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Original Article

Colonization by *Streptococcus pneumoniae* among children in Porto Velho, Rondônia, Western Brazilian Amazon

Colonização por *Streptococcus pneumoniae* em crianças na Amazônia Ocidental Brasileira, Porto Velho, Rondônia

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Abstract

Streptococcus pneumoniae is one of the primary pathogens that are associated with acute respiratory infections (ARI) that cause high rates of morbidity and mortality among children under five years of age in developed and developing countries. This study aimed to determine the prevalence of nasopharyngeal colonization, the antimicrobial resistance profile, and the capacity for biofilm formation by *S. pneumoniae* isolated from children aged 0-6 years with ARI throughout the Porto Velho-RO. A total of 660 swabs were collected from children with ARI. Molecular and biochemical tests were performed to characterize the isolates. The disk-difusion method and the E-test were used for antimicrobial sensitivity testing (TSA). Biofilm formation capacity was assessed using microtiter plate assays, and serotype detection was acheived using polymerase chain reaction (PCR) analyses. The colonization rate for *S. pneumoniae* was 8.9% (59/660) and exhibited a high prevalence in children under 23 months of age 64.4% (38/59). The observed serotypes were 9V and 19F with frequencies of 1.7% (1/59) and 13.6% (8/59), respectively. The antimicrobial susceptibility test revealed 100% (59/59) sensitivity to vancomycin. In contrast, trimethoprim and oxacillin exhibited high resistance rates of 76.3% (45/59) and 52.5% (31/59), respectively. Of the biofilm-forming isolates, 54.8% (23/42) possessed resistance to some antimicrobials. In this study, *S. pneumoniae* showed high rates of antimicrobial resistance and the ability to form biofilms, as these are factors that favor bacterial persistence and can cause serious damage to the host.

Keywords: antimicrobial resistance, children infections, colonization, nasopharyngeal, *Streptococcus pneumoniae*.

Resumo

Streptococcus pneumoniae é um dos principais patógenos associados a infecções respiratórias agudas (IRAs) que causam altas taxas de morbidade e mortalidade entre crianças menores de cinco anos de idade em países desenvolvidos e em desenvolvimento. Este estudo teve como objetivo determinar a prevalência de colonização da nasofaringe, o perfil de resistência antimicrobiana e a capacidade de formação de biofilme dos *S. pneumoniae* isolados de crianças de 0 a 6 anos com IRA na cidade de Porto Velho-Rondônia. Um total de 660 swabs foi coletado de crianças com IRA. Testes moleculares e bioquímicos foram realizados para identificar os isolados bacterianos. O método de disco-difusão e o E-test foram utilizados para o teste de sensibilidade antimicrobiana (TSA). A capacidade de formação de biofilme foi avaliada por meio de ensaios em placas de microtitulação e a detecção de sorotipos foi obtida por meio de análises de Reação em Cadeia da Polimerase (PCR). A taxa de colonização por *S. pneumoniae* foi de 8,9% (59/660) e apresentou alta prevalência em menores de 23 meses de idade 64,4% (38/59). Os sorotipos identificados foram 9V e 19F com frequências de 1,7% (1/59) e 13,6% (8/59) respectivamente. O teste de sensibilidade aos antimicrobianos revelou 100% (59/59) de sensibilidade à vancomicina. Em contraste, trimetoprima e oxacilina apresentaram altas taxas de resistência de 76,3% (45/59) e 52,5% (31/59) respectivamente. Dos isolados formadores de biofilme 54,8% (23/42) possuíam resistência a alguns dos antimicrobianos. Neste estudo, *S. pneumoniae* apresentou altas taxas de resistência antimicrobiana e capacidade de formar biofilmes, pois são fatores que favorecem a persistência bacteriana e podem causar sérios danos ao hospedeiro.

Palavras-chave: resistência antimicrobiana, infecções em crianças, colonização, nasofaringe, *Streptococcus pneumoniae*.

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1. Introduction

Acute respiratory infections (ARI) are among the major causes of morbidity and mortality worldwide that affect children under the age of five years in developing countries (Tchidjou et al., 2010). Among the major bacterial pathogens related to ARI, *Streptococcus pneumoniae* with invasive pneumococcal disease (IPD) exhibits an incidence rate of 11.3% and 13.8% per 100,000 individuals within the population and is responsible for 11% of deaths in children under five years of age (O'Brien et al., 2009; ECDC, 2018).

