Streptococcus pneumoniae **in medical districts of Burkina Faso: serotype distribution and antibiotic susceptibility, from 2010 to 2012.**

Abstract

Introduction: *Streptococcus pneumoniae* is a highly virulent pathogen. Despite the recent introduction of vaccines in the program of prevention in Burkina Faso, treatment by antibiotics has remained in use. The aim of this study was to determine the serotypes implied in invasive pneumococcal infections and their resistance to antibiotics used for routine treatment in Burkina Faso.

Methods: from March 2010 to December 2012, a total of 6,102 cerebrospinal fluid (CSF) and pleural fluid (PF) specimens were collected in Burkina Faso. The specimens were analyzed by latex agglutination assay, culture, and real time polymerase chain reaction (rt-PCR) for species identification. Serotyping was performed by rt-PCR and minimum inhibitory concentrations were determined by the E-test.

Results: among 562 *S. pneumoniae* strainsidentified from 6,102 specimens, 440 isolates were serotyped by rt-PCR. Serotypes 1 and 5 were the most common (50%); however, 70 (15.9%) strains were not detected by rt-PCR methodology. Among patients of less than 20 years of age, serotype 1 was the most frequent strain identified (9.3% to 13.2%). Ampicillin and ceftriaxone were the most active antibiotics *in vitro* against the strains identified. The rate of *S. pneumoniae* strains susceptible to chloramphenicolwas 83%. Serotypes 1 (7/49) and 5 (5/7) were non-susceptible to chloramphenicol.

Conclusion: serotypes 1 and 5 were the common serotypes identified. Conjugate vaccines introduced in Burkina Faso should take in account these valences, to decrease the incidence of pneumococcal infections efficiently. ~~This study demonstrated that ampicillin and ceftriaxone can be used to treat clinical cases of pneumococcal infections.~~

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Keywords: *Streptococcus pneumoniae*, serotypes, susceptibility, antibiotics, Burkina Faso

Introduction

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Streptococcus pneumoniae (pneumococcus) is a major cause of pneumonia, meningitis, and otitis media with a mean mortality rate of 0.7 to 1.2 million deaths among children less than 5 years old each year worldwide, including 90% in developing countries [1, 2]. More than 90 serotypes of *S. pneumoniae* have been identified based on the composition of the capsular polysaccharide of the specific strains. Distribution of these serotypes varies according to geographic region and the age of the patients [3,4]. Serotype 1 accounts for 40 and 76% of serotypes isolated in Mozambique and Ghana, respectively, whereas it was not found among 294 strains serotyped in Portugal in 2003 [5,6,7]. Prevention by vaccination is an important intervention that could reduce the incidence of pneumococcal infections and their complications [8]. The pneumococcal conjugate vaccine 7 (PCV7, Prevnar, Pfizer) licensed in the USA and in Europe does not include the serotypes 1 and 5 that are involved in meningitis epidemics in West Africa [9, 10]. Currently, there are two conjugate vaccines available: PCV10 (Synflorix, GlaxoSmithKline Inc.) and PCV13 (Prevnar13, Pfizer). Both vaccines include serotypes 1 and 5, which are mainly found in Africa. In Burkina Faso, only PCV13 is available and was introduced by the national immunization program in October 2013. Therefore, PCV13 might be an alternative to reduce pneumococcal infections (pneumonia, bacteremia, meningitis, and otitis media) in Africa. Among all the known serotypes, only a small number are responsible for

pneumococcal infections [11,12]. Johnson and*al.* (2010) previously reported that seven serotypes (1, 5, 6A, 6B, 14, 19F, 23F) caused over 70% of invasive pneumococcal disease (meningitis, pneumonia and bacteremia) in a literature review of studies from the USA, Canada, Australia and Europe [9].

S. pneumoniae is naturally sensitive to antibiotics, particularly beta-lactams. Since the discovery of penicillin G non-susceptible *S. pneumoniae* in 1967 [13], the prevalence of *S. pneumoniae* resistance to antibiotics such as beta-lactams, macrolides, tetracycline and chloramphenicol has increased worldwide particularly in developing countries where self-medication is significant [14]. Furthermore, the pneumococcal vaccine is very expensive and is unaffordable for developing countries. Thus, there is a critical need to develop pneumococcal vaccines against new serotypes not included in the current PCVs and against pneumococcal strains that are non-susceptible to antibiotics.

