International Journal of Microbiology and Biotechnology
2022; 7(2): 51-57
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20220702.11
ISSN: 2578-9678 (Print); ISSN: 2578-9686 (Online)

Genetic Diversity of Circulating Genotypes of PCR Confirmed *Neisseria meningitidis* Serogroups Amongst Cerebrospinal Meningitis Patients in Parts of Northern Nigeria

Iseimokumo Christopher Peletiri[1, 2, *], Grace Mebi Ayanbimpe[1], Eugene Ifeanyichukwu Ikeh[1]

[1]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Health Sciences, University of Jos, Jos, Nigeria

[2]Medical Microbiology & Parasitology Laboratories, National Hospital, Abuja, Nigeria

Email address:

kumochris@hotmail.com (I. C. Peletiri)

[*]Corresponding author

To cite this article:

Iseimokumo Christopher Peletiri, Grace Mebi Ayanbimpe, Eugene Ifeanyichukwu Ikeh. Genetic Diversity of Circulating Genotypes of PCR Confirmed *Neisseria meningitidis* Serogroups Amongst Cerebrospinal Meningitis Patients in Parts of Northern Nigeria. *International Journal of Microbiology and Biotechnology*. Vol. 7, No. 2, 2022, pp. 51-57. doi: 10.11648/j.ijmb.20220702.11

Received: February 24, 2022; **Accepted**: March 17, 2022; **Published**: April 9, 2022

Abstract: The genetic potential of an organism is carried in the base sequence of its DNA according to the genetic code. Just as we have defined genotypes in humans, bacterial genotypes are specific and defined too. However, information on circulating *N. meningitidis* genotypes from northern Nigeria are unavailable in literature. Molecular genotyping methods are used to address two very different kinds of problems associated with disease outbreak (short term or local epidemiology and long term or global epidemiology). The multilocus sequence typing scheme developed for *N. meningitidis* which is based on DNA sequencing of segments of seven housekeeping loci was used being the gold standard. The PubMLST.org database and Bacterial Isolate Genome Sequence Database software were equally used. Of the 40 genotyped samples, 31 (77.5%) had detectable genotypes; genotype *abcZ* in three cases (7.5%); *adk*, four (10%); *aroE*, three (7.5%); *fumC*, 16 (40%); *pdhC*, two (5%); and genotype *pdh* in three cases (7.5%). The six genotypes encountered were found to be circulating amongst the various serogroups in parts of northern Nigeria which is now publicly reported in literature for the first time in Nigeria. Genotype *abcZ* was found circulating amongst serogroups A (*sacB*) and Y (*synF*); genotype *adk* amongst serogroups A (*sacB*), W135 (*synG*), and Y (*synF*); genotype *aroE* amongst serogroups C (*synE*), W135 (*synG*), and Y (*synF*); genotype *fumC* amongst serogroups A (*sacB*), B (*synD*), C (*synE*), W135 (*synG*), X (*xcbB*), and Y (*synF*); genotype *pdhC* amongst serogroups B (*synD*), and X (*xcbB*); and genotype *pgm* amongst serogroups B (*synD*), C (*synE*), and Y (*synF*). The utilization of our findings by health authorities would be of great importance for public health strategies such as vaccination. Our knowledge has been widened to acknowledge the presence of bacterial genotypes circulating amongst various *N. meningitidis* serogroups.

Keywords: Genetic Diversity, Bacterial Genotypes, Serogroups, *N. meningitidis*, CSM, Northern Nigeria

1. Introduction

Neisseria meningitidis, often called meningococcus is a Gram-negative diplococcus of about 0.8 µm in diameter that resemble coffee beans when viewed microscopically. *N. meningitidis* is typically found in the upper respiratory tract and has been identified and implicated as one of the leading causes of bacterial meningitis [1]; and the aetiologic agent of meningococcal septicaemia. Bacterial meningitis is a life-threatening condition that requires prompt recognition and treatment [2], and has remained a serious global health problem [3]. Over 1.2 million cases of bacterial meningitis are estimated to occur worldwide each year [4]. In Nigeria, about 5000 cases of meningitis occur every year and has been linked to the cause of several deaths [5]. As at 2017, the meningitis belt in Nigeria had covered 25 of the 36 States of the Federation (all the 19 northern States including the Federal Capital Territory (FCT), and some southern States such as Anambra, Cross River, Ebonyi, Enugu, Imo, and Oyo) as shown in Figure 1 as reported by the Nigeria Centre

for Disease Control [6]. Just as we have defined genotypes in humans, same is applicable to microorganisms; known as bacterial genotypes. However, information on circulating *N. meningitidis* genotypes in Northern Nigeria are sketchy in literature.