Streptococcus pneumoniae (pneumococcus) is an opportunistic Gram-positive pathogen that uses the upper respiratory tract as its reservoir and can colonize the nasopharynx of human beings from the first day of life (Donkor, 2013; Faust et al., 2012). The transport rate of pneumococcus varies widely and can average from 20 to 40% in immunocompetent children, and its prevalence decreases with age, where is can reach 5%–10% in adults (Loughran et al., 2019).

In most cases, pneumococci colonize the mucosal surface and result in individuals acting only as healthy carriers. However, in the presence of bacterial and host predisposing factors, these bacteria can invade adjacent sterile sites or the bloodstream and cause severe localized or systemic infections such as otitis media, pneumonia, sepsis, and meningitis. In addition, to being a prerequisite for invasive disease, nasopharyngeal colonization also allows for continued transmission and dissemination of pneumococcus within the Community (Chen et al., 2014; Weiser et al., 2018).

Low socioeconomic status and inadequate environmental conditions such as family overcrowding, nutritional conditions, and passive exposure to smoke have been identified as the primary risk factors that are involved in the occurrence of respiratory diseases in childhood (Duse et al., 2010; Macedo et al., 2007; Van Gageldonk-Lafeber et al., 2007).

The presence of numerous virulence factors associated with the invasiveness of different tissues and evasion of the host immune system allow pneumococcus to be a successful pathogen. Pneumococci can be classified into several different serotypes based on the structure of their capsular polysaccharide, and there are approximately 48 serogroups and 93 serotypes (Hyams et al., 2010). The capsular polysaccharide as the main virulence factor in these bacteria and acts as the primary defense against the host immune system where it can inhibit complement activity and prevent phagocytosis by polymorphonuclear cells during the invasion process (Loughran et al., 2019). In addition to actign as an important epidemiological marker, the capsule is the target structure in the production of vaccines against this microorganism. In March 2010, the 10-valent PCV10 conjugate pneumococcal vaccine was included in the Brazilian Immunization Program that contains the most prevalent serotypes in invasive pneumococcal disease (IPD) to reduce the burden of pneumococcal diseases (Brandileone et al., 2018).

Despite the great advance the insertion of the pneumococcal vaccine, treatment of infections caused by *S. pneumoniae* has become problematic and is limited due to the development of resistance to the antimicrobials used. Over the past 40 years, increased resistance to penicillin and to macrolides by *S. pneumoniae* has been reported in many regions of the world, thus threatening the advances made during the post-vaccine period (Karcic et al., 2015; Safari et al., 2014; Cherazard et al., 2017).

Studies have also demonstrated the potential capacity for biofilm formation *in vitro* by certain strains of *S. pneumoniae*, and this process is associated with the colonization of the nasopharynx and subsequently with a decrease in susceptibility to antimicrobials and escape from host defense systems (Chao et al., 2015; Vermee et al., 2019).

The study of colonization of the nasopharynx by *S. pneumoniae* has contributed to the surveillance of antimicrobial resistance and allows for monitoring of the distribution of circulating serotypes in the post-PCV10 era (Devine et al., 2015; Menezes et al., 2016; Zhou et al., 2015). In Brazil, there are few published studies regarding this subject, and this is particularly true in the North region, where no studies have been reported to date. In this context, this study aimed to determine the prevalence of nasopharyngeal colonization, the epidemiological data, the antimicrobial resistance profile, and the ability to form biofilms by *S. pneumoniae* isolated from children aged 0-6 years with ARI in Porto Velho-RO.

2. Methodology, Location and Study Population

This study was carried out in the city of Porto Velho-Rondônia located in the Brazilian Amazon region (see Figure 1). Samples of nasopharyngeal secretions were obtained from 660 children that were admitted to the Hospital Infantil Cosme e Damião (HICD) located in the city of Porto Velho that is the main children's medical center in the state of Rondônia. Children aged 0 to 6 years who presented with a clinical profile suggestive of ARI such as cough, fever, runny nose, nasal discharge, wheezing, dyspnea, itchy eyes, and otalgia were included. After a clinical evaluation was performed by the HICD physician and signed Informed Consent Form (FICF) were obtained, the children were admitted to the study. Sociodemographic characteristics were obtained at the completion of the epidemiological investigation.

