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ST of *Streptococcus pneumoniae* Circulating in Burkina Faso before the Introduction of PCV-13, 2013

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Abstract

Introduction: Burkina Faso experiences regular cases of *Streptococcus pneumoniae* meningitis. As part of the strategy to reduce cases of meningitis, the 13-valent pneumococcal conjugate vaccine (PCV-13) has been introduced in the Expanded Programme on Immunisation (EPI). Despite these efforts, there are some cases of pneumococcal meningitis including both vaccine and non-vaccine serotypes. The objective of this study was to describe the pneumococcal sequence types (ST) circulating in Burkina Faso before the introduction of the 13-valent pneumococcal conjugate vaccine (PCV-13). **Methods:** It was a descriptive cross-sectional study that took place from 27th October 2013 to 7th January 2014. *S. pneumoniae* strains were collected in Burkina Faso and Multi Locus Sequence Typing (MLST) was performed at the Pneumococcal Laboratory at the Centers for Disease Control and Prevention (CDC) in the USA (United States of America). MLST consists of 4 steps: amplification, purification, sequencing and interpretative reading of the results. The amplification used 7 primers consisting of sequences of *aroe*, *gdh*, *gki*, *recP*, *spi*, *xpt*, *ddl* genes. **Results:** Of 37 strains tested, 10 serotypes were identified. Serotype 1 was prevalent in 48.7% (18/37) followed by serotype 25F in 10.8% (4/37). Serotypes 5 and 12F/12A/12B/44/46 were 8.1% (3/37) each. Serotype 1 contained 5 STs including ST303 24.3% (9/37), ST217 8.1% (3/37) and ST618 8.1% (3/37); followed by serotype 25F with ST105 10.8%

(4/37), serotype 5 with ST289 8.1% (3/37) and serogroup 12F/12A/12B/44/46 with ST 989 8.1% (3/37). **Conclusion:** Pneumococci are characterised by their great variability both in number of serotypes and in ST within the same serotype. Thus, 10 serotypes have been identified. Also, within serotype 1, 5 different STs have been described. These data indicate the complexity of the pneumococcus which is strongly involved in purulent bacterial meningitis at national level. This requires continuous surveillance of pneumococcal meningitis through laboratory capacity building.

Keywords

Streptococcus pneumoniae, Burkina Faso, PCV-13, Sequence Types

1. Introduction

The African meningitis belt is the area of Africa south of the Sahara that extends from Senegal in the west to Ethiopia in the east that periodically experiences acute or purulent bacterial meningitis [1] [2] [3]. These purulent meningitises are mainly caused by three etiological agents, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b and *Neisseria meningitidis*, and are characterised by a predominantly neutrophilic leukocytosis [4] [5]. Therefore, these pathogens cause meningitis epidemics especially in the meningitis belt of Lapeyssonia [1] [6]. Furthermore, these meningitis epidemics are always accompanied by high mortality and morbidity rates [1] [4] [7].

Burkina Faso, a country located in the heart of West Africa, regularly records cases of acute bacterial meningitis [5] [7] [8]. As part of its strategy to reduce meningitis cases, Burkina Faso introduced vaccines such as the *Haemophilus influenzae* type b vaccine in January 2006, MenAfriVac® in December 2010 and PCV-13 in October 2013 [7] [8] [9]. The recently introduced PCV-13 significantly reduces the occurrence of meningitis and other pneumococcal infections in relevant targets [8] [9]. As part of an overall strategy to monitor the actions of this vaccination campaign, the World Health Organization and Burkina Faso's partners have supported the relevant departments of the Ministry of Health in moving from enhanced surveillance to case-by-case surveillance. Indeed, case-by-case surveillance is fundamentally different from enhanced surveillance because any suspected case of meningitis must be subject to a cerebrospinal fluid (CSF) sample that is analysed to identify the microorganisms involved, including pneumococci [5] [8] [9].