The aim of this study was to determine the serotypes of strains implied in invasive pneumococcal infections and their resistance to antibiotics used in routine treatment in Burkina Faso.

Methods

Specimen collection and *S. pneumoniae* identification

A total of 6,102 specimens including 3,970 CSF and 2,132 and PF samples were received at the Bacteriology and Virology Laboratory of the Teaching Hospital YalgadoOuedraogo (Ouagadougou) from March 2010 to December 2012, from nine medical regions of Burkina Faso. The CSF samples were received for confirmation of suspected cases of acute bacterial meningitis. *S. pneumoniae* isolates were received from the Center-South and Central Plateau regions exclusively; PF specimens were collected from the Center medical region only. The specimens were collected in compliance with standard operating procedures defined by the Ministry of Health from Burkina Faso. A case of suspected meningitis was defined by rapid onset of fever (temperature, ≥38.5°C rectal or ≥38°C

axillary) and at least one of the following symptoms: stiff neck, altered and/or reduced level of consciousness, petechial or purpural rash, photophobia, vomiting, convulsions, restlessness; and for infants, fever with bulging fontanel, poor sucking and irritability were also included in the case definition. A lumbar puncture was performed systematically for patients with suspected acute bacterial meningitis, regardless of age. Each sample came with an analysis bulletin including the patient's documented socio-demographic and clinical data, and a personal form of case notification.

The detection of *S. pneumoniae* in CSF and PF was carried out by Gram stain (Gram-positive lanceolate diplococci), by latex agglutination assay with the PASTOREX™ Meningitis kit (Bio-Rad, France) on the supernatant of turbid CSF, according to the manufacturer's instructions, and by culture. One to 3 drops of CSF (well-homogenized purulent CSF or sediment of slightly centrifuged CSF) were inoculated onto chocolate or blood agar (Trypcase-soya base supplemented with 5% sheep blood) and the plates were incubated in a humid atmosphere overnight at 37°C, with 5-10% CO_2. Suspect colonies appeared moist or sometimes mucoid, grey, centrally depressed, and α-hemolytic on blood agar or surrounded by a greenish-yellow color on chocolate agar. They were used for *S. pneumoniae* identification by negativity of the catalase test, sensitivity to optochin, and bile solubility. Pneumococci are sometimes optochin-resistant, therefore, the bile solubility test is used to distinguish *S. pneumoniae*from all other alphahemolytic streptococci. *S. pneumoniae*is bile soluble whereas all other alpha-hemolytic streptococci are bile resistant [15]. In addition to these tests, real-time polymerase chain reaction assays using a specific segment of autolysin A gene (*lyt*A) were performed to detect *S. pneumoniae*. This gene segment is highly conserved within the species and it allows the best separation of *S. pneumoniae* from *S. mitis*, *S. oralis* and *S. pseudopneumoniae* [15,16].

Antimicrobial susceptibility tests

Susceptibility tests of *S. pneumoniae* to antibiotics were performed using the disk diffusion method on Mueller-Hinton agar media supplemented with 5% defibrinated horse blood, according to the recommendations of the antibiotic susceptibility testing committee of the French Society of Microbiology[17]. The antibiotics tested were oxacillin (5 µg),ampicillin (10 µg), ceftriaxone (30 µg), erythromycin (15 UI), and chloramphenicol (30 µg). The MICs of oxacillin, ampicillin, and ceftriaxone were determined by the E-test method according to the Antibiogram Committee of the French Microbiology Society (AC-FMS) recommendations [17]. Inhibition diameters and minimum inhibitory concentrations of the antibiotics were interpreted according to AC-FSM recommendations. Intermediate resistant and resistant strains were considered non-susceptible to antibiotics and multidrug resistant strains were non-susceptible to three different classes of antibiotics.

Serotyping by rt-PCR

DNA extraction

Bacterial DNA was extracted from 200 µl of CSF or bacterial suspension according to the Qiagen DNA mini protocol (QIAamp® DNA Mini Kit, Ref.51306, Qiagen SA, France). Enzyme treatment was performed in advance using a Qiagen kit: 100 µl of TE buffer containing 0.04 g/ml of lysozyme (Sigma-L 6876) and 75 U/ml of mutanolysin (Sigma-M9901) was pipetted into the microcentrifuge tube.