2.1. Clinical collection and sample handling

Collections occurred between February and December of 2013. The combined swab technique of the oropharynx and nasopharynx was used to obtain the samples. The swabs were then placed in the same bottle containing 3 ml of physiological saline solution. The samples were transported to the Microbiology Laboratory of the Tropical Medicine Research Center (CEPEM) and stored under refrigeration (4°C) to ensure the survival of the microorganisms.

Initially, the samples were homogenized and seeded into blood agar and subsequently incubated at 37 °C for 24 h in an atmosphere containing 5% CO2. To obtain isolates suggestive of *S. pneumoniae*, the morphological characteristics, hemolytic activity, catalase activity, Gram staining, sensitivity to optoquine and bacitracin (5µg disk), and bile solubility were evaluated (WHO, 2011).

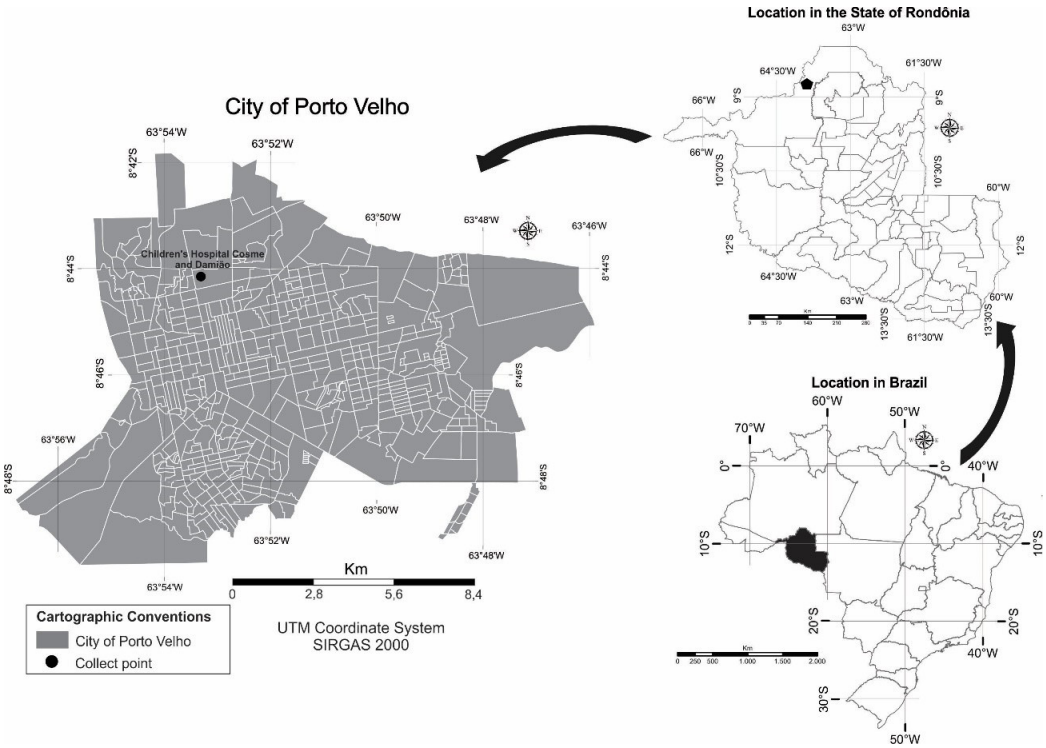

Figure 1. Location of the study area.

The study was approved by the Ethical Committee of Rondônia Tropical Medicine Research Center (protocol 17/11 of 31/08/2011) and CAAE 0007.046.000-11.2010).

