco smoke doubles the risk of infection (AOR = 2.2), which is consistent with global data linking indoor air pollution to colonization by respiratory pathogens [28, 37–39]. Furthermore, it was also reported in the present study that inadequate sanitation systems increase the risk (AOR = 1.7), highlighting the role of environmental hygiene in transmission [35, 40, 41]. Similarly, in the present study, fever was strongly associated with infection (AOR = 2.21), aligning with its role as a marker of invasive bacterial disease [38, 42]. Unvaccinated children faced nearly triple the risk (AOR = 2.67), reinforcing the efficacy of PCV in reducing carriage, as demonstrated globally [28, 35, 43, 44].

Study Limitations

Despite its contributions, this study has limitations: the cross-sectional design prevents causal inference between risk factors and *S. pneumoniae* infection. Given the relatively small sample size (430 participants), the study's results might not be applicable to the broader Gabonese population or to areas with different socioeconomic contexts. Finally, the lack of data on serotypes: The study does not specify the *S. pneumoniae* serotypes involved, which would be useful for evaluating the effectiveness of available vaccines.

CONCLUSION

Our findings highlight substantial *S. pneumoniae* prevalence among Gabonese schoolchildren and pinpoint modifiable risk factors for potential public health action. The results underscore the need to strengthen prevention strategies in Gabon, including improving vaccine coverage by introducing and expanding pneumococcal vaccination programs, particularly with conjugate vaccines such as PCV13, which could significantly reduce the prevalence of pneumococcal infections. Raising awareness of environmental risk factors through campaigns on the dangers of tobacco smoke and the importance of adequate sanitation could reduce children's exposure to identified risk factors. Strengthening healthcare systems to improve access to healthcare and education, especially for mothers, which could have a significant impact on reducing pneumococcal infections among children in this region and beyond.

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Author Contributions

Conceptualization: T.N.-M. and H.K.-M.; Methodology: T.N.-M., H.K.-M., A.J.-E.N., S.M., and B.A.P.P.; Validation: T.N.-M., H.K.-M., and S.M.; Formal analysis: M.A.-H.; Investigation: S.M., T.N.-M., and A.J.-E.N.; Writing – original draft: T.N.-M.; Writing – review and editing: T.N.-M. and B.A.P.P.; Supervision: T.N.-M. and H.K.-M. All authors have approved the final manuscript.

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