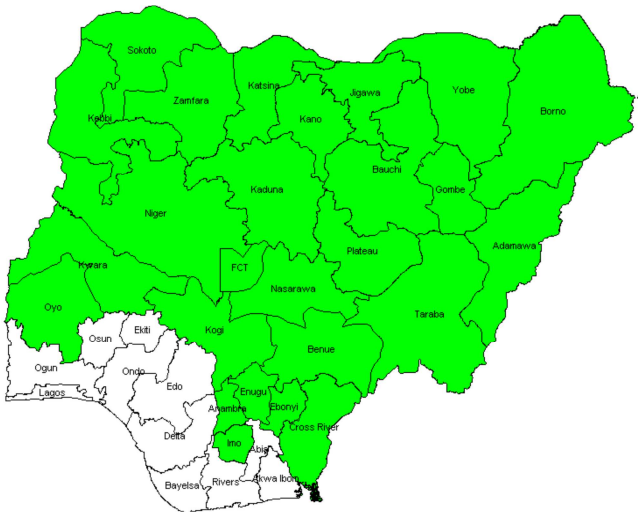

Figure 1. *States within the Meningitis belt in Nigeria [6].*

Molecular genotyping methods are used to address two very different kinds of problems associated with disease outbreak [7]. Firstly, are the isolates or mDNA/gDNA recovered from a localized outbreak of disease the same or different strains? (short term or local epidemiology) [7]. Short-term or local epidemiology focuses on genetic variations or microvariations that may occur during an individual outbreak or epidemic event in a particular geographic location [8]. Secondly, how are strains causing disease in one geographic area related to those isolated world-wide? (long term or global epidemiology) [7]. The goal of long-term or global epidemiology is to measure the accumulation of genetic variation overtime and establish a linkage between strain lineage and disease on a global scale [8]. Genotyping, which refers to the discrimination of bacterial strains based on their genetic content is now the method of choice used for bacterial strain typing due to its high resolution [9]. The genetic profile of a given strain generated by a specific genotyping method can be as unique as a fingerprint. Thus, genotyping is also referred to as DNA fingerprinting [9].

The multilocus sequence typing (MLST) is a DNA sequencing-based genotyping method which generates the original sequence of nucleotides and discriminate among bacterial strains directly from polymorphisms in their DNA; and has been developed with a global epidemiology perspective [10]. MLST indexes the sequences of seven internal (representative) housekeeping gene fragments to identify bacterial genotypes and associate them with biological properties [7, 10 – 11]. MLST scheme is available for *N. meningitidis* [7] which is based on DNA sequencing of segments of seven housekeeping loci (*abcZ, adk, aroE, fumC, gdh, pdhC,* and *pgm*), and is recognized as the gold standard for accurate strain characterization and epidemiological surveillance of this organism [12]. Meningococci are characterized at the genetic level by multilocus sequence typing (MLST), which uses sequences of the seven housekeeping genes to determine isolate sequence type (ST) [7]. Groups of closely related ST are termed clonal complexes (cc) and these are good surrogates of bacterial lineage. A subset of clonal complexes, known as hyperinvasive (or hypervirulent) lineages were responsible for the majority of disease worldwide [13]. We aimed at determining the genetic diversity of circulating genotypes of *N. meningitidis* serogroups in parts of Northern Nigeria.

2. Materials and Methods

2.1. Ethics Approval and Consent to Participate

Ethical approvals were obtained from the Health Research Ethics Committees of National Hospital, Abuja (NHA/EC/034/2015), Federal Capital Development Authority Health Services (FHREC/2017/01/27/03-04-17), Kebbi State Ministry of Health (MOH/KSREC/VOL.1/56/No101.3/2015), Plateau State Ministry of Health (MOH/MIS/202/VOL.T/X, 2017), Sokoto State Ministry of Health (SMH/1580/V.IV, 2017), and Zamfara State Ministry of Health (ZSH REC/ 02/03/2017) [14-15]. Written informed consent for storage and future use of unused sample, and sample material and data transfer agreement were also obtained [14-15].