2.2. 16S rRNA amplification, and sequencing

All colonies with a suggestive profile of *Streptococcus spp*. were subjected to polymerase chain reaction (PCR) to amplify the 16S ribosomal gene, using the primers 16S08F (GYCCADACWCCTACGG) and 16S08R (CACGACTGACGAC) developed by Luiz Shozo Ozaki (from Virginia Commonwealth University (VCU). From a bacterial colony of blood agar, a PCR reaction was performed containing: 16.8 µL of Milli Q H2O, 2.0 µL of DNTP (2.5 mM), 2.0 µL of Buffer (10xPCR, 2 .5 mM), 2.0 µL of MgCl2 (50 mM), 2.0 µL of Primers (10 p/Mol), and 0.2 µL of Taq polymerase (5 U/µL) at a final volume of 25 µL. PCR parameters were 94°C for 5 min, 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, and extension at 72°C for 8 min. Amplification verification was performed using 1% agarose gel electrophoresis and visualization with a UV transilluminator. The amplification products were purified using the Qiagen commercial kit (Sample & Assay Technologies) according to the manufacturer's protocol. Sequencing was performed using an automatic DNA sequencer (ABI 3100, Center and Research Gonçalo Moniz-Fiocruz). The sequencing results were extracted to a specific file in FASTA format and submitted to the Human Oral Microbiome Database (HOMD) program that identifies the closest results of 16S rRNA sequences sent by users between the HOMD or other 16S rRNA gene sequences (http: //www.homd.org/index.php).

2.3. S. pneumoniae serotyping through multiplex PCR

The *S. pneumoniae* isolates were subjected to genomic DNA extraction using the phenol/chloroform method and then stored at -20 °C for further use in the investigation of serotypes (Sambrook et al., 1989).

Capsular serotyping of *S. pneumoniae* was performed using multiplex PCR described previously by Pai et al. (2006). For each reaction, the following components were used: 1 µL of DNA from the bacteria of interest in the research, 15.8 µL of autoclaved MiliQ water, 2.0 µL of DNTP (2.5 mM), 2.0 µL of Buffer (10x PCR 2.5 mM), 2.0 µL of MgCl2 (50 mM), 2.0 µL (10 p/mol) of primers specific for each serotype and 0.2 µL (50 mM) of Taq DNA polymerase (Invitrogen) at a final volume of 25 µL. Amplification conditions for each serotype were performed as described previously by Coskun-Ari et al. (2012).

2.4. Antimicrobial susceptibility test

Streptococcus pneumoniae isolates were screened for penicillin susceptibility assessment using the oxacillin disc diffusion method (1µg) as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013). Isolates that presented an inhibition zone ≤ 19 mm in diameter were presumed to be resistant to penicillin and subsequently subjected the minimum inhibitory concentration (MIC) determination. The MIC was determined using the epsilometer-test (E-test) technique. The *S. pneumoniae* ATCC 49619 strain was used as the test quality control. The MIC cutoff points for benzylpenicillin were recommended by the CLSI (CLSI, 2014). To determine

the phenotypic profile of bacterial sensitivity *in vitro*, other antimicrobials were tested and included vancomycin (30µg), azithromycin (15µg), tetracycline (30µg), trimethoprim (30µg), chloramphenicol (30µg), rifampicin (5 µg), and clindamycin (2 µg).

2.5. Biofilm detection by spectrophotometry

Biofilm detection on polystyrene was performed using 96-well polystyrene microtiter plates (Costar, USA), as previously described for Stepanovic et al. (2007), with slight modifications.

For interpretation of the results, the strains were classified as follows: no biofilm producer (NBP) (D.O. 0,120 nm), weak biofilm producer (WBP) (D.O. 0,120 a 0,240 nm), and strong biofilm producer (SBP) (D.O. 0,240 nm) (Christensen et al., 1985). For control quality of the biofilm assay, strains of the were used *Escherichia coli* enteroagregative 042 that is a strong biofilm producer, and the non-pathogenic *E. coli* strain HB101 was used as a negative control.

2.6. Statistical analysis

The statistical analyses applied in this study were performed according to non-parametric tests using the GRAPHPAD PRISM 6.0 program, and these tests included Fisher's exact test and odds ratio analysis. Statistical significance was defined as $p < 0.05$. Tests parametrics such as Student t test were also used.

3. Results

A total of 660 samples of nasopharyngeal secretions were obtained, and 315 bacterial isolates were identified according to genus and species after being subjected to PCR and amplification of the 16S ribosomal gene. Of this total, 41.3% (130/315) of isolates were identified as belonging to *Streptococcus* sp., 45.4% were *S. pneumoniae* (59/130), 20% were *S. agalactiae* (26/130), 19.2% were *Streptococcus* spp. (25/130), 8.5% were group D *Streptococcus* (11/130), and 6.9% were *S. viridans* (9/130) (as shown in Table 1).