Species identification by real-Time PCR

*lyt*A PCR was performed as described previously [16], with the following modification. The *lyt*A PCR was carried out using 25 µl reaction mix containing 12.5 µl of TaqMan® Universal Master Mix (AppliedBiosystems), 2 µl of primers and probe, 4.5 µl of water quality PCR (Applied Science) and 2 µl of DNA template. In every run of rt-PCR, 2 µl of no-template control (NTC) and 2 µl of *S.*

pneumoniae-positive DNA control were included. The forward and reverse oligonucleotide primers and probe used were described previously [16]. All primers and probes were obtained from CDC Atlanta laboratories. The PCR cycling conditions were 1 cycle of 50°C for 2 min, 1 cycle of 95°C for 10 min followed by 50 cycles of 95°C for 1 min and 60°C for 15 s. The positive cutoff values were ≤35 cycles, equivocal values were >35 and <40, and the negative values were ≥40 cycles. Template DNA with equivocal values was diluted four-fold and ten-fold and the amplification was repeated.

Serotyping

Triplex rt-PCR was used to determine 21 pneumococcal serotypes into seven triplex PCR reactions. The serotyping was performed on *S. pneumoniae*-positive DNA extracted from CSF and PF specimens. The PCR was performed in a 25 µl volume reaction mix containing 12.5 µl of Invitrogen Platinum® Super Mix-UDG and 50 µM of $MgCl_2$, 10 µM of forward and reverse primers (StrepLab), 10 µM of probes (FAM, HEX, ROX) (StrepLab), water quality PCR (negative control) according to the step of reaction, 5 µl of *S. pneumoniae*-positive DNA template, and 5 µl of no-template control (NTC), and 5 µl of *S. pneumoniae*-serotype DNA positive control was included in every run. PCR cycling conditions were 1 cycle of 95°C for 10 min followed by 40 amplification cycles of 95°C for 1 min and 60°C for 15 s. A Stratagene Mx3005P™(Agilent Technologies La Jolla, California, USA) thermal cycler was used for PCR amplification. The amplification data were analyzed by MxPro QPCR (Mx3005P) software. The specimens were recorded as positive if the cycle threshold (Ct) values were less than 35 cycles. Indeterminate samples were defined as those with cycle threshold (Ct) values greater than 35 cycles: each indeterminate specimen was retested and if the result remained the same, it was transmitted to the Pneumococcus laboratory of the CDC and Prevention in Atlanta (GA, USA) for a more elaborate analysis.

Ethical aspects

All specimens were collected as part of the routine clinical management of patients, according to the national guidelines in Burkina Faso. The study was approved by the medical establishment committee of the Teaching Hospital YalgadoOuedraogo.

Statistical analyses

XLSTAT 7.1 was used to determine the 95% CI of MICs. Differences were considered significant when $p < .05$.

Results

Sample characteristics

Most *S. pneumoniae* strains that have been identified to date were isolated from the cerebrospinal fluid (CSF) of patients tested (98%; 551/562). Among them, 55% (311/562) were identified in male patients. The age of patients varied from 5 days to 90 years with a mean age of 167.3±8.4 months, 95% confidence interval [CI] 150.7–183.8. Children under 15 years old represented 48.3% of the study population.

Geographic distribution of *S. pneumoniae* showed that the North (126/562), Center-West (107/562) and East (106/562) medical districts had the highest number of cases. These medical districts are situated in the Sahelian and Sudanian zone of the country (**Table 1**).

Table 1: Number of *S. pneumoniae* serotypes detected in samples collected in medical districts of Burkina Faso.

Medical regions	**CSF**		**PF**		**Total**	
	Number	***Sp+**	**Number**	***Sp+**	**Number**	***Sp+**
Sahel	313	77	0	0	313	77
Center	1747	45	2132	12	3879	57
Center-West	586	107	0	0	586	107
Center-North	238	68	0	0	238	68
Center-South	6	6	0	0	6	6
North	601	126	0	0	601	126
East	446	106	0	0	446	106
Boucle of Mouhoun	21	3	0	0	21	3
Central Plateau	12	12	0	0	12	12
Total	**3,970**	**550**	**2,132**	**12**	**6,102**	**562**

*number of *S. pneumoniae* serotypes detected by latex agglutination assay, culture and rt-PCR; Sp+: presence of *S. pneumoniae*; CSF: cerebrospinal fluid; PF: pleural fluid.