S. pneumoniae occurs as gram-positive, candle-flame lanceolate diplococci and is strongly implicated in purulent and fatal meningitis due to its capsule [1] [8] [10]. This pneumococcal capsule is the source of a wide variety of serotypes that may or may not be vaccine serotypes [5] [8] [9]. The vaccine serotypes included in the pneumococcal conjugate vaccine (PCV13) are serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F or 23F [5] [8]. Non-vaccine serotypes include

serogroup 12F/12A/12B/44/46, serotypes 35B, 25F and 2. The serotypes also contain several standard sequences that are important for epidemiological surveillance [11] [12]. Indeed, the determination of the different serotypes before the introduction of PCV-13 provided the government of Burkina Faso with scientific data to defend to World health organization (WHO) and its technical partners the interest of introducing PCV-13 and not pneumococcal conjugate vaccine seven valent (PCV7) or PCV10 [5] [8] [9]. Therefore, it is also important to have information on *S. pneumoniae* standard sequences (ST) from the same period. It is in this context that we propose to describe the type sequences of pneumococcal strains circulating in Burkina Faso before the introduction of PCV-13 in Burkina Faso.

2. Methods

2.1. Setting, Type and Period of Study

This study was carried out concurrently in the meningitis reference laboratory (Charles De Gaulle Paediatric University Hospital), the national level laboratories (Yalgado Ouédraogo University Hospital and the National Public Health Laboratory) in Burkina Faso and in the pneumococcal laboratory of the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, United States of America. This was a descriptive cross-sectional study that took place from 27 October 2013 to 7 January 2014.

2.2. Samples

The samples consisted of thirty-seven (37) strains of *S. pneumoniae* isolated from the cerebrospinal fluid (CSF) of patients with meningitis and came from the Central, Central West, Central South, Central North, Eastern and Northern regions.

2.3. Laboratory Tests

S. pneumoniae strains isolated from the above laboratories in Burkina Faso were aliquoted in 0.5 mL of Greaves solution in a cryotube and transported to the CDC laboratory for testing. Once at the CDC, the strains were plated in fresh blood agar (FBA) and re-tested in the optochine with an inhibition diameter ≥ 14 mm. Next, the strains were sequenced in MLST which involves four main steps of amplification, purification, sequencing and interpretative reading of the results. The primer amplification used seven primers that carried the corresponding genes. The genes used in the sequencing of *S. pneumoniae* were *aroe* (shikimate dehydrogenase), *gdh* (glucose-6-phosphate dehydrogenase), *gki* (glucose kinase), *recP* (transketolase), *spi* (signal peptidase I), *xpt* (xanthine phosphoribosyltransferase), *ddl* (D-alanine-D-alanine ligase) (See **Table A4**). The preparation of the *aroe*, *gdh*, *gki*, *recp* and *xpt* gene mix for the amplification of the 7 regions for MLST was done according to **Table A1**. For the *ddl* gene, the preparation of the mix was done according to **Table A2**. After the various preparations and dis-

tributions, the plate was placed in a thermocycler. Table 1 below gives the primers used for amplification of each of the seven genes.

Polymerase Chain Reaction (PCR) amplification for MLST sequencing of pneumococci followed the following conditions for the *aroe*, *gdh*, *gki*, *recp*, *spi* and *xpt* genes: [1X (94˚C, 1 min)]; [30X (94˚C, 15 sec); (54˚C, 30 sec); (72˚C, 45 sec)]; [1X (72˚C, 10 min)]; and [1X (4˚C, ∞]. For the ddl gene, the amplification conditions were as follows: [1X (94˚C, 1 min)]; [30X (94˚C, 15 sec); (50˚C, 1 min); (72˚C, 45 sec)]; [1X (72˚C, 10 min)] and [1X (4˚C, ∞]. Once the PCR was completed, the success of the amplification had to be verified. To do this, 2 µL of the amplicons underwent rapid migration on 1% agarose gel. Also, purification of the PCR product was performed. For this purpose, the ExoSAP-IT™ protocol was used according to the manufacturer's instructions. For this purpose, in a clean 0.2 mL tube, 4 µL of amplicon + 3 µL of ExoSAP-IT™ was placed. This mixture was incubated at 37˚C for 15 minutes to degrade the primers and residual deoxyribonucleotide triphosphate (dNTP). Finally, incubate at 80˚C for 15 minutes to inactivate residual ExoSAP-IT™ reagents. The resulting PCR products are ready for amplification. For pneumococcal Deoxyribonucleic acid (DNA) sequencing by MLST, the composition of the reagents used in the preparation of the sequencing mix is given in Table A4. Plates are introduced into the thermocycler under these conditions: [1X (96˚C, 1 min)]; [25X (96˚C, 10 sec); (55˚C, 5 sec); (60˚C, 4 min)]; [1X (4˚C, 7 min)] and [1X (4˚C, ∞)]. Table A3 gives the AB sequencer used for sequencing the samples.