Sociodemographic data showed the rate of *S. pneumoniae* carriers among the children studied was 8.9% (59/660), and 50.8% (30/59) were detected in males. A higher rate of isolation was observed in children under 23 months of age 64.4% (38/59) (as shown in Table 2).

In relation to environmental factors, even with a high number of people living with children, we did not observe a statistical correlation between agglomeration of people and colonization, nor in relation to the place of housing. The electric fan was the main means of ventilation in the houses observed in 56,63% (374/660), and most rooms had no or only one window for environmental ventilation (as shown in Table 2).

Of the children colonized with *S. pneumoniae*, 18.6% (11/59) belonged to the passive smoker group, and 16.9% (10/59) were associated with others variables (asthma and passive smokers, and asthma and rhinitis). Among the symptoms, cough 89.8% (53/59), runny nose 81.3% (48/59), and nasal obstruction 81.3% (48/59) were the most prominent symptoms, and there was a statistically significant difference in nasal obstruction between colonized children and children with ARI (p=0.0343). Regarding environmental factors, we observed that ARI occurred throughout the year, but with a higher prevalence of colonization in the period of decreased precipitation (p=0.0049) (see Figure 2).

All 59 *S. pneumoniae* isolates were serotyped using multiplex PCR. Serotypes 9V and 19F were found at frequencies of 1.7% (1/59) and 13.6% (8/59), respectively. Of the isolates, 84.7% did not possess typing based on the serotypes evaluated in the study.

In regard to the susceptibility profile of *S. pneumoniae* to antimicrobials, we observed a higher frequency of resistance to trimethoprim in 76.3% (45/59) of the isolates, to oxacillin in 52.5% (31/59) of the isolates, and to azithromycin in 37.2% (22/59) of the isolates. The antimicrobials that exhibited a high sensitivity rate were vancomycin 100% (59/59), chloramphenicol 98.3% (58/59), rifampicin 93.2% (55/59), clindamycin 86.4% (51/59), and tetracycline 67.8% (40/59) (as shown in Table 3).

Table 1. Results of 16S ribosomal gene sequencing.

Species	Number of isolates	%
Streptococcus pneumoniae	59	45.4
Streptococcus agalactiae	26	20
Streptococcus spp (NC)	25	19.2
Streptococcus group D	11	8.5
Streptococcus viridans	09	6.9
Staphylococcus sp.	48	15.2
Staphylococcus epidermis	24	7.6
Granulicatella adiaces	23	7.3
Neisseria sp.	18	5.7
Rothia mucilaginosa	12	3.8
Agrobacterium tumefaciens	08	2.5
Moxarella catarrhalis	08	2.5
Staphylococcus aureus	08	2.5
Corynebacterium sp.	06	1.9
Rolstonia sp.	06	1.9
Microbacterium sp.	06	1.9
Kocuria sp.	04	1.3
Acinetobacter baumannii	02	0.6
Bacillus subtilis	02	0.6
Entrobacter sp.	02	0.6
Escherichia coli	02	0.6
Pseudomonas aeruginosa	02	0.6
Abiotrophia defectiva	02	0.6
Haemophilus sp.	01	0.3
Lactobacillus fermentum	01	0.3
Total	**315**	**100**

% = Frequency.

Table 2. Factors associated with *Streptococcus pneumoniae* colonization.

