ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 15, Issue, 01, pp. 67384-67390, January, 2025
https://doi.org/10.37118/ijdr.29054.01.2025

RESEARCH ARTICLE OPEN ACCESS

ASYMPTOMATIC CARRIAGE, KNOWLEDGE, AND RISK FACTORS ASSOCIATED WITH STREPTOCOCCUS PNEUMONIAE INFECTION AMONG PUBLIC TRANSPORT USERS (TAXI-BUS) IN FRANCEVILLE, SOUTH-EAST GABON

Thiéry Ndong Mba*[1, 2], Hilaire Moundounga Kenguele[1], Simon Mapasu[1] and Arnaud Brice Pambo-Pambo[3]

[1]Laboratory of Molecular and Cellular Biology (LABMC), University of Science and Technology of Masuku (USTM), Franceville, Gabon; [2]Laboratory of Research in Biochemistry (LAREBIO), University of Sciences and Techniques of Masuku (USTM) Franceville,Gabon; [3]Laboratory of Animal Physiology, University of Science and Technology of Masuku (USTM), Franceville, Gabon

ARTICLE INFO

Article History:

Received 17th November, 2024
Received in revised form
14th December, 2024
Accepted 20th December, 2024
Published online 24th January, 2025

Key Words:

Asymptomatic carriage. Public transport, Streptococcus pneumoniae, Franceville, Gabon.

***Corresponding Author: Thiéry Ndong Mba,**

ABSTRACT

Background: Despite the existence of a vaccine, pneumococcal bacteria remain a major health problem, particularly in Africa, where they cause many deaths, especially among young children and the elderly. The present study looked at asymptomatic carriage, knowledge and risk factors associated with *Streptococcus pneumoniae* infection, among public transport users (Taxis-Bus) in Franceville, south-east Gabon. ***Materials and Methods:*** This prospective cross-sectional study conducted from April 19 to July 23, 2024 on 241 adults, analyzed their sputum samples for *Streptococcus pneumoniae* bacteria by culture on nutrient agar supplemented with 5-10% defibrinated sheep blood. Suspect colonies were identified by a biochemical optochin sensitivity test. Factors increasing the risk of carrying the bacteria were identified by statistical analysis, including bivariate analysis and multivariate logistic regression. Within a 95% confidence interval, all $p \leq 0.05$ were considered statistically significant. ***Results:*** A total of 241 users of public transport in Franceville, commonly known as "Taxis-Bus", with a mean age of 37 ± 13.75 years, were registered for the present study. The overall prevalence of *Streptococcus pneumoniae* infection was 24.5% (95% CI: [0.19-0.30]). Male gender (adjusted Odds Ratio = 2.88; 95% CI [1.69 ;4.92], p≤0.001*), age group 60 and over (adjusted Odds Ratio = 2.12; 95% CI [1,3 ;1.42], p=0.032*), regular taxi-bus use (adjusted Odds Ratio = 2.22; 95% CI [1.27 ; 3.88], p= 0.045*), not being vaccinated (Adjusted Odds Ratio = 2.78; 95% CI [1.6 ; 4.1 p=0.041*), and smoking (Adjusted Odds Ratio = 5.23; 95% CI [2.77 ; 9.85] ; p=0.012*), significantly exposed study participants to *Streptococcus pneumoniae* infection. ***Conclusion:*** The results obtained in the present study underline the importance of pneumococcal vaccination, particularly for the most vulnerable people such as men, the elderly and smokers. They also highlight the need for preventive measures on public transport to limit the spread of the bacterium.

Citation: Thiéry Ndong Mba, Hilaire Moundounga Kenguele, Simon Mapasu and Arnaud Brice Pambo-Pambo, 2025. *"Asymptomatic Carriage, Knowledge, and risk factors Associated with Streptococcus Pneumoniae infection among public Transport users (Taxi-Bus) in Franceville, South-East Gabon". International Journal of Development Research*, 15, (01), 67384-67390.