2.2. Sample Size Determination

Sample size was calculated using the Cochran formula [16] for calculating simple proportion. At 0.05 alpha level of significance, 95% confidence level and a patient population size of seventy-seven and previous prevalence 13.7%, a sample size of 181.7 which was adjusted to 210 samples after calculating for 10% attrition [14-15]. The subjects were recruited consecutively until the sample size was attained [14-15].

2.3. Sample Collection

CSF samples were collected as previously reported [14].

2.4. Extraction and Quality Check of Metagenomic DNA

Metagenomic DNA extraction and quality check was as previously reported [14].

2.5. Multiplex Real-Time PCR for N. meningitidis Detection

Multiplex Real-time PCR for molecular detection of *N. meningitidis* was as previously reported [15].

2.6. Singleplex Real-time PCR for N. meningitidis Characterization

Singleplex Real-time PCR for molecular characterization of *N. meningitidis* was as previously reported [15].

2.7. Sample Selection

Of the appropriately characterized 67 *N. meningitidis* serogrouped strains with singleplex Real-time PCR as reported by Peletiri and co-authors [15], 40 (59.7%) were properly selected for genotyping to ensure both geographical coverage (spread) and serogroup representation.

3. Multilocus Sequence Typing (MLST) Protocol for *N. meningitidis* Genotyping

This assay protocol describes a Real-time PCR method using SYBR chemistry, designed to genotype selected genotypic markers of *N. meningitidis.* The markers include *abcZ, adk, aroE, fumC, gdh, pdhC*, and *pgm* as shown in Table 1. The primers used for amplification by Real-time PCR were: *abcZ*-F, 5' AATCGTTTATGTACCGCAGG 3', and *abcZ*-R, 5' GTTGATTTCTGCCTGTTCGG 3'; *adk*-F, 5' GGTGCACCGGGTGCAGGTAA 3', and *adk*-R, 5' CCTAAGATTTTATCTAACTC 3'; *aroE*-F, 5' ACGCATTTGCGCCGACATC 3', and aroE-R, 5' ATCAGGGCTTTTTTCAGGTT 3'; *fumC*-F, 5' TCCCCGCCGTAAAAGCCCTG 3', and *fumC*-R, 5' GCCCGTCAGCAAGCCCAAC 3'; *gdh*-F, 5' ATCAATACCGATGTGGCGCGT 3', and *gdh*-R, 5' GGTTTTCATCTGCGTATAGAG 3'; *pdhC*-F, 5' GGTTTCCAACGTATCGGCGAC 3', and *pdhC*-R, 5' ATCGGCTTTGATGCCGTATTT 3'; and *pgm*-F: 5' CTTCAAAGCCTACGACATCCG 3', and *pgm*-R, 5' CGGATTGCTTTCGATGACGGC 3'. All the primers were synthesized by Eurofins, Germany. Primers were supplied lyophilized; first reconstituted to 100 µM following manufacturer's instructions and working concentrations of 10 µM prepared using 1 x TE buffer as diluent [14-15].

3.1. MLST Protocol Set-up

Sample Requirement

Metagenomic DNA (mDNA) samples of serogrouped *N. meningitidis* with singleplex Real-time PCR, stored at -20°C (or -80°C) until required for testing.

Reagents and Materials

10 µM *N. meningitidis* primer mixes labelled *abcZ, adk, aroE, fumC, gdh, pdhC*, and *pgm* mix respectively. Promega qPCR Master mix; PCR water (Nuclease free water); ABI One Step Plus Real-time PCR System (Thermofisher, UK); ABI 96 well qPCR plate; P10, P100, and P1000 pipettes and tips; Thermal seal for PCR plate; Cold rack; Refrigerated centrifuge with plate holder (Heraeus, UK).

Table 1. *N. meningitidis MLST scheme, including gene locus, amplicon length, and trimmed length of sequence used for allelic determination on http://www.mlst.net platform [17].*

Housekeeping genes	Gene locus	Trimmed length
Putative ABC transporter	*abcZ*	433
Adenylate kinase	*adk*	465
Shikimase dehydrogenase	*aroE*	490
Fumurate dehydrogenase	*fumC*	465
Glucose-6-phosphate dehydrogenase	*gdh*	501
Pyruvate dehydrogenase subunit	*pdhC*	480
Phosphoglucomutase	*pgm*	450

Setting up Reaction.