Socioepidemiological data	ARI N=660	Colonization N=59	p-value
Age months [mean (min-max)]	23.0 (0.1 - 83.84)	23.7 (0.3 - 74.4)	0.6733
Gender			
Male	362 (54.8)	30 (50.8)	0.5839
Female	298 (45.1)	29 (49.2)	
Main place of living [n (%)]			
Own home	262 (39.6)	49 (83.0)	
school	21 (3.1)	8 (13.5)	0.1182
Not informed	377 (57.1)	2 (3.3)	-
Persons living with child [n (%)]			
Not informed	15 (2.2)	3 (5.0)	-
1-2	197 (29.8)	23 (38.9)	1.1837
3-5	346 (52.4)	29 (49.1)	0.6843
>6	102 (15.4)	4 (6.7)	0.0837
Windows / rooms [n (%)]			
0-1	464 (70.3)	48 (81.3)	0.09676
>1	196 (29.6)	11 (18.6)	
Type of ventilation [n (%)]			
No type	87 (13.1)	6 (10.1)	-
Fan	374 (56.6)	34 (57.6)	0.8809
air conditioning	199 (30.1)	19 (32.2)	
Family environmental exposition [n (%)]			
Not informed/No diseases	271 (41.0)	29 (49.1)	-
Asthma Only	80 (12.1)	8 (13.5)	0.6817
Passive smoker only	106 (16.0)	11 (18.6)	0.5828
Rhinitis Only	55 (8.3)	1 (1.6)	0.0753
Tuberculosis	6 (0.9)	0	1.0000
In association	142 (21.5)	10 (16.9)	0.5064
Vaccination (only full) [n (%)]			
DTP/Hib	161 (24.4)	21 (35.5)	0.0147*
Pneumococci	187 (28.3)	25 (42.4)	0.0034*
Influenza	15 (2.3)	1 (1.7)	1

P-value not obtained for variable 'not informed'. *There was significant relation between vaccination with the presence of ARI and colonization.

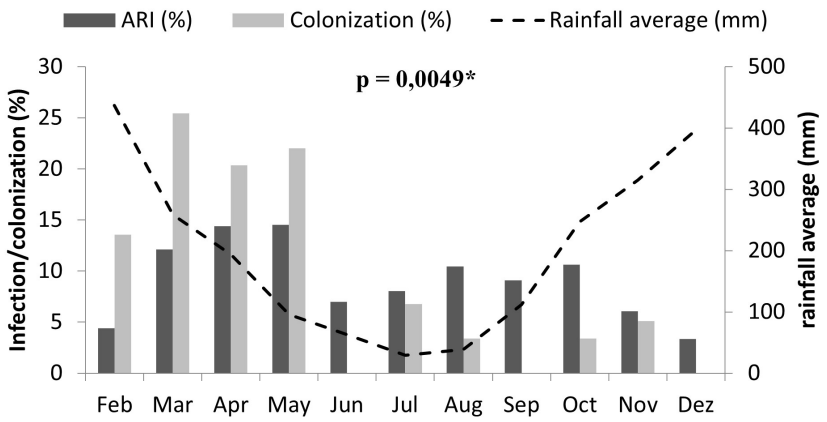

Figure 2. Percetage of ARI records and colonization by *S. pneumoniae*, and average rainfall (mm) during the study period.

Table 3. Antimicrobial susceptibility profile of 59 *S. pneumoniae* isolates isolated from children with ARI.

ANTIMICROBIAL	(n = 59)					
	Sensitive	%	Intermediate	%	Resistant	%
Oxacilin (OXA)	28/59	47.5	-	-	31/59	52.5
Vancomycin (VAN)	59/59	100	-	-	-	-
Azithromycin (AZI)	20/59	33.8	17/59	28.8	22/59	37.2
Tetracycline (TET)	40/59	67.7	15/59	25.4	04/59	6.7
Trimethoprim (TRI)	02/59	3.4	12/59	20.3	45/59	76.3
Chloramphenicol (CLO)	58/59	98.3	01/59	1.7	-	-
Rifampicin (RIF)	55/59	93.2	02/59	3.4	02/59	3.4
Clindamycin (CLI)	51/59	86.4	-	-	08/59	13.6
Benzylpenicillin	52/59	88.1	06/59	10.2	01/59	1.7

In general, the susceptibility to the tested antimicrobials was high, where 88.1% (52/59) were sensitive to Benzylpenicillin. The percentage of non-susceptibility to penicillin was 10.2% (06/59) intermediate and 1.7% (01/59) full resistance (as shown in Table 3).

In regard to the biofilm formation capacity of the *S. pneumoniae* isolates, 71.2% (42/59) exhibited a strongly adherent phenotype (FMA), 15.3% (9/59) were weakly adherent (FCA), and 13.5% (8/59) were non-adherent (NA).

When correlating the resistance profile and biofilm formation, it was observed that of the 42 isolates that exhibited a strong biofilm-forming phenotype, 54.8% (23/42) possessed resistance to antimicrobials according to the disk diffusion test (p = 0, 0008).

