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Antibiotic Resistance Profile of Serotypes of *Streptococcus pneumoniae* Strains in Bangui, from 2017 to 2022: Case of Serotype 1

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Abstract

Goals: The aim of this study was to determine the antibiotic resistance profile of serotypes of *Streptococcus pneumoniae* strains circulating in Bangui. **Methodology:** A prospective and analytical analysis was carried out at the National Laboratory of Clinical Biology and Public Health from 2017 to 2022. The strains came from our study on the contribution to the study of antibiotic sensitivity of *Streptococcus pneumoniae* strains. The multiplex PCR test was used for its cost-effectiveness in terms of amplifiers which can be purified in order to be sequenced. It also makes it possible to detect several germs as well as their serotypes. For a PCR reaction, several elements are involved in the reaction medium or Master Mix. These are the desoxyribonucleotides (dNTPs), the magnesium ions ($MgCl_2$) and the primers. A set of 14 primers divided into 3 classes were used. Class 1 primers served as an internal control by targeting the cpsA gene. It is a highly conserved gene found in capsular loci characterized to date. The primers of the second class were used to target specific serotypes by specific reactions (out of six possibilities). The group reaction was carried out using the primers of the third class in order to carry out an initial screening of the samples and to classify the pneumococcal isolates. Related serotypes were grouped based on the amplification of common genes. Using the technique of electrophoresis on agarose gel and an ultraviolet radiation device, the migration bands are then visualized and analyzed.

The data collected had been entered into Excel 2010 and analyzed with Epi info 7. The exact Fischer chi2 test at the 5% threshold, the relative risk and its 95% confidence interval were used to compare the proportions and determine the associations. **Results:** 187 antibiotic-resistant strains of *Streptococcus pneumoniae* were collected. The average frequency of serotypes 1, 9A, 4 and untypeable identified were 43.59%, 18.18%, 18.27% and 39.57% respectively. The frequency of serotype 1 was predominant for the age group over five years old with 56.88%. The male sex was predominant with 55.08% for serotype 1. Resistance to penicillin and gentamicin for serotype 1 during this study, for the age group under 5 years old, was 77%. For serotypes 19A and 4, tetracycline resistance was predominant with 20% for the age group under 5 years. The resistance to penicillin and gentamicin of non-typeable serotypes was 33% for the age group under 5 years old. For the age group over 5 years old, resistance to erythromycin predominated at 37%. The distribution of serotypes by sex depending on antibiotic resistance was variable. There was a statistically significant association between identified serotypes and antibiotic resistance ($p < 0.05$). **Conclusion:** The study determined serotypes 1, serotypes 19A, serotypes 4 and non-typeable serotypes. These results would be due to the quality of vaccination or poor protection of vaccines.

Keywords

Streptococcus pneumonia, Serotype, Antibiotic Resistance, Bangui

1. Introduction

Streptococcus pneumoniae infections are a major public health problem worldwide [1] [2]. *Streptococcus pneumoniae* belongs to the Streptococcacae family of the genus Streptococcus. It is facultative aeroanaerobic gram-positive cocci [3]. It is one of the most common and virulent pathogens in invasive and non-invasive infections [4] [5]. There are 9 million cases of infection at *Streptococcus pneumoniae* in the world, including 2.4% million in Africa. In Mali, out of 58 cas of méningitidis, 36 cases or 62.1% are to *Streptococcus pneumoniae.* Early studies carried out in Central Africa Republic had ascended only on 218 suspicious cases of meningitidis, 68.42% were awarded at *Streptococcus pneumoniae*. The virulence of *Streptococcus pneumoniae* is due to the presence of a polysaccharide capsule, more than 90 of which determine distinct serotypes [6] [7]. The incidence of the serotype varies according to age, race, geographic region, as well as the severity of the disease and the presence of antibiotic resistance genes. The WHO has recommended the introduction of pneumococcal conjugate vaccines in Expanded Programs on Immunization (EPI) to prevent and reduce the incidence of pneumococcal infections caused by vaccine serotypes and reduce the spread of antibiotic resistance [8]. The heptavalent pneumococcal conjugate vaccine (PCV-7) is much more widely used in Western countries [8]. Studies carried out in France show cases of failure after vaccina-