Worksheet was created according to the number of samples to be tested. The ABI 96 well plate placed into a plate holder on a cold rack. Note: Each genotypic marker was tested alone. So, for each sample, there were seven (7) separate reactions. Into each well, 15 µL of Promega qPCR Master mix/ Primer was dispensed. Five (5) µL of mDNA, Standard or QC, or Negative control (PCR water) was added into appropriate well. The plate was sealed with thermal seal and centrifuged at 1000 rpm for one minute in refrigerated centrifuge (2 – 8°C). The plate was placed into the holder in the ABI One Step Plus Real-time PCR machine. The manufacturer's instruction was followed for setting up template – *N. meningitidis* genotyping. SYBR Chemistry and Standard mode, with Absolute Quantification were selected. The run was started and saved correctly. The thermal profile comprised of initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5s, 60°C for 30s, 72°C for 10s and a final melt cycle of 72°C to 95°C at ramp rate of 0.3°C/s.

Result Analysis

After the run, both the amplification curve and cycle threshold (Ct) values were inspected. Ct values of < 35 were positive; Ct values of 35 – 40 were equivocal; Ct values > 40 were negative. For the equivocal ones, the amplification curve and melting curve were checked to make the call. If it melts at same specific temperature as the positive cases, it would be positive.

3.2. Sequenced Results from MLST

The genome sequences obtained from genotyping (MLST protocol) were uploaded to the publicly available *Neisseria* PubMLST.org database (http://pubmlst.org/neisseria) [18], which is powered by the Bacterial Isolate Genome Sequence Database (BIGSdb) software [19] for the determination of sequence type (ST), clonal complex (cc), the outer membrane proteins (OMPs) (*PorA, PorB, FetA, fHbp, NadA*, and *NHBA*), core genome MLST (cgMLST), and antibiotic resistance genes (*gyrA, penA*, and *rpoB*).

3.3. Extracting Typing Information from a Local Genome File

The MLST scheme was selected from a drop-down box of the PubMLST.org database for *Neisseria,* and the analysis run was started by clicking the submit button. Individual allelic matches were identified along with the sequence type (ST) if the combination of alleles has been previously defined [20]. Typing information can be readily extracted from whole genome sequence assemblies using sequence query page. Genome assembly contids were pasted into the sequence query form and the required scheme or locus (in this case MLST) selected. Any locus exact matches are displayed and, if this corresponds to a defined combination of alleles, the profile definition (Sequence type (ST)/ clonal complex (cc) for MLST) was displayed [20].

4. Results

Of the 40 genotyped samples with Multilocus Sequence Typing (MLST) protocol, 31 (77.5%) were detected for one genotype or the other; while 9 (22.5%) were undetectable genotype strains. Of the seven genotypes tested for, six were encountered. MLST result revealed genotype *abcZ* in 3 cases (7.5%); genotype *adk*, 4 cases (10%); genotype *aroE*, 3 cases (7.5%); genotype *fumC*, 16 cases (40%); genotype *pdhC*, 2 cases (5%); and genotype *pgm,* 3 cases (7.5%) as shown in Table 2.

Table 2. *Prevalence of circulating N. meningitidis genotypes amongst the various identified serogroups in parts of Northern Nigeria.*

Gene Locus	Allele	Number encountered	%	Circulating amongst serogroups	No. of serogroups
abcZ	433	3	7.5	A (sacB) 1; Y (synF) 2	2
adk	465	4	10.0	A (sacB) 2; W135 (synG) 1; Y (synF) 1	3
aroE	490	3	7.5	C (synE) 1; W135 (synG) 1; Y (synF) 1	3
fumC	465	16	40.0	A (sacB) 2; B (synD) 6; C (synE) 1; W135 (synG) 3; X (xcbB) 1; Y (synF) 3	6
pdhC	480	2	5.0	B (synD) 1; X (xcbB) 1	2
pgm	450	3	7.5	B (synD) 1; C (synE) 1; Y (synF) 1	3
Undetectable genotype strains		9	22.5		
Total		40	100.0		

Key: Gene locus - Housekeeping genes
abcZ - Putative ABC transporter; *adk* - Adenylate kinase; *aroE* - Shikimase dehydrogenase;
fumC - Fumurate dehydrogenase; *pdhC* - Pyruvate dehydrogenase subunit; *pgm* – Phosphoglucomutase.