Detection rate of *S. pneumoniae*

In total, 562 *S. pneumoniae* cases were detected by latex agglutination assay (251), culture (156) and rt-PCR (529), respectively. The classic bacteriological methods (latex agglutination assay and culture) detected fewer cases than the rt-PCR method. Among156 strains identified by culture, 147 were used for the susceptibility testing to antibiotics and the nine remaining strains were lost during subculture.

Serotype distribution

Among 529 *S. pneumoniae* cases detected by rt-PCR, 440 were tested for serotyping, including 431 collected from CSF and 9 from pleural fluid (PF). Before rt-PCR was introduced to the Bacteriology and Virology Laboratory of the Teaching Hospital, specimens were sent to a national reference laboratory, which explains why 89 cases of *S. pneumoniae* were not serotyped among the total pneumococci detected. In total, 370 (84.1%) *S. pneumoniae* cases were typeable whereas 70 (15.9%) were not detected by 7 serotyping rt-PCR reactions (**Table 4**). Seven serotypes represented 75.4% of all the *S. pneumoniae* cases tested: 1(40.9%), 5 (9.3%), 12F (7.5%), 6A/6B (6.1%), 14 (4.8%), 23F (4.1%) and 2 (2.7%). The other serotypes represented 8.5% of total cases (**Table 2**).

Table 2: Distribution of pneumococcal serotypes isolated from cerebrospinal fluid and pleural fluid.

Serotypes/ serogroups	CSF		PF		Total	
	Number of cases	%	Number of cases	%	Number of cases	%
1	177	40.2	3	0.7	180	40.9
5	40	9.1	1	0.2	41	9.3
12F/(12A/44/46)	34	7.7	0	0.0	34	7.5
6A/6B	29	6.6	2	0.5	31	6.1
14	20	4.5	1	0.2	21	4.8
23F	16	3.6	2	0.5	18	4.1
2	12	2.7	0	0.0	12	2.7
19F	9	2.0	0	0.0	9	2.0
6A/6B/6C/6D	4	0.9%	1	0.2%	5	1.1%
7A/7F	4	0.9	0	0.0	4	0.9
4	4	0.9	0	0.0	4	0.9
Other serotypes	16	3.6	0	0.0	16	3.6
ND	70	15.9	0	0.0	70	15.9
Total	**431**	**98.0**	**9**	**2.0**	**440**	**100**

CSF: Cerebrospinal Fluid; PF: Pleural Fluid; ND: no strains detected.

Other serotypes: 3 (0.68%), 33F/33A/37 (0.68%), 18 (0.68%), 19A (0.45%), 16F (0.45%), 15A/15F (0.23%), 22A/22F (0.23%), and 9A/9V (0.23%).

The typeable strains of *S. pneumoniae* were found in 56.1% of male patients (**Table 3**); there was no statistically significant difference in the number of serotype 1 cases between the two genders (p=.26).

Table 3: Distribution of *S. pneumoniae* serotypes/serogroups according to patient gender.

Serotypes/serogroups	**Female (%)**	**Male (%)**	**Total (%)**
1	82 (18.6)	98 (22.3)	180 (40.9)
5	18 (4.1)	23 (5.2)	41 (9.3)
12F/(12A/44/46)	12 (2.7)	22 (5.0)	34 (7.5)
6A/6B	16 (3.6)	15 (3.4)	31 (6.1)
14	6 (1.4)	15 (3.4)	21 (4.8)
23F	7 (1.6)	11(2.5)	18 (4.1)
2	7 (1.6)	5 (1.1)	12 (2.7)
19F	2 (0.5)	7 (1.6)	9 (2.0)
Others St	11 (2.5)	13 (3.0)	24 (6.5)
ND	32 (7.3)	38 (8.6)	70 (15.9)
Total	**193 (43.9)**	**247 (56.1)**	**440 (100)**

Other serotypes: 9V/9A (1), 7A/7F (4), 6A/6B/6C/6D (5), 4 (4), 33A/33F/37 (3), 3 (3), 22F/22A (1), 19A (2), 18 (3), 16F (2), and 15A/15F (1). ND: no strains detected.