Table 1. The primer amplification used seven primers that carried the corresponding genes [13] [14].

Genes	Primers sequences
aroe	*aroe*-F: TCC TAT TAA GCA TTC TAT TTC TCC CTT C (26)
	aroe-R: ACA GGA GAG GAT TGG CCA TCC ATG CCC ACA CTG (33)
gdh	*gdh*-F: ATG GAC AAA CCA GC(G/A/T/C) AG(C/T) TTC ACA AAA G (28)
	gdh-R: ACG ATA GGT GAT ATC TGG TTG CCA AGT CCA TTT G (34)
gki	*gki*-F: TCG TTT GGA CTT GCT TGG ATT GGC AG (26)
	gki-R: AGA TGT GCG TAC TTG TGG GAA ACT ATT TTC ATC G (34)
recp	*recp*-F: GAA TGT GTG ATT CAA TAA TCA CCT CAA ATA GAA GG (35)
	recp-R: TGC TGT TTC GAT AGC AGC ATG GAT GGC TTC C (31)
spi	*spi*-F: CGC TTA GAA AGG TAA GTT ATG AAT TT (26)
	spi-R: GAA GAG GCT GAG ATT GGT GAT TCT CGG CC (29)
xpt	*xpt*-F: TTA ACT TTT AGA CTT TAG GAG GTC TTA TG (29)
	xpt-R: CGG CTG CTT GCG AGT GTT TTT CTT GAG (27)
ddl	*ddl*-F: TAA AAT CAC GAC TAA GCG TGT TCT GG (26)
	ddl-R: AAG TAG TGG GTA CAT AGA CCA CTG GG (26)

Legend: number in front of each gene is the number of bases.

2.4. Ethical Considerations

The study received approval from the ethical committee for health research of the Ministry of Health of Burkina Faso (Deliberation N˚2014-10-116).

2.5. Data Analysis

For the descriptive statistics of the patients, the Stata 14.0 software was used to describe the different serotypes identified as well as the corresponding standard sequences (ST). Also, the STs were analysed with the CDC Fret programme using EditSeq/Seqman software and Codon Code Aligner software.

3. Results

The 37 *S. pneumoniae* strains were from the Centre (31.3%), East (28.1%), Centre-West (18.8%), North (12.5%), Centre-North (6.3%) and Centre-South (3.1%) regions. The youngest patient was 1 month old while the oldest was 55 years old. Of all the *S. pneumoniae* strains tested, 10 different serotypes were identified. Serotype 1 was the most common at 48.6% (18/37), followed by serotype 25F at 10.8% (4/37). Serotypes 5 and 12F/12A/12B/44/46 were 8.1% (3/37) each. Serotypes 4, 6B and 23F were 5.4% (2/37) each while serotypes 2, 14 and 35B were 2.7% (1/37) each. Table 2 gives the description of the different STs of the identified serotypes according to the 7 genes tested.

After the description of pneumococcal STs according the 7 genes used in MLST sequencing, it's suitable to have more details of the proportion each ST described. Thus, the main ST described is ST303 of serotype 1 for 24.3% followed by ST105 of serotype 25F for 10.8%. Table 3 below gives for each serotype, the corresponding ST.

Table 2. Description of the different STs of the identified serotypes according to the 7 genes tested.