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is a pathogenic bacterium responsible for a wide range of invasive infections, such as pneumonia, meningitis, and septicemia [1]. These infections represent a major public health problem, particularly in developing countries where they mainly affect children and the elderly [2]. Transmission of *S. pneumoniae* occurs through the air, particularly via respiratory droplets emitted during coughing or sneezing [3]. Pneumococcus is one of the leading causes of death in children under 5 years old worldwide. The World Health Organization (WHO) estimates that it is responsible for more than 250,000 child deaths each year, mainly in developing countries [4]. Studies have shown that nasopharyngeal carriage of *S. pneumoniae*among children in sub-Saharan Africa can reach 50 to 80% in some regions [5]. *S. pneumoniae* is one of the main causes of pneumonia among young children (under 2 years old) and the elderly (over 65 years old) in sub-Saharan Africa. The WHO estimates that it is responsible for about 15% of pneumonia deaths in this region [6]. This bacterium is also a frequent cause of bacterial meningitis in sub-Saharan Africa, particularly in the "meningitis belt" [7]. An important aspect of pneumococcal epidemiology is asymptomatic carriage. Indeed, the bacteria can colonize the nasopharynx of individuals without causing symptoms. These asymptomatic carriers constitute a significant reservoir for the bacteria and play a crucial role in its spread within the community [8]. Public transportation, with its high population density and close contact, represents an environment conducive to the transmission of respiratory pathogens [9]. In Franceville, a city in southeastern Gabon experiencing rapid demographic and urban expansion, the use of public transportation is constantly increasing. It was therefore crucial to assess the extent of asymptomatic carriage of *S. pneumoniae* in this specific context.This study aimed to determine the prevalence of asymptomatic carriage of *S. pneumoniae* among public transportation users in Franceville, to estimate the level of knowledge about it, and to identify the factors associated with this carriage. The results obtained will allow for a better understanding of the dynamics of pneumococcal transmission in this population and will guide prevention strategies, particularly vaccination.

MATERIALS AND METHODS

Type, Period, and Location of the Study: This prospective and cross-sectional study was conducted from April 19 to July 23, 2024, at the Potos Taxi-Bus Station and the Biomedical Analysis Laboratory of the "Bonne Espérance" Clinic in the "Ongali" district of Franceville.

Study Population: The target population consisted of public transportation users (Taxi-Bus) of any gender in Franceville.

Study Eligibility Criteria

Inclusion Criteria: The study included all individuals aged 18 years and older who regularly used the "Potos - Quartier Sable" taxi-bus line as a means of public transportation at least once a week in Franceville. They had to agree to participate in the study by signing an informed consent form, not present any clinical signs of respiratory infection (cough, fever, runny nose, sore throat) at the time of sampling, and have correctly answered the questionnaire submitted to them.

Exclusion Criteria: Due to certain factors that could influence the results, individuals who had been on antibiotics in the 4 weeks preceding the sampling, or those who had received a pneumococcal vaccine (conjugate or polysaccharide) in the past 5 years, those who suffered from chronic respiratory diseases such as asthma, chronic bronchitis, and emphysema, etc., and those who refused to participate were excluded from this study.

Sampling Method and Sample Size Determination: To reflect the diversity of users, a stratified random sampling method was used based on the days of sample collection. To determine the number of participants needed for this study on asymptomatic carriage, knowledge, and factors associated with *Streptococcus pneumoniae* among public transportation users (Taxi-Buses) in Franceville, Southeast Gabon, the standard sample size calculation formula for a single proportion was used:

$$n = [(Z\alpha / 2)^2 \times (P \times (1 - P)] / d^2 \Longrightarrow n = [(1.96)^2 \times 0.5\ (1–0.5)]/(0.05)^2 = 181$$

This formula, already used in other studies [10], allows for the calculation of the sample size (n) with a 95% confidence level ($Z\alpha/2 = 1.96$). In the absence of precise data on the prevalence of asymptomatic carriage of *S. pneumoniae* in the literature in Franceville, a conservative value of 50% was chosen for this study. To compensate for possible dropouts, the initial sample size (181 people) was increased by 10%, in accordance with the recommendations of Mba et al. 2023. Thus, this study finally included 241 participants.

Data Collection: Rigorous data collection was essential to obtain reliable and interpretable results during this study. It required careful planning, adequate training of the study personnel, and adherence to ethical research principles.

Collection of Sociodemographic Information: A structured and pre-established questionnaire was administered to the participants to collect information on their age, sex, residence, profession, and frequency of public transportation use.

Microbiological Sampling and Analysis

Sample Collection and Transport: After signing the informed consent form, a sputum sample was collected from each participant by qualified medical personnel using sterile nylon-shafted swabs. After carefully collecting each sample following the recommended techniques, the tube was correctly labeled with the participant's information, and the swab was immediately placed in a viral transport medium (VTM). VTM is an isotonic buffered solution (PBS) containing stabilizing proteins (albumin), antifungals, and antibiotics to prevent bacterial and fungal growth. This medium maintains the viability of viruses and bacteria for several hours, even days, at room temperature.