The distribution of serotypes according to the age of patients showed that children under 5 years old were infected mostly by serotypes 1 (9.3%), 5 (4.8%), 6A/6B (5.0%), 14 (2.7%) and serogroup12F

(2.3%), whereas 7% of *S. pneumoniae* serotypes were not detected in this age group. Serotype 1 was the most common *S. pneumoniae* serotype in patients 11–20 years old (13.2%), 6–10 years old (10.9%) and 0–5 years old (9.3%) (**Table 4**).

The frequency of *S. pneumoniae* typeable cases was higher in the age groups 0–5years old, 6–10years old and 11–20 years old but there was no statistically significant difference between the values of these age groups (p = .037 and .073, respectively). However, a comparison of the frequency of *S. pneumoniae* typeable cases in patients < 5 years old were significantly different (p< 0.001) compared with other age groups (**Table 4**).

Table 4: Rates of *S. pneumoniae* serotypes/serogroups according to the patient age group.

		Rates of serotypes/serogroups (%)										
Years	**Number of cases**	**1**	**5**	**12F/(12A/44/46)**	**6A/6B**	**14**	**23F**	**19F**	**2**	**Other serotypes**	**ND**	**Total**
0–5	167	9.3	4.8	2.3	5.0	2.7	2.0	0.9	1.4	2.5	7.0	38.0
6–10	91	10.9	1.4	1.6	0.2	0.7	0.9	0.0	0.5	1.1	3.4	20.7
11–20	102	13.2	1.4	1.6	0.9	0.7	0.5	0.5	0.5	0.5	3.6	23.2
21–30	25	3.2	0.7	0.5	0.2	0.0	0.0	0.0	0.0	0.7	0.5	5.7
31–40	19	1.8	0.2	0.9	0.0	0.2	0.2	0.0	0.2	0.2	0.5	4.3
41–50	9	0.9	0.5	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.2	2.0
51–60	13	0.9	0.2	0.2	0.0	0.0	0.2	0.5	0.0	0.5	0.5	3.0
≥61	14	0.7	0.2	0.5	0.5	0.5	0.2	0.2	0.2	0.0	0.2	3.2
Total	**440**	**40.9**	**9.3**	**7.7**	**7.0**	**4.8**	**4.1**	**2.0**	**2.7**	**5.5**	**15.9**	**100.0**

ND: no strains detected; other serotypes: 9V/9A (0.2%), 7A/7F (0.9%), 6A/6B/6C/6D (1.1%), 4 (0.9%), 33A/33F/37 (0.7%), 3 (0.7%), 22F/22A (0.2%), 19A (0.5%), 18 (0.7%), 16F (0.5%), and 15A/15F (0.2%).

S. pneumoniae susceptibility to antibiotics

According to the disk diffusion method, all isolates were susceptible to ampicillin (100%) and ceftriaxone (100%). However, reduced susceptibilities were recorded with oxacillin (8.9%). High rates of susceptibility were found with erythromycin (94.6%) and chloramphenicol (83%) (**Table 5**).

The E-test method revealed that all isolates were susceptible to ampicillin (MIC50: 0.032 µg/ml; MIC90: 0.047 µg/ml) and ceftriaxone (MIC50: 0.047 µg/ml; MIC90: 0.25 µg/ml (**Table 5B**).

Table 5: Susceptibility of pneumococcal isolates to various antibiotics

A : susceptibility to antibiotics

Antibiotics	Inhibition diameter (mm)		Number	Susceptible	Non-Susceptible	
	S	R			Intermediate	Resistant
Erythromycin 15UI	≥ 26	< 24	147	139 (94.6%)	4 (2.7%)	4 (2.7%)
Chloramphenicol 30µg	> 23	< 23	147	122 (83%)	0	25 (17%)

B : MIC values

	MIC (µg/mL)							
	Susceptible	Resistant	S (%)	I (%)	R (%)	MIC50 (µg/mL)	MIC90 (µg/mL)	MIC range
AM	≤0.5	>2	147 (100)	0	0	0.032	0.047	<0.016-0.38
CRO	≤0.5	>2	147 (100)	0	0	0.047	0.25	<0.016-0.5

S: Susceptible; I: Intermediate; R:Resistant MIC: Minimal inhibitory concentration; AM: Ampicillin; CRO: Ceftriaxone

The typed strains showed a good susceptibility to beta-lactam. For other antibiotics, a decreased susceptibility to several serotypes was noted: serotypes 1, 5, 6A/6B and 14 were resistant to chloramphenicol, erythromycin. The serotypes not detected by the seven reactions of rt-PCR were resistant to all these antibiotics except for chloramphenicol (**Table 6**).