tion with PCV7 and PCV13 in children in the pediatric department [9]. Antibiotic treatment failure is one of the reasons for antibiotic resistance in recent years among *Streptococcus pneumoniae* strains [10] [11] [12]. Some emerging serotypes show reduced sensitivity to several antibiotics including ceftriaxone [13]. The results of vaccination with PCV appear to reduce the number of cases of invasive and invasive infections [14] [15]. According to the report from the National Reference Center for Pneumococci, changes in the distribution of serotypes are observed both in children and adults with a virtual disappearance of vaccine serotypes except serotype 3, and the appearance of non-vaccinated serotypes vaccines. Since 2016, the emergence of serotype 24F has been the cause of the number of cases of meningitis in children under 5 years old. Worldwide, there are a wide variety of *Streptococcus pneumoniae* serotypes. Serotypes circulating in developed countries may be different from those in developing countries. The most common serotypes are 23, 14, 19 and 6 and are the most resistant to penicillin. In Africa, the trend of pneumococcal infection is decreasing [16]. In the Central African Republic, there is little data on the impact of pneumococcal vaccines. Data on the profile of pneumococcal serotypes are less documented. PCV 13 vaccination coverage in the Central African Republic is estimated at 90%. Despite the introduction of PCV, cases of *Streptococcus pneumoniae* infection are still reported.

The objective of this study is to evaluate the profile of antibiotic resistance of serotypes of *Streptococcus pneumoniae* strains circulating in Bangui at the Central African Republic.

2. Materials and Methods

From January 2017 to December 2022, the National Laboratory of Clinical Biology and Public Health (NLCBPH) received samples of CSF, pus, pleural fluid, joint fluid, from the four hospitals of Bangui in CAR (Le Complexe Hospitalo-Pediatric University, the Maman Elisabeth University Hospital, the Community University Hospital, the Central African Sino Friendship University Hospital Center). These health establishments are Central reference Hospitals with services different specialities. They receive all cases referred from peripheral health facilities that do not have an adequate technical platform. The patients presented meningeal syndromes for the CSF [17]. For CSF, samples were systematically collected and analyzed in hospital laboratories. Based on the leukocyte count (White blood cells ≥5 mm^3), an aliquot was transferred to Trans-Isolate medium and sent to the LNBCSP. A total of 187 samples were isolated for the presence of gram-positive diplocus in chains on fresh or cooked blood agar media. An antibiogram was carried out to determine the sensitivity profile of the strains of *Streptococcus pneumoniae.* Serotyping was carried out on the strains of *Streptococcus pneumoniae* known to be resistant to antibiotics. The swamplons had been routed to NLCBPH. Data were collected from laboratory records on a computer by the principal investigator. The data were the socio-demographic

characteristics and the different laboratory results concerning our subject.

2.1. Analysis of *Streptococcus pneumoniae* Strains

The strains of *Streptococcus pneumoniae* were those of our study carried out on the contribution to the study of the antibiotic sensitivity of strains of *Streptococcus pneumoniae* [18]. For serotyping, the multiplex PCR test was used because of its cost-effectiveness in terms of amplicon and it hight sensitivity. It also makes it possible to detect several germs as well as their serotypes. A set of 14 primers divided into 3 classes were used. Class 1 primers served as an internal control by targeting the cpsA gene. The primers of the second class were used to target specific serotypes by specific reactions (out of six possibilities). The group reaction was carried out using the primers of the third class in order to carry out an initial screening of the samples and to classify the pneumococcal isolates. Related serotypes were grouped based on amplification of common genes. Each reaction used two primers which define, by limiting it, the sequence to be amplified. Amplification is carried out using an enzyme, Taq polymerase, capable of synthesizing DNA from the nucleotides present in the reaction using the products of each synthesis step as a template for the following steps. For a PCR reaction, several elements are needed in the reaction medium or Master Mix. Free deoxyribonucleotides (dNTPs) 5 mM are used during the polymerization of the DNA strand complementary to the template DNA strand. dATP, dTTP, dCTP and dGTP are added successively. It is important that the concentration of the various deoxyribonucleotides be identical in order to avoid Taq polymerase errors. Buffers and magnesium ions ($MgCl_2$) define a medium with an optimal pH and an optimal salt concentration for the proper functioning of the enzyme. Primers delineate the DNA sequence to be amplified for each specific reaction. To begin with, they each hybridize at the end of the fragment to be amplified thanks to the complementarity of the nucleotide sequences. The "Reward" primer extends the strand in the 3' to 5' direction and the Forward primer, in the 5' to 3' direction. Thanks to their free 3' OH end, they serve as an anchor point for Taq polymerase. For serotyping, the Multiplex PCR technique which consisted of using 6 specific reactions with pairs of specific primers corresponding to *Streptococcus pneumoniae*. Tag polymerase, by first attaching to the primers, synthesizes the corresponding DNA strand. The reaction mixture is transferred into a 2 ml tube and distributed into the strips according to the calculation made taking into account the number of samples and the Multiplex PCR sequence program. We then obtain two single strands of DNA. The primers hybridize to the target DNA strands by base complementarity. After being fixed, they serve as a starting point for the polymerization of the strand complementary to the template DNA. The last step, also called elongation, at a temperature of 72˚C, the DNA polymerase begins to polymerize by adding free deoxyribonucleotides. Polymerization takes place in the 5' to 3' direction. These different steps make it possible to obtain an exponential amplification rate of around 2n. The agarose