Microbiological Analysis of Samples: The samples were transported to the Biomedical Analysis Laboratory of the "Bonne Espérance" Clinic in the "Ongali" district of Franceville as soon as possible, respecting the recommended temperature conditions (generally between 2°C and 8°C). At the laboratory, the samples were inoculated on nutrient agar supplemented with 5 to 10% defibrinated sheep blood, which provides essential nutrients for the growth of *S. pneumoniae*. Then, the cultures were incubated at 35-37°C in a CO_2-enriched atmosphere (5-10%) for 24 hours. Suspect colonies were identified by a biochemical optochin sensitivity test. This is a rapid and reliable method for differentiating *Streptococcus pneumoniae* (pneumococcus) from other alpha-hemolytic streptococci, particularly Streptococcus viridans, often present in sputum.

Principle: Optochin (ethylhydrocupreine hydrochloride) is a derivative of quinine that inhibits the growth of *S. pneumoniae* by interfering with the enzymatic activity of membrane ATPase. *S. pneumoniae* is sensitive to low concentrations of optochin, while other alpha-hemolytic streptococci are generally resistant.

Protocol

- ***Culture Preparation:*** From a pure culture of the suspect *S. pneumoniae* colony obtained on blood agar (after 24h incubation at 37°C in a CO_2-enriched atmosphere), a bacterial suspension was prepared in sterile saline solution adjusted to a turbidity equivalent to 0.5 McFarland. The bacterial suspension was inoculated onto blood agar using a sterile swab to obtain a lawn culture.
- ***Optochin Disk Application:*** An optochin disk (containing 5 µg of optochin) was placed in the center of the inoculated agar. Light pressure was applied to the disk on the agar to ensure good contact.
- ***Incubation:*** The agar was incubated at 35-37°C in a CO_2-enriched atmosphere (5-10%) for 24 hours.
- ***Interpretation:*** The diameter of the growth inhibition zone around the optochin disk was measured. An inhibition zone diameter ≥ 14 mm indicated optochin sensitivity and strongly suggested the presence of *S. pneumoniae*. An inhibition zone diameter < 14 mm suggested optochin resistance and indicated that it was likely not *S. pneumoniae*.

Quality Assurance: Using a clinical data sheet, analysis results, medical history, and risk factors, data quality was ensured through preliminary testing of questionnaires on 5% of participants, after appropriate training of personnel for data collection and management of an integrated quality control system of the Biomedical Analysis Laboratory of the "Bonne Espérance" clinic in the Ongali district of Franceville. All laboratory procedures were performed in accordance with standardized operating procedures.

Ethical Considerations: Before starting this study, ethical approval was obtained from the Regional Director of Health of the Southeast in Franceville, by letter n° 0345/PHO/SG/DRSSE/SGP/D, and the approval of the municipal authorities of the commune of Franceville. Written informed consent was obtained from each participant, who was informed in advance not only of the procedure and reasons for nasopharyngeal swabbing or sputum collection but also of their right to terminate their participation in this study at any time. Confidentiality of information was maintained through codes and storage in a locked cabinet. Patients diagnosed with *Streptococcus pneumoniae* benefited from regular follow-up.

Statistical Analysis: The data collected during this study were entered into a Microsoft Excel 2013 spreadsheet. Once cleaned and secured, they were analyzed using R software version 3.6.1. Descriptive statistical analyses were performed to determine means with standard deviation for continuous data and frequencies with percentages. The association between independent and dependent variables was determined using bivariate analysis, and all variables recognized as risk factors for *Streptococcus pneumoniae* infection were further analyzed by multivariate binary logistic regression to adjust for confounding factors. Results were considered significant for any p-value less than or equal to 0.05 within a 95% confidence interval.

RESULTS

Overall Prevalence of Streptococcus pneumoniae among Study Participants (N=241): A total of 241 participants with an average age of 37 ± 13.75 years were enrolled in this study. Of these, 59 (24.5%) (95% CI: [0.19-0.30]) were positive for *Streptococcus pneumoniae*, compared to 182 (75.5%) negative participants.

The highest proportion of this pathology was observed in men (38/59) (64.41%), in the age group of 60 years and older (40/59) (67.87%), followed by that of 60 years and older.