4. Discussion

S. pneumoniae is one of the major causes of respiratory tract diseases, and its study in the context of public health is relevant due to its high rate of morbidity and mortality in childhood in both developed and developing countries (Weiser et al., 2018).

The pre-requisite for the development of pneumococcal disease is the presence of nasopharyngeal colonization. The present study obtained a rate of 8.9% (59/660) of children admitted with characteristic ARI with colonization by *S. pneumoniae* in the nasopharynx. No correlation was observed between sex and carrier status, and this was in agreement with other previous studies (Bogaert et al., 2004; Ozdemir et al., 2008; Velasquez et al., 2009). In Brazil, studies have demonstrated that the prevalence of colonization in children ranges from 13.9 to 72%, particularly among those who attend day care centers or nurseries (Rey et al., 2002; Laval et al., 2006; Brandileone et al., 2016).

Petraitiene et al. (2015) conducted a study of 900 children under 6 years of age and observed a 40.8% (367/900) rate of colonization with *S. pneumoniae* with little difference between sexes, and they noted that 73.5% (270/367) of these children attended day care centers (Petraitiene et al., 2015). Several studies examining colonization by *S. pneumoniae* have been conducted in schools and day care centers in Brazil, both of which constitute risk factors for colonization (Laval et al., 2006; Neves et al., 2013). Studies by Reis et al. (2008), Pimenta et al. (2011) and Neves et al. (2013), revealed that the prevalence rate was 55% in Fortaleza, 49% in Brasília, 49% in Rio de Janeiro, and 66% in Salvador.

We demonstrated that 18.6% of the cases were related to living with smokers, and in 16.9% of the cases, we observed that the association between others variables, as asthma and smoking was statistically significant (p = 0.028). Previous studies have revealed that passive smokers are more susceptible to bacterial infections. Lesions within the respiratory epithelium, connective tissue, and vascular endothelium are predisposing factors for the development of pneumococcal bacteremia caused by tobacco consumption, even at low concentrations (Strulovici-Barel et al., 2010; Almirall et al., 2014; Strzelak et al., 2018).

In the present study, we observed a higher prevalence of colonization by *S. pneumoniae* accompanying the period of lower rainfall. However, few studies have investigated the correlation between climatic factors and colonization by *S. pneumoniae* (Liu et al., 2017).

The hospital environment has high rates of antimicrobial resistance due to the large handling of hospital antimicrobials in these places, causing a global health problem with estimates of thousands of deaths in the next years (Wu et al., 2021; Melo et al., 2021). In 2017, *S. pneumoniae* joined the list of 12 priority pathogenic bacteria that was released by the World Health Organization due to of the increase in the rate of infections and resistance to antimicrobials (WHO, 2017). The majority of studies have correlated carrier rates with antimicrobial resistance and also with resistance in general and the most common serotypes (Rey et al., 2002; Fabio et al., 2001; Malfroot et al., 2004; Bayraktar et al., 2005; Cardozo et al., 2006; Matsumoto et al., 2007; Artan et al., 2008; Zhao et al., 2019).

Resistance of *S. pneumoniae* to trimethoppine was observed along with resistance to penicillin in the 1980s, and this resistance was enhanced by the use of this drug in the treatment of otitis media in children and as an HIV prophylaxis in adults. Globally, the resistance of *S. pneumoniae* to antimicrobials varies, particularly to

trimethopin, and this resistance is a prevalent feature in developed and developing countries (Cherazard et al., 2017).

In this study, the isolates exhibited high rates of resistance to trimethoppine 76.3%, oxacillin 52.5%, and azithromycin 37.3%. Regarding the sensitivity profile, the isolates were 100% sensitive to vancomycin, 98.3% sensitive to chloramphenicol, 93.2% sensitive to rifampicin, 86.4% sensitive to clindamycin, and 67.8% sensitive to tetracycline.

It was also demonstrated that 88.1% of the isolates were sensitive to penicillin, while 10.2% exhibited intermediate resistance and 1.7% posessed full resistance. This was lower than the rates reported by other studies that demonstrated high resistance to penicillin that ranged from 55% to 63.3% (Velasquez et al., 2009; Rey et al., 2002; Yu et al., 2001; Monteros et al., 2007; Masuda et al., 2002). This high sensitivity to penicillin has also been demonstrated in other Brazilian and international studies (Marchese et al., 2011; Rocha et al., 2017).