Of the 31 genotyped strains as shown in Table 2, six different sequence types (ST) with corresponding clonal complexes (cc) were extracted. From genotype *abcZ*, ST 6956, cc ST-41/44 complex and ST 9013, cc ST-5 complex were found; from genotype *aroE*, ST 6921, cc ST-196 complex and ST 12030, cc ST-213 complex were found; from genotype *fumC*, ST 7577, cc ST-23 complex was found; and from genotype *pgm*, ST 6796, cc ST-269 complex was found as shown in Table 3. Details of Sequence types (ST) and clonal complexes (cc) are reflected in Table 3. The genotype *abcZ* strains identified were assigned to four different sequence types (ST): ST 6956, cc ST-41/44 complex, BURST analysis displayed Singleton with frequency of one. ST 9013, cc ST-5 complex, BURST analysis also displayed Singleton with a frequency of one. The remaining two sequence types, ST 8716, and ST 10820 had no clonal complexes assigned as shown in Table 3. ST and cc results for the remaining five genotypes (*adk, aroE, fumC, pdhC*, and *pgm*) are shown in Table 3.

Table 3. *Genotypes of N. meningitidis allelic profile for Sequence type (ST) and clonal complex (cc) extracted from PubMLST.org powered by BIGSdb software.*

Gene Locus	Allele	Sequence Type (ST)	Clonal complex (cc)	BURST analysis	Singleton			
				ST	Frequency			
abcZ	433	6956	ST- 41/44 complex	6956	1			
		9013	ST- 5 complex	9013	1			
		8716	Nil		Nil			
		10820	Nil		Nil			
adk	465	11087	Nil		Nil			
aroE	490	6921	ST-198 complex	6921	1			
		12030	ST-213 complex	12030	1			
fumC	465	7577	ST-23 complex	Not available				
					Frequency	SLV	DLV	SAT
pdhC	480	8724	Nil	8724	1	1	1	-
		10882	Nil	10882	1	1	1	-
		10883	Nil	10883	1	-	2	-
				* Singletons – None				
pgm	450	6796	ST-269 complex	6796	1			
		7144	Nil	7144	1			
		10820	Nil	10820	1			

Footnote: Extracting typing information from a local genome file.

Table 4. *Epidemiological characterization of genotyped strains of N. meningitidis recovered from CSM patients in parts of Northern Nigeria during the 2017 and 2018 meningitis seasons.*

Genotype[a]	Serogroup[b]	Sequence Type (ST)[c]	Strain designation[d]
abcZ	A, Y	6959	A: P1.22-42, 4-20, F3-53: ST-6959 (cc41/44)
			Y: P1.22-42, 4-20, F3-53: ST-6959 (cc41/44)
		9013	A: P1.22-42, 4-20, F3-53: ST-9013 (cc5)
			Y: P1.22-42, 4-20, F3-53: ST-9013 (cc5)
		8716	A: P1.22-42, 4-20, F3-53: ST-8716
			Y: P1.22-42, 4-20, F3-53: ST-8716
		10820	A: P1.22-42, 4-20, F3-53: ST-10820
			Y: P1.22-42, 4-20, F3-53: ST-10820
adk	A, W135, Y	11087	A: P1.18-51, 2-72, F5-75: ST-11087
			W135: P1.18-51, 2-72, F5-75: ST-11087
			Y: P1.18-51, 2-72, F5-75: ST-11087
aroE	C, W135, Y	6921	C: P1.7-9, 2-23, Δ: ST-6921 (cc198)
			W135: P1.7-9, 2-23, Δ: ST-6921 (cc198)
			Y: P1.7-9, 2-23, Δ: ST-6921 (cc198)
		12030	C: P1.7-9, 2-23, Δ: ST-12030 (cc213)
			W135: P1.7-9, 2-23, Δ: ST-12030 (cc213)
			Y: P1.7-9, 2-23, Δ: ST-12030 (cc213)
fumC	A, B, C, W135, X, Y	7577	A: P1.12-5, 2-41, F4-8: ST-7577 (cc23)
			B: P1.12-5, 2-41, F4-8: ST-7577 (cc23)
			C: P1.12-5, 2-41, F4-8: ST-7577 (cc23)
			W135: P1.12-5, 2-41, F4-8: ST-7577 (cc23)
			X: P1.12-5, 2-41, F4-8: ST-7577 (cc23)
			Y P1.12-5, 2-41, F4-8: ST-7577 (cc23)
pdhC	B, X	8724	B: P1.21-10, 13-44, F5-58: ST-8724
			X: P1.21-10, 13-44, F5-58: ST-8724
		10882	B: P1.21-10, 13-44, F5-58: ST-10882
			X: P1.21-10, 13-44, F5-58: ST-10882
		10883	B: P1.21-10, 13-44, F5-58: ST-10883
			X: P1.21-10, 13-44, F5-58: ST-10883
pgm	B, C, Y	6796	B: P1.21-21, 2-21, Δ: ST-6796 (cc269)
			X: P1.21-21, 2-21, Δ: ST-6796 (cc269)
			Y: P1.21-21, 2-21, Δ: ST-6796 (cc269)
		7144	B: P1.21-21, 2-21, Δ: ST-7144
			C: P1.21-21, 2-21, Δ: ST-7144
			Y: P1.21-21, 2-21, Δ: ST-7144
		10820	B: P1.21-21, 2-21, Δ: ST-10820
			C: P1.21-21, 2-21, Δ: ST-10820
			Y: P1.21-21, 2-21, Δ: ST-10820