Overall Prevalence of Streptococcus pneumoniae Infection, According to Sociodemographic Information of Study Participants (N=241): From the sociodemographic variables tested for the presence of an association with *Streptococcus pneumoniae* infection among study participants, a multivariate logistic regression test showed that there was a statistically significant association between the overall prevalence of *Streptococcus pneumoniae* and factors such as male gender (adjusted Odds Ratio = 2.88; 95% CI [1.69; 4.92], p≤0.001*), and the age group of 60 years and older (adjusted Odds Ratio = 2.12; 95% CI [1.3; 1.1.42], p=0.032*), as shown in Table 1.

Overall Prevalence of Streptococcus pneumoniae in Relation to the Frequency of Public Transportation Use by Study Participants (N=241): Table 2 presents a logistic regression analysis of the frequency of public transportation use by study participants. Participants who regularly used taxi-buses (adjusted Odds Ratio = 2.22; 95% CI [1.27; 3.88], p=0.045*) were significantly more likely to be infected with *Streptococcus pneumoniae* than other users.

Overall Prevalence of Streptococcus pneumoniae Infection in Relation to Participants' Knowledge of the Bacteria (N=241): Table 3 indicates that not being vaccinated (adjusted Odds Ratio = 2.78; 95% CI [1.6; 4.1], p=0.041*) significantly exposed individuals to *Streptococcus pneumoniae* infection.

Overall Prevalence of Streptococcus pneumoniae in Relation to Risk Factors to Which Study Participants Were Exposed (241): A logistic regression analysis indicated that smoking (adjusted Odds Ratio = 5.23; 95% CI [2.77; 9.85]; p=0.012*) significantly exposed study participants to *Streptococcus pneumoniae* infection (Table 4).

Table 1. Logistic Regression Analysis for Potential Sociodemographic Determinants of *Streptococcus pneumoniae* Infection

Variables	Total number of participants tested N(%)	Overall *prevalence of Streptococcus pneumoniae*	Bivariate analysis		Multivariate Analysis	
		Positive % (n/N)	Crude OR IC 95%	p-value	Adjusted OR IC 95%	p-value
Gender						
Female	127 (53.7)	29.92 (38/127)	Reference	-	1	-
Male	114(46.3)	18.42 (21/114)	2.51 [1.32;4.9]	0.003	2.88 [1.69;4.92]	≤0.001*
Age range (years)						
18 - 40	104(43.16)	8.66 (9/104)	Reference	-	1	-
41 - 60	78 (32.37)	12.82 (10/78)	0.34[0.15 ; 0.74]	0.004	-	-
61 and over	59 (24.67)	67.8 (40/59)	5.8[3 ; 11.46]	≤0.001	2.12[1.3;1.42]	0,032*
Marital status						
Married	52(43.9)	3.33 (9/52)	0.58[0.23 ; 1.32]	0.20	-	-
Single	151(52.93)	15.67 (45/151	2.3[1.14 ; 4.87]	0.014	0.34[0.13 ;0.92]	0.41
Divorced	38 (3.17)	46.15 (5/38)	Reference	-	1	-
Professional status						
Employed	85 (66.59)	19.78 (15/85)	Reference	-	1	-
Unemployed	156 (33.41)	7.3 (44/156)	1.83[0.92 ; 3.81]	0.084	-	-
Place of residence						
Franceville	203 (77.32)	18.3 (39/203	Reference	-	1	-
Other	38 (22.68)	6.45 (20/38)	4.63[2.11 ; 10.29]	≤0.001	1.14[0.58 ; 0.26]	0.27

OR = Odds Ratio; CI = Confidence Interval; * = Significant Test

Table 2. Logistic Regression Analysis of the Overall Prevalence of *Streptococcus pneumoniae* in Relation to the Frequency of Public Transportation Use by Study Participants

Variables	Total number of participants tested n (%)	Overall *prevalence of Streptococcus pneumoniae*	Bivariate analysis		Multivariate Analysis	
		Positive% (n)	Crude OR IC 95%	p-value	Adjusted OR IC 95%	p-value
Do you regularly use the taxi-bus ?						
Yes	202 (83.82)	17.33 (35/202)	0.133 [0.06;0.29]	≤0.001	1.52 [0.86 ;2.70]	0.23
No	39 (16,18)	61.54 (24/39)	Reference	-	1	-
How often do you regularly use the taxi-bus?						
Every day	154(63.90)	32.47 (50/154)	4.15 [1.9 ;10.17]	≤0.001	2.22 [1.27 ;3.88]	0.045*
Several times a week	87 (36.1)	10.35 (9/87)	Reference	-	1	-
Do you often take the taxi-bus during rush hour?						
Yes	198 (82.16)	10.69 (37/198)	0.17 [0.27 ; 1.4]	0.21	-	-
No	43(17.84)	27.91 (12/43)	Reference	-	1	-