Table 6: *S. pneumoniae* isolates susceptible to various antibiotics.

Serotypes/ serogroups	Total tested	OX5		AM		CRO		C		E	
		S	Total	S	Total	S	Total	S	Total	S	Total
1	49	46	49	49	49	49	49	42	49	47	49
4	1	1	1	1	1	1	1	1	1	1	1
5	7	7	7	7	7	7	7	2	7	1	7
14	7	6	7	7	7	7	7	6	7	7	7
12F/(12A/44/46)	10	10	10	10	10	10	10	4	10	9	10
19F	3	2	3	3	3	3	3	3	3	3	3
23F	5	3	4	5	5	5	5	5	5	5	5
33F/33A/37	1	1	1	1	1	1	1	1	1	1	1
6A/6B	4	3	4	4	4	4	4	4	4	4	4
9A/9V	1	1	1	1	1	1	1	1	1	1	1
Not detected	9	9	9	9	9	9	9	9	9	7	9
Total	**97**	**89**	**96**	**97**	**97**	**97**	**97**	**78**	**97**	**86**	**97**

S: number of susceptible isolates; OX5: Oxacillin 5 µg; AM: Ampicillin; CRO: Ceftriaxone; C: Chloramphenicol; E: Erythromycin

Discussion

The distribution of *S. pneumoniae* serotypes according to the frequency of positive cases detected in CSF showed (14%, 550/3970) a significant difference compared with the positive cases from PF (1%, 12/2132) (p = .002). These overall rates are different from the 7% reported by Antonio and *al*. (2008) in Gambia [18] and 14% reported by Cornick and*al.* (2011) in Malawi [19]. However, in those countries, the frequencies of pneumococcal meningitis were lower than for pulmonary infections (21% in Gambia; 79% in Malawi).

In Burkina Faso, there is a rigorous policy for the treatment of acute bacterial meningitis, which recommends the collection of CSF before any antibiotic treatment, which allows avoiding beheading these infections. However, the recommendations are not formal for respiratory infections: generally the collection of samples arises after one or several episodes of presumptive antibiotic treatment with one dose of ceftriaxone (100 mg/kg). Such a practice might explain the low rate of detection of pneumococcus among respiratory infections in comparison with meningitis.

The rate of typeable strains of *S. pneumoniae* was higher in male patients (56.1%). Similar results were reported in Togo and in Burkina Faso (56%) [20] and in Germany (55.5%) [21].

Among *S. pneumoniae* isolates, serotypes 1 and 5 were the most commonly detected with a frequency of 40.9% and 9.3%, respectively. These two serotypes represented more than 50% of all serotypes identified. These results are similar to those reported in Mozambique [6] and in Niger [22]. Another study from North Ghana, country that borders Burkina Faso, 76% of cases were serotype 1 [7]. Our serotype findings are comparable to those of several African meningitis belt countries (Ghana, Nigeria, Mali, Senegal, and Gambia) where serotype 1 was the most prevalent (32%), followed by serotype 5 (15%), serogroup 6 (7%), serotype 2 (6%), serotype 3 (6%) and serogroup 12 (5%) [23]. The most frequent serotypes in Burkina Faso were among the most common in Kenya, with the exception of serotypes 2 and 7A/7F [24].However, the serotype frequencies in invasive infections in West Africa

and East Africa are different from those identified in North Africa, notably Tunisia [25], in Asian countries [26] and European countries [27,28].

Our results showed that the most affected age group was 0-5 years (37%) followed by 11-20 years (23%) and 6-10 years (22%); however, there was no statistically significant difference between these age groups. Serotype 1 was more frequent in all age groups than the other serotypes. These results are comparable to those reported in African meningitis belt[29] that showed the children older than 5 years were the most commonly infected with serotype 1 (60% to 80%).