Footnote:

a – Genotypes were obtained from sequencing of seven housekeeping genes using the Multilocus Sequence Typing (MLST) protocol.

b - Serogroups were obtained by characterization of identified *N. meningitidis* from metagenomic DNA (mDNA) with Singleplex Real-time PCR.

c - Sequence Type (ST) was extracted from PubMLST.org powered by BISGdb software.

d - Strain designation was based on the previously recommended nomenclature [21], comprising of serogroup, *porA* type (Px), *fetA* type (Fx), and sequence (STx) (clonal complex (cc)).

5. Discussion

Genetic diversity data obtained from the housekeeping genes is commonly used to establish phylogenetic relationships among members of a population [22]. Phylogeny can be used as a framework to which pathogenicity-related characteristics such as virulence, virulence genes, and host specificity may be mapped. With this mapping, important insights can be obtained on the origin of pathogenicity and host adaptations of a pathogen [23]. Of the 31 samples, six genotypes were encountered as shown in Table 2; as well as six clonal complexes. The six genotypes encountered were found to be circulating amongst the various *N. meningitidis* serogroups. Genotype *abcZ* was identified amongst two serogroups, A (*sacB*) and Y (*synF*); genotype *adk* amongst three serogroups, A (*sacB*), W135 (*synG*), and Y (*synF*); genotype *aroE* amongst three serogroups, C (*synE*), W135 (*synG*), and Y (*synF*); genotype *fumC* amongst six serogroups, A (*sacB*), B (*synD*), C (*synE*), W135 (*synG*), X (*xcbB*), and Y (*synF*); genotype *pdhC* amongst two genotypes, B (*synD*), and X (*xcbB*); and genotype *pgm* amongst three genoypes, B (*synD*), C (*synE*), and Y (*synF*) as shown in Table 2. Our results revealed that genotype *fumC* was the most predominant circulating genotype occurring in all the six serogroups.

Of the six clonal complexes (cc) found as shown in Table 3, literature search revealed four (ST-41/44 complex, ST-5 complex, ST-23 complex, and ST-269 complex) as hypervirulent or hyperinvasive [24 – 26]. The ST-41/44 complex has been found to cause disease in various European countries as well as the USA and in New Zealand where it

has been responsible for an epidemic since 1991 [13, 27 – 28]. However, seven other sequence types, ST 8716, 10820, 11087, 8724, 10882, 10883, and 7144 had no clonal complex assigned as shown in Table 3.

Of the 120 *N. meningitidis* isolates from Nigeria that are captured in the PubMLST.org database between 1996 and 2017, only three serogroups A (7), C (112), and W135 (1) were identified and the results are available as such. All the seven serogroup A cases had the same Sequence Type, ST 5 and clonal complex (cc) ST-5 complex. The only W135 isolate (id: 17862) from Bauchi in 2010 had ST 8637, cc11; with strain designation of W: P1.5-2, Δ: F1-7: ST-8637 (cc11). Surprisingly, all the 112 serogroup C isolates had the same Sequence Type, ST 10217, cc-10217, with strain designation of C: P1.21-15, 16: F1-7: ST-10217 (cc10217) including the report by Kwambana-Adams and colleagues [29] which reported ccST-10217 outbreak in Zamfara State, Nigeria. Meanwhile, Peletiri and co-authors [15] had implicated *N. meningitidis* serogroups X and Y as identified causes of CSM for the first time in parts of Northern Nigeria; and is now presented here with diverse but defined strain designations of: X: P1. 12-5, 2-41, F4-8: ST-7577 (cc23); X: P1. 21-10, 13-44, F5-58: ST-8724; X: P1.21-10, 13-44, F5-58: ST-10882; and Y: P1. 22-42, 4-20, F3-53: ST-6959 (cc41/44); Y: P1. 22-42, 4-20, F3-53: ST-9013 (cc5); and Y: P1. 21-21, 2-21, Δ: ST-6796 (cc269). However, of the Niger Republic isolates submitted at the PubMLST.org database, *N. meningitidis* serogroups A, C, W155, X, and Y are being reported with varying ST and cc.