OR = Odds Ratio; CI = Confidence Interval; * = Significant Test

DISCUSSION

Also known as pneumococcus, *Streptococcus pneumoniae* is a bacterium responsible for a wide range of infections, including pneumonia, meningitis, and septicemia. Despite the availability of effective vaccines, pneumococcus continues to pose a significant threat to public health, particularly in developing countries. This study, conducted in Franceville, Gabon, focused on the prevalence of asymptomatic carriage of *Streptococcus pneumoniae* and sought to identify risk factors associated with this carriage among public transportation users. This population group, in frequent contact with a large number of people in often confined spaces, could play an important role in the transmission of the bacteria within the community. Our results highlight key risk factors and underscore the importance of targeted prevention strategies to combat the spread of pneumococcus.

variability of prevalences. The prevalence of *Streptococcus pneumoniae* infection is generally lower in regions where pneumococcal vaccine coverage is high [20]. The climatic conditions in which the different studies were conducted can also be noted, since pneumococcal infections are more frequent during cold and humid seasons [21]. Exposure to risk factors influences the variability of prevalences. The prevalence of *Streptococcus pneumoniae* infection may be higher in areas where populations are exposed to risk factors

Table 3. Logistic Regression Analysis of the Overall Prevalence of *Streptococcus pneumoniae* in Relation to Participants' Knowledge of the Bacteria

Variables	Total number of participants tested n (%)	Overall *prevalence of Streptococcus pneumoniae*	Bivariate analysis		Multivariate Analysis	
		Positive% (n)	Crude OR IC 95%	p-value	Adjusted OR IC 95%	p-value
Have you ever heard of Streptococcus pneumoniae (pneumococcus)?						
Yes	193 (80,09)	20.73 (40/193)	Reference	-	1	-
No	48 (19.91)	39.58 (19/48)	2.5 [1.19;5.16]	0.009	1.10 [0.80;2.46]	0.13
Do you know how pneumococcus is transmitted?						
Yes	76 (31.54)	30.27 (23/76)	Reference	-	1	-
No	48 (19.91)	21,82 (36/165))	0.64 [0.33 ;1.25]	0.2	-	-
Do you know how to prevent pneumococcal infections?						
Yes	96 (39.83)	17.71 (17/96)	Reference	-	1	-
No	145(60.17)	28.97 (42/145)	1.37 [0.71 ; 2.75]	0.37	0,33 [0,01 ;10,0]	0,53
Have you ever been vaccinated against pneumococcus?						
Yes	227 (94.19)	21.56 (49/227)	Reference	-	1	-
No	14 (5.81)	71.43 (10/14)	8.97 [2.46 40.94]	0.000	2.78 [1.6 ; 4.1]	0.041*

OR = Odds Ratio; CI = Confidence Interval; * = Significant Test

Table 4. Bivariate and Multivariate Logistic Regression of the Overall Prevalence of *Streptococcus pneumoniae* in Relation to Risk Factors to Which Study Participants Were Exposed

Variables	Total number of participants tested n (%)	Overall *prevalence of Streptococcus pneumoniae*	Bivariate analysis		Multivariate Analysis	
		Positive % (n)	Crude OR CI 95%	p-value	Adjusted OR CI 95%	p-value
Smoking						
Yes	34 (14,11)	61.77 (21/34)	0.14 [0.06;0.31]	≤0.001	5.23 [2.77 ;9.85]	0.012*
No	213(85.89)	17.84 (38/213)	Reference	-	1	-
Regular alcohol consumption						
Yes	23 (54,46)	16,39 (11/23)	3.23 [1.21 ;8.6]	0.010	-	-
No	218 (45,54)	5.88 (48/218)	Reference	-	1	-
History of respiratory disease						
Yes	44 (79,47)	6,74 (32/44)	0.11 [0,04 ; 0,27]	≤0.001	-	-
No	197(15,17)	23.53 (27/197)	Reference	-	1	-
Chronic health problems (diabetes, hypertension, etc.)						
Yes	4 (55,36)	8,07 (3/4)	9.58 [0,75 ;510,1]	0.047	-	-
No	237(44,64)	16.0 (56/237)	Reference	-	1	-
Proximity to people with frequent respiratory infections						
Yes	18 (9.8)	7 (7/18)	2.09[0,65 ;6,25]	0,15	-	-
No	223(29.3)	21,74 (52/223)	Reference	-	1	-