In our study, 15.9% (70/440) of *S. pneumoniae* strains were not detected by our rt-PCR methodology. This high rate could be due to the use of 7-limited reactions in rt-PCR that only detect 21 different serotypes. In a study in Niger, the authors used a sequential multiplex PCR (SM-PCR) assay with 8 reactions covering 32 different serotypes of *S. pneumoniae*[22]. This assay allowed them to distinguish 26 different serotypes in confirmed meningitis cases, with 11.3% (98/867) of *S. pneumonia* strains detected that were not detected by our rt-PCR method. Therefore, using a sequential multiplex PCR (SM-PCR) assay in our study might have reduced the rate of *S. pneumoniae* not detected in our study.

In addition to the diversity of *S. pneumoniae* serotypes, the emergence of strains not susceptible to antibiotics is another problem of pneumococcal infection treatment, particularly in developing countries where extensive use of antibiotics is significant. Since the first description of *S. pneumoniae* with reduced susceptibility to penicillin in Australia in 1967 [13], the number of isolates resistant to penicillin has increased worldwide. However, the introduction of conjugate vaccines in several countries has decreased this resistance to antibiotics indirectly. According to our results, β-lactam antibiotics, particularly ampicillin and ceftriaxone, were active against the pneumococcal strains tested. These two antibiotics were very active against *S. pneumoniae;* therefore, it should be prescribed for pneumococcal infection even if conjugate vaccines are introduced into the national immunization program.

Among all the tested antibiotics was the least active as 96.5% of the *S. pneumoniae* strains were resistant to it. However, low resistance rates of were reported in the southeast of Austria (0.2%) [30] and in the elderly population in all regions of Canada (11.6%) [31].

Serotypes 1, 5, 6A/6B and 14 were not susceptible to the antibiotics tested. Other studies performed in Algeria and Tunisia reported a decreased susceptibility of these serotypes to antibiotics except serotype 5 [24**,** 32].

The 13-valent pneumococcal conjugate vaccine, recently introduced in Burkina Faso, is suitable for the reduction of pneumococcal infection prevalence and to prevent resistance to antibiotics. The 13-valent conjugate vaccine covered 72.1% of serotypes identified in the current study and might have more impact on the serotypes that are non-susceptible to antibiotics. Our result was agreed from Arjun and al. in India [33]. Serogroup 12 represented 7.7% of serotype in our studies was identified in other studies in Ghana and New Caledonia [3,7]; it must be taken into account for the design of future pneumococcal conjugate vaccines to use in Burkina Faso and other countries.

Conclusions

The results of this study show that serotypes 1, 5, 6A/6B, 12F, 14, 23F and 2 were the most frequent in Burkina Faso. However, 15.9% of *S. pneumoniae* serotypes were not detected by our rt-PCR methodology. Therefore, the identification of such strains requires the development of new primers. Beta lactams that are commonly used for the treatment of the pneumococcal infections have maintained strong antimicrobial activities on the identified *S. pneumoniae* serotypes; however. The most frequent serotypes identified in the present study are included in the formulation of the 13-valent conjugate vaccine currently used in Burkina Faso. The use of the 13-valent conjugate vaccine including serotypes 1 and 5 might decrease the incidence of pneumococcal infections in Burkina Faso. However, other

serotypes, including serogroup 12, deserve particular attention and should be taken into account in the development of future vaccine formulations.

If I found more recent references, that would be better, especially the early references.

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Abbreviations

rt-PCR: real time-Polymerase Chain Reaction; SM-PCR: sequential multiplex PCR; CHU-YO: Centre HospitalierUniversitaireYalgadoOuedraogo; CSF: Cerebrospinal Fluid; PF: Pleural Fluid; Ct: Cycle threshold; NTC: No Template Control; lytA: autolysin A gene; CI: confidence interval; UFR-SDS: Unité de Formation et de la Rechercheen Sciences de la Santé; CDC: Centers for Disease Control and Prevention; MIC: Minimum Inhibitory Concentrations; S: susceptible; OX5: Oxacillin 5µg; AM: Ampicillin; CRO: Ceftriaxone; C: Chloramphenicol; E: Erythromycin; AC-FSM: Association Comity of French Society of Microbiology.