The outer membrane proteins (OMPs)/ vaccine candidates' results obtained through BLAST protocol revealed much. The genome sequences of the 31 genotyped strains queried for the vaccine candidate antigens: NadA, NHBA, fHbp, PorA, and fetA showed revealing results. Based on the query outcomes, NadA_peptide matched two genotypes (*abcZ* and *aroE*), 33.3% (2/6). Our findings are in tune with earlier report by Bambiri and colleagues [30] that identified approximately 30% of meningococci with NadA. NHBA_peptide matched three genotypes (*adk*, *fumC*, and *pgm*), 50% (3/6). fHbp_peptide matched four genotypes (*abcZ*, *adk*, *fumC*, and *pdh*), 66.7% (4/6). PorA (VR_1 and VR_2) matched all six genotypes (*abcZ*, *adk*, *aroE*, *fumC*, *pdhC,* and *pgm*), 100% (6/6).

6. Conclusion

The high level of genetic diversity of circulating genotypes of PCR confirmed *N. meningitidis* serogroups in parts of Northern Nigeria is being reported here as baseline data for reference. Using the MLST protocol, we identified the circulating genotypes of *N. meningitidis* in parts of Northern Nigeria which is now publicly reported in literature for the first time in Nigeria. Our knowledge has been widened to acknowledge the presence of bacterial genotypes circulating amongst various *N. meningitidis* serogroups. As shown in Table 4, various strain designations were revealed. Based on these findings, vaccine agents to be administered in our environment should include all the circulating vaccine candidates. The utilization of our findings by health authorities would be of great importance for public health strategies such as vaccination [12]. Finally, commencement of preventive vaccination programmes in Nigeria will certainly do us a lot of good as against the current reactive vaccination programmes being implemented.

Acknowledgements

We acknowledge the Federal Ministry of Health, Nigeria and the ethics committee members of National Hospital, Abuja, Nigeria; Kebbi State Ministry of Health, Nigeria; Plateau State Ministry of Health, Nigeria; Sokoto State Ministry of Health, Nigeria; and Zamfara State Ministry of Health. All staff of the various laboratories where the samples were received and phenotypically processed are appreciated [14 -15]. Also, we wish to appreciate the MD/ CEO of Safety Molecular Pathology Laboratory (SMPL), Enugu, Dr. Emmanuel Nna (PhD) for training the principal investigator in the practical application of metagenomic protocol and general orientation in molecular diagnostic techniques in the PCR laboratory of his institute. Also appreciated are all staff of SMPL, Enugu for the various roles they played in the course of running of all samples for preparation of metagenomic DNA template and actual molecular diagnostic procedures. This research utilized the PubMLST database (https://pubmlst.org) developed by Keith Jolley [19].

References

[1] Brooks, G. F., Butel, J. S., Carroll, K. C., & Morse, S. A. (Eds.). (2007). The *Neisseriae*: In: Jawetz, Melnick & Adelberg's Medical Microbiology (24th Edition), The McGraw Hill Companies, Inc.

[2] Centers for Disease Control and Prevention (CDC) (2011). Epidemiology of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae.* In CDC Laboratory Methods for the Diagnosis of meningitis. (pp. 3 - 9), Second Edition. http://www.cdc.gov/meningitis/lab-manual/index.html. cdcinfor@cdc.gov.

[3] Centers for Disease Control and Prevention (CDC) (2011). Introduction. In CDC Laboratory Methods for the Diagnosis of meningitis. (p. 1), Second Edition. http://www.cdc.gov/meningitis/lab-manual/index.html. cdcinfor@cdc.gov.

[4] World Health Organization (1998). Control of epidemic meningococcal disease: WHO practical guidelines, 2nd edition, WHO/EMC/BA/98, 3: 1–83. who.int/iris/handle/10665/64467

[5] Nnadozie, C. (2013). Nigeria records 5000 cases of cerebrospinal meningitis annually. Daily Independent Newspaper, 3rd November, 2013.

[6] Nigeria Centre for Disease Control (NCDC) (2017). Preparedness and response to cerebrospinal meningitis outbreaks. A guide for health workers and authorities in Nigeria. FMoH, NCDC, 2017.