Sérotype	ST	*aroe*	*gdh*	*gki*	*recp*	*spi*	*xpt*	*ddl*
1	**303**	10	5	4	1	7	19	9
6B	**12,693**	8	134	367	5	10	4	6
1	**217**	10	18	4	1	7	19	9
1	**618**	13	8	4	1	7	19	14
5	**289**	16	12	9	1	41	33	33
5	**289**	16	12	9	1	41	33	33
35B	**12,694**	5	13	4	8	6	22	18
1	**12,695**	10	5	4	1	7	723	9
23F	**802**	10	13	53	1	72	38	31
1	**303**	10	5	4	1	7	19	9
23F	**802**	10	13	53	1	72	38	31
1	**217**	10	18	4	1	7	19	9
1	**303**	10	5	4	1	7	19	9
12F	**989**	12	5	89	8	6	112	14
4	**5504**	8	88	103	18	36	142	161

Continued

1	**303**	10	5	4	1	7	19	9
25F	**105**	5	15	4	1	6	1	6
1	**303**	10	5	4	1	7	19	9
12F	**989**	12	5	89	8	6	112	14
1	**1316**	2	5	4	1	7	19	9
25F	**105**	5	15	4	1	6	1	6
5	**289**	16	12	9	1	41	33	33
1	**2830**	10	5	4	1	155	19	9
4	**12,696**	8	5	103	1	9	14	161
1	**303**	10	5	4	1	7	19	9
1	**303**	10	5	4	1	7	19	9
1	**618**	13	8	4	1	7	19	14
6B	**8052**	7	20	1	2	6	1	17
1	**303**	10	5	4	1	7	19	9
1	**618**	13	8	4	1	7	19	14
25F	**105**	5	15	4	1	6	1	6
12F	**989**	12	5	89	8	6	112	14
1	**303**	10	5	4	1	7	19	9
25F	**105**	5	15	4	1	6	1	6
2	**12,697**	2	13	36	12	6	6	14
14	**12,698**	2	5	4	1	17	21	14
1	**217**	10	18	4	1	7	19	9

Table 3. Proportion of STs according the serotypes determined.

Serotypes	ST	Frequencies	Proportion
25F	**105**	4	**10.8**
1	**217**	3	8.1
5	**289**	3	8.1
1	**303**	9	**24.3**
1	**618**	3	8.1
23F	**802**	2	5.4
12F	**989**	3	8.1
1	**1316**	1	2.7
1	**2830**	1	2.7
4	**5504**	1	2.7
6B	**8052**	1	2.7
6B	**12,693**	1	2.7
35B	**12,694**	1	2.7
1	**12,695**	1	2.7
4	**12,696**	1	2.7
2	**12,697**	1	2.7
14	**12,698**	1	2.7
Total		37	100

4. Discussion

The cases of *S. pneumoniae* meningitis that occurred in Burkina Faso before the introduction of PCV13 were due to a variety of serotypes. Thus, from Table 2, we were able to determine 10 different serotypes in the present study. From Table 3, we notice a single serotype may consist of one or more STs. This is the case for serotype 1, which is made up of 5 STs, with ST303 (9/17), ST217 (3/17) and ST618 (3/17) in the foreground. Results from other work in West Africa show a high prevalence of ST303 (7/9) within serotype 1 (1). This differs from previous work in the West African region which reported ST618 as 72.7% (92/127) [11] [15]. In contrast, in South Africa, serotype 1 ST217 is reported at 96% (872/912) with three subclasses ST217 C1 at 92% (353/382), ST217C2 at 4% (15/382) and ST217C3 at 4% (14/382) [16]. Moreover, within this same serotype, there are various STs such as ST1316, ST2830, ST12695. These data show that there is great genetic diversity of pneumococcal strains circulating within the same country, from one country to another in the West African sub-region; and this, in time and space. This great genetic diversity could be explained by mutations in pneumococcal specie [16] [17] [18].