gel electrophoresis technique made it possible to separate DNA according to its molecular weight. Nucleic acids migrate in an electric field. The amplicons are revealed by an agentfluorescent intercalator called BET (ethidium bromide). An ultraviolet radiation device also called UGENUS made it possible to visualize and analyze the migration bands. LThe 1X TAE buffer is mixed with agarose at a rate of 2 g of agarose per 100 ml of buffer (the proportion of agarose depends on the size of the DNA molecules to be separated). The agarose gel was prepared in a microwave oven to homogenize the mixture. The mixture is cooled (around 45˚C) and deposited a few drops of BET, then placed the seals supplied with the tank to close the gel support. The level was adjusted so that the gel holder was horizontal. The gel was poured slowly to a thickness of 3 to 5 mm, ensuring that it surrounded the teeth of the comb. We let the gel cool and removed the comb and gaskets. The gel is ready for amplicon deposition. The support containing the gel was placed in the electrophoresis tank which previously contains the TAE buffer, the wells were placed on the negative pole side. 3 µl of loading dye and 10 µl of DNA were mixed on a piece of parafilm; the mixture was taken with a micropipette to fill the wells. A molecular size marker is then deposited in its well, closed and turned on the tank, finally the migration takes place until the charge dye arrives near the edge of the gel for approximately 55 min at 120 V. connections were disconnected and we removed the support containing the gel. The gel is illuminated under an apparatus in order to observe the fluorescent DNA bands. The estimation of the size of the fragments is made through comparison with the molecular weight marker scale (100 base pairs).

2.2. Data Analysis

The software Microsoft Excel (Redmond, Washington, USA) was used to collect the data, then exported and analyzed using the Epi-Info 7.2 software (WHO, Geneva, Switzerland and CDC, Atlanta USA), the texts were entered using using World 2010 software (Redmond, Washington, USA). The quality of the data was assessed and corrected. The Chi square test was used to compare two or more qualitative variables; the Student and the analysis of variance (ANOVA) test to compare two or more means; logistic regression was used to estimate the relative risk. A significance threshold of 5% was used for all tests ($p < 0.05$).

3. Results

From January 1, 2017 to December 31, 2022, we collected 187 antibiotic-resistant strains of *Streptococcus pneumoniae* at the LNBCSP. They were used for serotyping. The age of the patients varied from 0.8 to 60 years with an average of 10 ± 9 years. The most common age was 4 years. The male gender (n = 112) predominated and the male/female sex ratio was 1.49.

Table 1 shows the distribution of serotypes from 2017 to 2022. The frequency of serotypes 1 was higher in 2020 with 93.05%, followed by non-typable serotypes in 2018 with 86.1% in 2018.

Figure 1 shows the frequency of serotypes of *Streptococcus pneumoniae* strains according to age groups from 2017 to 2022. The frequency of serotypes 1 is higher (56.88%) for the age group over five years than the other serotypes during the study period.

Figure 2 shows the frequency of serotypes of *Streptococcus pneumoniae* strains by sex from 2017 to 2022. The serotype frequency of antibiotic-resistant strains of *Streptococcus pneumoniae* was predominant for males and for all serotypes.