In Brazil, studies performed in Salvador between the years 2000 to 2007 and between 1996 to 2012 revealed penicillin resistance rates of 22% and 20.3%, respectively (Menezes et al., 2016; Santos, 2015). Other studies performed in Rio de Janeiro revealed that the rate was 27% and that 20% of isolates were not susceptible to penicillin (Neves et al., 2013; Pinto et al., 2017). In other locations around the world, including Suzhou, China, studies by Geng et al. (2014) examining children under 5 years of age with respiratory infections revealed that 39.4% of the strains were resistant to penicillin. Torres et al. (2013) conducted a survey between 2007 and 2009 in seven regions of Peru incorporating healthy children under two years old and observed a 58% resistance rate to cotrimoxazole, a 52.2% resistance rate to penicillin, a 29.1% resistance rate to tetracycline, a 28.9% resistance rate to azithromycin, and a 26.3% resistance rate to erythromycin.

Similar to other microorganisms that colonize the respiratory tract, the persistence of *S. pneumoniae* in this niche is related to its ability to form adherent biofilms (Chao et al., 2019). The present study demonstrated that 71.2% of the isolates possessed the ability to form biofilms with a strongly adherent phenotype. When correlating biofilm formation and resistance to oxacillin, it was observed that 54.8% of the isolates were not susceptible to the antimicrobial-formed biofilm, and this was a statistically significant correlation (p=0.0008).

According to Sanchez et al. (2011) and Simell et al. (2012), the clinical significance of biofilm formation is still unknown; however, it has been suggested that it aids in the growth of the pathogen during colonization and contributes to the development of invasive diseases. In a review performed to discuss the properties of pneumococcal biofilms and their role during the colonization of these pathogens, Chao et al. (2015) suggested that the biofilm is a mechanism that pneumococci use to resist exposure to antimicrobials during colonization in the human host. In this context, biofilms not only influece the metabolism of the microorganism but also allow for communication between the various species present in a givern location, ultimately leading to the sharing of antimicrobial resistance genes (Chao et al., 2019).

In the present study, 59 *S. pneumoniae* isolates were subjected to PCR to identify serotypes 1, 3, 4, 5, 6A/B, 7A, 9V, 14, 18C, 19A, 19F, and 23F, and success was acheived in the identification of serotype capsulars of only nine isolates, where serotypes 9V and 19F F were identified in 1.7% and 13.6% of the isolates, respectively. These are among the most frequently reported by authors in Brazil and Latin America (Rey et al., 2002; Fabio et al., 2001; Camargos et al., 2006).

The introduction of conjugated pneumococcal vaccines (PCVs) has resulted in a great impact on reducing the burden of invasive pneumococcal disease (IPD) associated with vaccine serotypes (Balsells et al., 2017; Masomian et al., 2020). In this study, the low rate of serotypes that were identified can be justified by the limitation of the research on vaccine serotypes that are PCV10 serotypes. In Brazil, PCV10 was introduced in 2010 in the National Public Immunization Program for Children, and since then, studies conducted throughout the country have demonstrated the impact of the vaccine in reducing IPD and vaccine serotypes and have also identified an increase in other non-vaccine serotypes (Brandileone et al., 2018; Brandileone et al., 2019). Santos et al. (2013) performed a study at the University Hospital of the University of São Paulo/Brazil to evaluate *S. pneumoniae* serotypes in patients with IPD before and after the vaccine, and they observed a reduction in the incidence from 20.3 to 3.94% in children under 2 years of age, and this represented a reduction of greater than 80% in cases. Brandileone et al. (2018) revealed that at 5 years after the introduction of PCV10, IPD cases caused by serotypes included in the vaccine exhibited a decrease of 85.6% among children aged 2 months to 4 years.

The present study was not designed to assess the effectiveness of the vaccine in the childhood population of Porto Velho; however, it does reveal a possible impact of the vaccine on the distribution of serotypes throughout the region. These findings highlight the need to monitor the frequency of isolates and post-vaccination genetic changes to detect potential replacements of disease by serotypes resulting from "vaccine escape", particularly in areas where colonization by non-typable isolates is frequent (Andrade et al., 2010).

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