Nevertheless, a certain homogeneity is observed in our work for non-dominant serotypes such as 25F which contains only ST105 (4/4), serotype 5 with ST289 (3/3) and serogroup 12F/12A/12B/44/46 with ST 989 (3/3). In contrast, in North America, within serogroup 12F/12A/12B/44/46, the ST218 clonal complex is reported to be the most involved in meningitis epidemics [12]. Thus, the diversity of STs of the same serotype from one continent to another could also be seen. Also, many other serotypes, such as 6B, 4, 2 and 14, yielded only one ST due to the limited number of strains. Such results do not allow any consistent conclusions to be drawn. In this context, it seems very difficult to fight against pneumococcal meningitis worldwide without laboratories diagnosis. Thus, the surveillance of these serotypes and STs could be an opportunity in the use of data for the introduction of new vaccines.

At the end of this work, we note the following limitations. Firstly, the limited number of sequenced pneumococcal strains per region does not allow us to have a distribution of ST. Secondly, it should be remembered that Burkina Faso has 13 regions, whereas we did this work with strains from 6 regions. Thirdly, the data date from 2013-2014, and may not correspond to the current situation.

However, the data reported in this study provide a basis for future work on molecular surveillance of meningitis and the impact of vaccination on the distribution of different serotypes of *S. pneumoniae*. Also, they reinforce the need to increase and extend the possibilities of culture of pneumococcal strains in bacteriology laboratories in our country for a better surveillance of the epidemiological profile of meningitis.

5. Conclusions

The aim of this work was to describe the typical sequences of *S. pneumoniae*

strains in Burkina Faso. Indeed, pneumococci are characterised by their great variability in terms of both the number of serotypes and the typical sequences within the same serotype. Thus, out of all the strains, ten serotypes have been identified. Also, within serotype 1, five different standard sequences have been described.

This information gives an idea of the complexity of this pathogen which is greatly involved in purulent bacterial meningitis at both national and sub-regional levels. Preventive measures such as vaccination should be encouraged to protect the vulnerable segments of our society such as children under five years of age as well as people over sixty years of age.

This also requires continuous surveillance of pneumococcal meningitis, which requires laboratory capacity building and staff training.

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Authors Contributions

Conceptualization: KD, OTR, SL
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Formal analysis: KD, OTR, ZAA, OO
Investigation: KD, SM, OTR
Resources: OTR, AF, MI
Supervision: OTR, KS, MI
Validation: OWHG, ZAA,
Visualization: OTR, KD, ASO
Writing ± original draft: KD, ASO, KBA
Writing ± review & editing: OO, CR, ST, ZS, SRST

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table A1. Preparation of mix for genes *aroe*, *gdh*, *gki*, *recp* and *xpt* for MLST amplification.

PCR Master Mix	x1 (Volum/µL)	x100
PCR Water	15.8	1580
Buffer10X Tampon	2.5	250
10 mM dNTPs	0.5	50
20 mM amorce sens	0.5	50
20 mM amorce anti-sens	0.5	50
Taq 5 U/µL	0.2	20
Total	20	
ADN	5	
Final volum	25.0	

Table A2. Mix Preparation for *ddl* gene amplification.

PCR Master Mix	x1 (Volum/µL)	x100
PCR Water	14.8	1480
Buffer10X	2.5	250
10 mM dNTPs	0.5	50
20 mM Primer Forward	1	100
20 mM Primer Reverse	1	100
Taq 5 U/µL	0.2	20
Total	20	
DNA	5	
Final Volum	25.0	

Table A3. Preparation of mix for gene *spi* amplification.

PCR Master Mix	x1 (Volum/µL)	x100
PCR water	14.8	1480
10X Buffer	2.5	250
10 mM dNTPs	0.5	50
20 mM Primer Forward	1	100
20 mM Primer Reverse	1	100
Taq 5 U/µL	0.2	20
Total	20	
DNA (Diluted 1:5)	5	From chelex extraction
Final volum	25.0	

Table A4. Mix preparation for MLST sequencing.

Sequencing Mix	x1 volum (µL)	x100
5X Sequencing Buffer	4	400
PCR Water	14.75	1475
Terminator nucleotides + Polymerase	1	100
Primer Forward (20 µM/pmol)	0.25	25
Primer Reverse (20 µM/pmol)	0.25	25
Purified DNA	0.5	
Final volum	20.5	