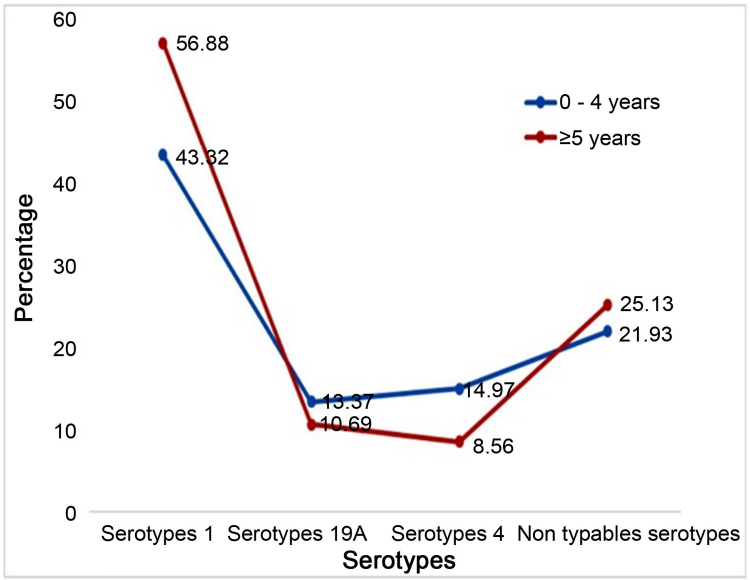

Figure 1. Frequency of serotypes of *Streptococcus pneumoniae* strains according to age groups from 2017 to 2022.

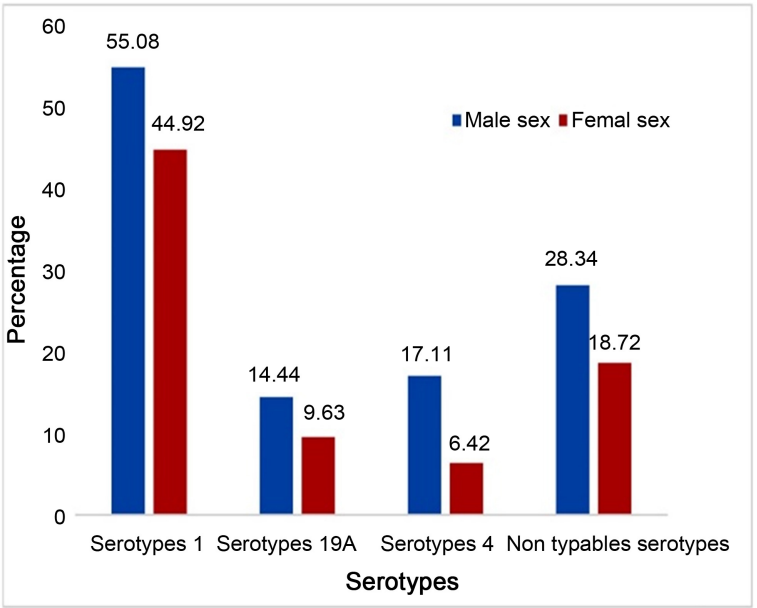

Figure 2. Frequency of serotypes of *Streptococcus pneumoniae* strains by sex from 2017 to 2022.

Table 2 shows the distribution of serotypes according to antibiotic resistance of *Streptococcus pneumoniae* strains from 2017 to 2022.

Table 3 shows the distribution of serotypes and antibiotic resistance of *Streptococcus pneumoniae* strains according to age groups from 2017 to 2022.

Table 1. Distribution of serotypes from 2017 to 2022.

Year (N = 187)	2017 (n = 9)	2018 (n = 68)	2019 (n = 18)	2020 (n = 28)	2021 (n = 20)	2022 (n = 44)
Percentage	%	%	%	%	%	%
Serotypes 1	10.16	15	51.87	**93.05**	67.38	24.06
Serotypes 19A	12.83	22.46	**26.74**	7.49	15.51	24.06
Serotypes 4	16.58	18.72	25.13	18.18	**20.86**	10.16
Non-typeable serotypes	19.25	**86.1**	26.2	31.02	22.46	52.41

Table 2. Distribution of serotypes according to antibiotic resistance of *Streptococcus pneumoniae* strains from 2017 to 2022.