OR = Odds Ratio; CI = Confidence Interval; * = Significant Test

Thus, contrary to previous studies that found lower prevalences, such as 6% in Hawassa, southern Ethiopia [11].18.5% in Addis Ababa,Ethiopia [12], 21.3% reported in Biratnagar, Nepal [13], and 23% in Beira, Mozambique [14], this study indicated an overall prevalence of *Streptococcus pneumoniae* infection of 24.5%. This result is lower than the 43% reported in Jimma, southwestern Ethiopia [15]. The variability in the results of studies on the prevalence of *Streptococcus pneumoniae* infection can be explained by several factors. First, methodological differences between studies, including the types of populations studied, age, socioeconomic status, general health status, and comorbidities of participants, can influence the prevalence of *Streptococcus pneumoniae* infection [16]. Studies on specific populations (children, the elderly, immunocompromised patients) will have different prevalences of *Streptococcus pneumoniae* infection than those conducted on the general population [17]. The prevalence of *Streptococcus pneumoniae* infection can also vary depending on the type of biological sample used (blood, urine, sputum, cerebrospinal fluid) [18]. The diagnostic techniques used for *Streptococcus pneumoniae* infection (culture, PCR, serology) have different sensitivities and specificities, which can affect the results [19]. The definition of asymptomatic carriage can vary from one study to another, which influences the observed prevalence.Second, geographical and temporal factors play an important role in the

such as air pollution, smoking, or overcrowding [22]. Finally, if the selection of participants in a study is not random, the results may not be representative of the general population. Errors in data collection or diagnosis can affect the results. Regarding sample size, it should be noted that studies with small samples may have less precise and more variable results [23]. Further investigation using multivariate logistic regression identified several factors that significantly increase the risk of infection. First, men were almost 3 times more likely to be infected than women. This result is consistent with the 2019 Global Burden of Diseases (GBD) study, which indicated that lower respiratory tract infections (LRTIs) caused more than 2.49 million deaths worldwide, including more than 1.29 million in men and nearly 1.2 million in women [24]. Several factors may contribute to men's greater susceptibility to *Streptococcus pneumoniae* infection, including biological factors. Some studies suggest that female sex hormones may confer some protection against respiratory infections. Estrogens, for example, could stimulate the immune response and reduce airway inflammation [25]. Genetic differences between men and women could influence susceptibility to infection and immune response. Moreover, the immune system of men could be generally less efficient than that of women, which would make them more vulnerable to infections [26]. Behavioral factors also play a role, including smoking, which is a major risk factor for respiratory infections, and men generally smoke more than women. Alcohol abuse, which can weaken the immune system and increase the risk of infection, and hygiene, as men may have less rigorous hygiene practices than women, which would promote the transmission of bacteria, are also contributing factors. Men tend to consult a doctor less often than women, which can delay the diagnosis and treatment of infections [27]. Regarding social factors, men often hold jobs that expose them to more polluted environments or more frequent contact with sick people (e.g., construction, transportation). Men may also live in more precarious or infection-prone conditions [28]. This study indicated that older people were nearly 6 times more at risk of pneumococcal infection than younger people. This observation is corroborated by another study that showed a higher than expected prevalence of pneumococcal colonization in the elderly [29]. However, this conclusion contradicts the generally accepted idea that adults over 60 are rarely carriers of pneumococcus [30]. Furthermore, the study showed that frequent use of public transportation increased the risk of Streptococcus pneumoniae infection by more than 2 times. This result is consistent with a study on "An overview of the bacterial microbiome of public transport systems: risks, detection and countermeasures" [31]. and another that indicated that frequent travel on public transportation increases the risk of transmission of microorganisms, particularly through contact with shared surfaces such as handrails and the proximity of passengers in an enclosed space [32]. Several reasons can justify this result. First, public transportation, by definition, brings together a large number of people in an enclosed and often confined space. This promiscuity facilitates the transmission of pathogens, including *Streptococcus pneumoniae*, by air (respiratory droplets) or by contact with contaminated surfaces [33]. Second, ventilation in public transportation is often insufficient to renew the air and effectively eliminate infectious particles in suspension.