	2017 (n = 9)	2018 (n = 68)	2019 (n = 18)	2020 (n = 28)	2021 (n = 20)	2022 (n = 44)	Total (N = 187)
Percentage	%	%	%	%	%	%	%
Serotypes 1							
Penicillin G	33.33	**73.53**	61.11	75	80	70.45	70.59
Ciprofloxacin	0	42.65	44.44	50	10	**81.82**	47.59
Tetracycline	22.22	64.71	44.44	75	45	40.91	54.54
Gentamycin	33.33	55.88	83.33	67.86	85	**93.18**	71.12
Erytromicin	11.11	79.41	55.56	75	**95**	59.09	70.05
Chloramphenicol	11.11	41.18	33.33	42.86	**55**	4.54	32.08
Cotrimoxazole	22.22	70.59	27.78	**82.14**	35	22.73	50.80
Serotypes 19 A							
Penicillin G	**44.44**	7.35	22.22	7.14	15	18.18	13.90
Ciprofloxacin	11.11	1.47	16.67	10.71	5	**18.18**	9.09
Tetracycline	**55.56**	11.76	44.44	3.57	25	18.18	18.72
Gentamycin	**55.56**	8.82	38.89	7.14	15	22.73	17.11
Erytromycin	11.11	10.29	**38.89**	10.71	20	11.36	14.43
Chloramphenicol	0	4.41	**33.33**	0	10	4.55	6.95
Cotrimoxazole	**11.11**	8.82	5.56	3.57	10	6.82	7.49
Serotype 4							
Penicillin G	**55.56**	7.35	0	25	20	4.55	12.30
Ciprofloxacin	0	2.94	**16.67**	14.29	5	6.82	6.95
Tetracycline	**55.56**	5.88	50	14.29	30	6.82	16.58
Gentamycin	**44.44**	1.47	38.89	14.29	35	6.82	13.90
Erytromycin	33.33	1.02	**38.89**	14.29	30	0	14.43

Continued

Chloramphenicol	**33.33**	5.88	27.78	3.57	10	0	8.02
Cotrimoxazole	22.22	2.94	**44.44**	10.71	10	2.27	9.63
Non-typeable serotype							
Penicillin G	**77.78**	27.94	33.33	25	10	25	27.81
Ciprofloxacin	22.22	17.65	22.22	32.14	5	**27.27**	21.39
Tetracycline	**77.78**	23.53	50	25	30	29.55	6.95
Gentamycin	**55.56**	22.06	50	28.57	30	31.82	30.48
Erytromycin	33.33	**33.82**	38.89	28.57	25	15.91	28.34
Chloramphenicol	11.11	**26.47**	11.11	14.29	20	11.36	18.18
Cotrimoxazole	11.11	17.65	5.56	7.14	10	**18.18**	13.90

Table 3. Distribution of serotypes and antibiotic resistance of *Streptococcus pneumoniae* strains according to age groups from 2017 to 2022.

Year	2017 (n = 9)		2018 (n = 68)		2019 (n = 18)		2020 (n = 28)		2021 (n = 20)		2022 (n = 44)		Total (N = 187)	
Age group	<5 n = 6	≥5 n = 3	<5 n = 30	≥5 n = 38	<5 n = 9	≥5 n = 9	<5 n = 15	≥5 n = 13	<5 n = 13	≥5 n = 7	<5 n = 13	≥5 n = 31	<5 n = 86	≥5 n = 101
Percentage	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Serotype 1														
Penicillin G	17	67	**93**	58	56	67	80	69	85	71	62	74	**77**	66
Ciprofloxacin	0	0	53	34	56	33	**73**	23	8	14	77	84	50	46
Tetracycline	17	0	63	66	33	56	73	**77**	54	29	54	35	56	53
Gentamycin	33	33	73	42	89	78	67	69	85	86	100	90	**77**	66
Erythromycin	0	33	80	79	44	67	80	69	92	100	31	7	65	**74**
Chloramphenicol	17	0	33	47	56	11	53	31	46	71	15	0	37	28
Cotrimoxazol	17	33	83	61	11	44	87	77	38	29	69	3	63	41
Serotype 19A														
Penicillin G	50	33	13	2	11	33	13	0	15	14	8	23	**15**	13
Ciprofloxacin	0	33	3	0	0	33	20	0	8	0	8	23	7	**11**
Tetracycline	50	67	20	5	22	67	7	0	23	29	15	19	**20**	18
Gentamycin	50	33	17	2	22	56	13	0	8	29	23	3	**19**	16
Erythromycin	17	0	13	8	11	67	13	8	15	29	8	13	13	**16**
Chloramphenicol	0	0	3	5	22	44	0	0	8	14	8	3	6	**8**
Cotrimoxazol	17	0	13	5	11	0	7	0	15	0	23	0	**14**	2
Serotype 4														
Penicillin G	83	0	7	8	0	0	40	8	23	14	0	6	**19**	8
Ciprofloxacin	0	0	0	5	11	22	27	0	8	0	8	6	**8**	7
Tetracycline	67	33	3	8	44	56	20	8	31	29	8	6	**20**	16

Continued

Gentamycin	67	0	0	3	33	44	20	7	31	43	8	6	**17**	13
Erythromycin	50	0	10	11	22	56	27	0	31	29	0	0	**19**	13
Chloramphenicol	50	0	3	8	22	33	7	0	15	0	0	0	**10**	7
Cotrimoxazol	33	0	3	3	22	67	13	8	15	0	8	0	**12**	9
Non-typeable serotype														
Penicillin G	83	67	33	24	33	33	33	15	31	14	31	23	**33**	24
Ciprofloxacin	0	67	7	26	11	33	40	23	31	0	31	26	16	**26**
Tetracycline	67	100	20	38	22	78	27	23	15	57	38	26	27	**35**
Gentamycin	67	33	23	21	44	56	33	23	15	57	46	26	**33**	29
Erythromycin	33	33	13	50	11	67	40	15	8	57	15	16	19	**37**
Chloramphenicol	17	0	33	45	11	11	7	23	0	57	15	10	7	**28**
Cotrimoxazol	17	0	1	21	0	11	0	15	8	14	54	3	**15**	13

Table 4 shows the distribution serotypes according to antibiotic resistance of *Streptococcus pneumoniae* strains according to sex from 2017 to 2022.

Table 5 shows the association between serotypes and antibiotic resistance of strains of *Streptococcus pneumoniae* from 2017 to 2022. *Streptococcus pneumoniae* strains resistant to penicillin G have 1.3 times the risk of carrying serotype 1. The others have a protective factor between antibiotic resistance and serotype 19A, serotype 4 and non-typeable serotype.

4. Discussions

Our study shows a male predominance with a sex ratio of 1.49. This result confirms that of the studies carried out by Indianara MG, De Moraes C *et al*., in Brazil in 2015 with a sex ratio 1.5 [19]. Monitoring the serotypes of antibiotic-resistant strains of *Streptococcus pneumoniae* is important to assess, above all, the relationship between circulating serotypes and vaccines as well as that of serotypes and antibiotics. This study grouped together 187 non-redundant strains of *Streptococcus pneumoniae* isolated in Bangui. During the six years, we identified four (04) different serotypes of *Streptococcus pneumoniae* strains. Serotypes 1 and non-typeable serotypes predominated with an average frequency of 43.57% and 39.57% respectively. This result is much higher than that found in a study carried out in Mozambique in 2013 by Nhantumbo AA [20]. Another study conducted by Kwambana-Adams BA *et al*. in 2016 in Ghana corroborates the findings that most strains of *Streptococcus pneumoniae* were serotype 1 [21]. The results of another study carried out in Burkina Faso by Kambire *et al*. between 2011 and 2012 showed that 15% of *Streptococcus pneumoniae* strains were non-typable, whereas in our study their frequency was doubled [22]. Serotypes 19A and 4 had an average frequency of 18.18% and 18.27%, respectively. A study carried out in Canada in 2012 by Demczuk *et al*. showed similar results where serotype 19 A was the most common with 19% [23]. Another study

Table 4. Distribution serotypes according to antibiotic resistance of *Streptococcus pneumoniae* strains according to sex from 2017 to 2022.