The lack of access to handwashing facilities or insufficient use of hydroalcoholic solutions promotes the transmission of bacteria. Users may touch contaminated surfaces and then bring their hands to their faces, facilitating the entry of pneumococcus into the respiratory tract [34]. Furthermore, stress, fatigue, and lack of sleep, often associated with frequent travel on public transportation, can weaken the immune system and increase susceptibility to infections [35]. Finally, public transportation is most often used by people of all ages and backgrounds, including those more vulnerable to infections such as children, the elderly, and immunocompromised individuals [36]. This study found that not being vaccinated increased the risk of *Streptococcus pneumoniae* infection by almost 3 times. This result, consistent with a previous study that indicated that the *S. pneumoniae* vaccine showed evidence of protection against invasive disease in adults [37], can be explained by the fact that pneumococcal vaccines stimulate the immune system to produce specific antibodies against the most common serotypes of the bacteria. Without vaccination, the body is more vulnerable to infection [38]. Vaccination reduces the circulation of pneumococcal strains in the population. Fewer vaccinated people means greater circulation of the bacteria, increasing the risk of exposure and infection for unvaccinated people [39]. Finally, some unvaccinated people may have risk factors that make them more susceptible to pneumococcal infection, such as a weakened immune system, chronic diseases, or age [40]. In this study, smokers had a 5 times higher risk of infection than non-smokers. This result is consistent with a narrative review, which indicated that smoking increases the risk of infectious diseases [27], and others in which smoking has also been shown to be a significant independent risk factor for invasive pneumococcal disease in the immunocompetent non-elderly adult population [41]. This can be explained by the fact that smoking has several adverse effects on the respiratory system, which may explain why smokers have a 5 times higher risk of pneumococcal infection than non-smokers [42].

Study limitations: Despite its contribution to research on *Streptococcus pneumoniae* infection, this study has some limitations. First, as the study focused only on public transport users (taxi-buses) in Franceville, the results may not be generalizable to the entire population of Franceville or to other regions of Gabon. Second, as this is a cross-sectional study, it can only establish associations and not cause-and-effect relationships between risk factors and *S. pneumoniae* infection.Third, the study focuses on asymptomatic carriage, which may not reflect the true incidence of the disease. Fourth, the study did not take into account other potential confounding factors, such as socioeconomic status, living conditions, or access to health care, which could influence the risk of infection. Although 241 participants is not a small sample, a larger sample size could increase the statistical power of the study and the precision of the estimates. The blood agar, even with 5 to 10% sheep blood, used in the study may not be the most sensitive method for detecting S. pneumoniae, particularly from samples with a low bacterial load or from patients who have recently received antibiotics. Some strains may also grow poorly or require longer incubation times. Finally, it is possible that the study participants are not representative of all taxi-bus users in Franceville, which could introduce selection bias.

CONCLUSION

This cross-sectional study conducted among taxi-bus users in Franceville revealed a high prevalence of asymptomatic *S. pneumoniae* carriage (24.5%). Risk factors associated with carriage included male sex, older age, regular use of taxi-buses, unvaccinated status, and smoking. These results underscore the importance of pneumococcal vaccination, particularly for the most vulnerable populations. Preventive measures, such as promoting hand hygiene and adequate ventilation in public transport, could help limit the spread of the bacteria. Further studies, particularly longitudinal ones, are needed to assess the impact of asymptomatic carriage on the occurrence of invasive pneumococcal diseases and to determine the effectiveness of public health interventions aimed at reducing the transmission of S. pneumoniae in the community.

Acknowledgements: The authors express their sincere gratitude to all respondents and data collectors for their invaluable contributions to this study. They also extend their thanks to the "Bonne Espérance" Clinic in the "Ongali" district of Franceville, Gabon, for their support.

Data Availability: However, raw data can be obtained from the corresponding author upon kind request.

Author Contributions: This study was a collaborative effort. TNM and HMK designed the study, while TNM, SM, and BAPP conducted it. Data analysis and interpretation involved HMK, BAPP, and TNM. HMK, BAPP, and SM conducted physical examinations and participant interviews. TNM took the lead on writing and editing the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of Interest: The authors have no conflicts of interest to disclose related to this publication.

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