Years	2017 (N = 9)		2018 (N = 68)		2019 (N = 18)		2020 (N = 28)		2021 (N = 20)		2022 (N = 44)	
Sex	M (n = 3)	F (n = 6)	M (n = 40)	F (n = 28)	M (n = 9)	F (n = 9)	M (n = 17)	F (n = 11)	M (n = 16)	F (n = 4)	M (n = 24)	F (n = 20)
Percentage	%	%	%	%	%	%	%	%	%	%	%	%
Serotype 1												
Penicillin G	67	16	70	79	67	56	76	73	**81**	75	63	80
Ciprofloxacin	**0**	0	15	14	11	11	18	**36**	13	75	25	10
Tetracycline	0	17	53	68	**100**	**100**	76	82	69	20	75	65
Gentamycin	17	17	55	43	78	89	88	91	56	20	**100**	85
Erythromycin	**67**	17	13	7	67	11	18	9	25	**50**	0	0
Chloramphenicol	33	33	32	4	**44**	11	0	9	13	0	0	0
Cotrimoxazol	**33**	17	0	7	22	0	6	18	13	0	4	0
Sérotype 19A												
Penicillin G	**67**	33	10	4	22	22	6	9	13	25	13	25
Ciprofloxacin	0	0	3	4	11	0	0	0	0	25	4	5
Tetracycline	**67**	**50**	15	7	56	33	0	18	19	50	21	15
Gentamycin	33	**50**	13	4	**44**	33	6	18	6	50	21	25
Erythromycin	0	17	10	11	**44**	**33**	12	18	13	50	21	10
Chloramphenicol	0	0	5	4	**56**	11	0	0	6	25	4	5
Cotrimoxazol	0	17	10	7	11	0	6	0	13	0	**21**	5
Sérotype 4												
Penicillin G	**100**	16	8	7	0	**0**	24	27	1	25	4	5
Ciprofloxacin	0	0	5	4	**22**	0	0	9	0	25	0	5
Tetracycline	67	50	5	14	**89**	11	12	19	31	25	8	5
Gentamycin	33	50	0	7	**67**	11	18	9	31	25	8	5
Erythromycin	**67**	17	13	7	**67**	11	18	9	25	50	0	0
Chloramphenicol	33	33	32	4	**44**	11	0	9	13	0	0	0
Cotrimoxazol	**33**	17	0	7	22	0	6	**18**	13	0	4	0
Non-typeable serotype												
Penicillin G	**100**	50	28	29	33	33	18	36	13	0	26	20
Ciprofloxacin	**33**	0	5	7	11	11	6	0	0	25	8	10
Tetracycline	**100**	67	25	21	44	56	18	36	25	50	38	20
Gentamycin	33	**67**	28	14	56	44	24	36	25	50	42	20
Erythromycin	**67**	17	35	32	22	56	29	27	19	50	25	5
Chloramphenicol	0	17	33	18	22	0	6	**27**	6	75	13	10
Cotrimoxazol	0	17	23	11	0	11	0	18	6	**25**	**25**	10

Table 5. Association between serotypes and antibiotic resistance of strains of *Streptococcus pneumoniae* from 2017 to 2022.

	RR	Chi 2	CI (95%)	*p*
Association between Serotype 1 and antibiotic resistance of *Streptococcus pneumoniae* strains				
Penicillin G Other antibiotics	1.3	16.52	[1.16 - 1.44]	10^{-5}
Association between Serotype 19A and antibiotic resistance of *Streptococcus pneumoniae* strains				
Penicillin G Other antibiotics	0.2	133.8	[0.13 - 0.27]	10^{-8}
Association between Serotype-4 and antibiotic resistance of *Streptococcus pneumoniae* strains				
Penicillin G Other antibiotics	0.18	124.27	[0.11 - 0.26]	10^{-8}
Association between non-typeable serotype and antibiotic resistance of *Streptococcus pneumoniae* strains				
Penicillin G Other antibiotics	0.36	119.4	[0.29 - 0.46]	10^{-8}

carried out in Spain in 2015 by Méndez-Lagea *et al*. showed that serotype 19A was common [24]. Serotype 1 had been identified more in the age group over five years old with a high frequency of 56.88% than that under five years old. Another study conducted by HECINI-HANNACHI in 2014 in Algeria showed similar results [25]. Contrary to our results, the study carried out by Kambire *et al*. between 2014 and 2015 showed that serotye 1 predominated in children under one year of age [26]. The frequency of serotypes was predominant for the male sex with respectively for serotype 1, serotype 19A, serotype 4 and the non-typable serotype 55.08% against 44.92%; 14.44% against 9.63%; 17.11% against 6.42% and 28.34% against 18.72%. The frequency of serotypes in relation to antibiotic resistance of *Streptococcus pneumoniae* strains varied according to age, sex and time. The frequency of serotype 1 identified in 2021 for the age group under five years was 93% in combination for penicillin G in 2018. It was 77% for ciprofloxacin in 2022. For tetracycline, it was 73% in 2020. For gentamicin, it was 100% in 2022. The frequency for erythromycin was 92% in 2021. Chloramphenicol was represented at 56%. For cotrmoxazol it was 87% in 2020. For the age group over five years, the frequency for penicillin G was 74% in 2022. It was 84% for ciprofloxacin in 2022. For tetracycline it was 77% in 2020. It was 90% for gentamicin. For erythromycin, it was 100% in 2021. For chloramphenicol, it was 71% in 2021. It was 77% for cotrimoxazol in 2020. The frequency of serotypes 19A was respectively depending on penicillin G of 50% in 2017 and ciprofloxacin by 20% in 2020 for the age group under five years. It was respectively 50% for tetracycline and gentamicin in 2017. It was 17% for erythromycin in 2017 then 22% for chloramphenicol in 2019 and finally 15% for cotrimoxazole in 2021. For the age group under five years, the frequency of penicillin G in association with serotypes 4 was 83% in 2017. It was also 27% for ciprof-

loxacin during the same period. For tetracyclines and gentamicin, it was 67% in 2017. It was 50% for erythromycin and chloramphenicol in 2017. It was 33% in 2017 for cotrimoxazole. The frequency of non-typable serotypes in relation to resistance to penicillin G, ciprofloxacin, tetracycline and gentamicin for the age group under five years was respectively 83% in 2017, 40% in 2020 and 67% in 2017. It was 40% for ciprofloxacin and erythromycin in 2020. It was 33% for chloramphenicol in 2018 and 54% for cotrimoxazol in 2022. For the age group over five years, it was respectively 67% and 100% in 2017 for penicillin, ciprofloxacin and tetracycline. It was respectively for tetracyclin, gentamicin, erythromycin, and chloramphenicole 57% in 2021. That of cotrimoxazole was 21% in 2018. Tetracycline and gentamicin for the age group under five years was respectively 83% in 2017, 40% in 2020 and 67% in 2017. It was 40% for ciprofloxacin and erythromycin in 2020. It was 33% for chloramphenicol in 2018 and 54% for cotrimoxazol in 2022. For the age group over five years, it was respectively 67% and 100% in 2017 for penicillin, ciprofloxacin and tetracycline. It was respectively for tetracyclin, gentamicin, erythromycin, and chloramphenicole 57% in 2021. That of cotrimoxazole was 21% in 2018. Tetracycline and gentamicin for the age group under five years was respectively 83% in 2017, 40% in 2020 and 67% in 2017. It was 40% for ciprofloxacin and erythromycin in 2020. It was 33% for chloramphenicol in 2018 and 54% for cotrimoxazol in 2022. For the age group over five years, it was respectively 67% and 100% in 2017 for penicillin, ciprofloxacin and tetracycline. It was respectively for tetracyclin, gentamicin, erythromycin, and chloramphenicole 57% in 2021. That of cotrimoxazole was 21% in 2018.

Antibiotic resistance of *Streptococcus pneumoniae* strains was associated with serotype 1. They had 1.3 times the risk of presenting resistance with penicillin G for serotype 1 (RR = 1.3; 95% CI [1.16 - 1.44]; $p < 0.05$). For serotypes 19A, serotypes 4 and non-typeable, there was a significant association and the *Streptococcus pneumoniae* strains had a protective factor (RR < 1; $p < 0.05$).

5. Conclusion

The study determined serotypes 1, serotypes 19A, serotypes 4 and non-typeable serotypes. The identification of vaccine serotypes would be due to the quality of vaccination or poor protection of vaccines.

Ethics Statement

The data collected was processed in accordance with the guidelines set forth by the Declaration of Helsinki. This study proposal was carried out with the agreement of Ministry of Health and Population N˚.1038MSP/DIRCAB/DR/SRH.19 of October 15, 2019.

Study Limitations

We were unable to carry out the latex agglutination test for serotyping *Strepto-*

coccus pneumoniae. The reagents were not delivered on time.

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Conflicts of Interest

The authors have no conflicts of interest to declare for this study.

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Abbreviations and Acronyms

CAR: Central African Republic;
DNA: Desoxyribonucleic Acid;
EPI: Expanded Programs on Immunization;
LNBCSP: National Laboratory of Clinical Biology and Public Health;
PCR: Polymérase Chain Reaction;
PCV: Antipneumococcal Conjugate Vaccine;
WHO: World Health Organisation.