[7] Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., et al. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America* 95: 3140–3145. doi: 10.1073/pnas.95.6.3140.

[8] Wang, X., Jordan, I. K., & Mayer, L. W. (2015). A phylogenetic perspective on molecular epidemiology. In Y. Yang, M. Sussman, D. Liu, I. Poxton, & J. Schwartzman (Eds.), pp. 517 – 536, *Molecular Medical Microbiology*, Volume 1 (2nd Edition), Elsevier Ltd. Academic Press, USA.

[9] Wenjun, L., Raoult, D., & Fournier, P. (2009). Bacterial strain typing in the genomic era. FEMS Microbiology Reviews 33: 892–916. doi: 10.1111/j.1574-6976.2009.00182.x.

[10] Chan, M., Maiden, M. C. J., & Spratt, B. G. (2001). Database-driven Multi Locus Sequence Typing (MLST) of bacterial pathogens. *Bioinformatics* 17: 1077–1083. doi: 10.1093/bioinformatics/17.11.1077.

[11] Jolley, K. A., Chan, M. S., & Maiden, M. C. J. (2004). MLSTdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics 5 (1): 86. doi.org/10.1186/1471-2105-5-86.

[12] Brehony, C., Jolley, K. A., & Maiden, M. C. J. (2007). Multilocus sequence typing for global surveillance of meningococcal disease. *FEMS Microbiology Reviews* 31: 15–26. doi.org/10.1111/j.1574-6976.2006.00056.x.

[13] Tan, A., Hill, D., Harrison, O., Srikhanta, Y. N., Jennings, M. P., Maiden, M. C. J., et al. (2016). Distribution of the type III DNA methyltransferases *modA, modB* and *modD* among *Neisseria meningitidis* genotypes: implications for gene regulation and virulence. *Science Reports* 6: 21015. doi.org/10.1038/srep21015.

[14] Peletiri, I. C., Ikeh, E. I., Nna, E., Ndike, U. P., Usman, Y. B., Durfa, L. D., et al. (2021a). Quality of metagenomic DNA extracted for molecular identification of microorganisms from CSF samples of patients with suspected cerebrospinal meningitis in Northern Nigeria. *African Journal of Clinical and Experimental Microbiology* 22 (2): 146–156. doi: 10.4314/ajcem.v22i2.6.

[15] Peletiri, I. C., Ikeh, E. I., Ayanbimpe, G. M., & Nna, E. (2021b). Molecular detection and characterization of bacteria from CSF samples of patients with suspected cerebrospinal meningitis in parts of northern Nigeria using metagenomic DNA extracts. *African Journal of Clinical and Experimental Microbiology* 22 (3): 365–376. doi: 10.4314/ajcem.v22i3.8.

[16] Cochran, W. G. (1977). Calculation of sample size when population is infinite – sampling proportions and percentages. In W. G. Cochran (author) (pp. 50 - 53), *Sampling Techniques*. Third Edition. John Wiley & Sons, Inc. New York, 1977. ISBN 0-471-16240-X.

[17] Centers for Disease Control and Prevention (2011). Characterization of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* by Molecular Typing Methods. In CDC Laboratory Methods for the Diagnosis of Meningitis, (pp. 205 - 258). Second edition. http://www.cdc.gov/meningitis/lab-manual/index.html. cdcinfor@cdc.gov.

[18] Jolley, K. A., & Maiden, M. C. J. (2014). Using multilocus sequence typing to study bacterial variation: prospects in the genomic era. Future Microbiology 9 (5): 623 – 630. doi.org/10.2217/fmb.14.24.

[19] Jolley, K. A, & Maiden, M. C. J. (2010). BIGSdb. Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11 (1): 595. doi.org/10.1186/1471-2105-11-595.

[20] Jolley, K. A., Bray, J. E., & Maiden, M. C. J. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Research* 3: 124. doi.org/10.12688/wellcomeopenres.14826.1.

[21] Jolley, K. A., Brehony, C., & Maiden, M. C. J. (2007). Molecular typing of meningococci: recommendations for target choice and nomenclature, FEMS Microbiology Reviews 31 (1): 89– 96. doi: 10.1111/j.1574-6976.2006.00057.x.

[22] Virdi, J. S., & Sachdeva, P. (2005). Genetic diversity of pathogenic microorganisms: Basic insights, public health implications and the Indian initiatives. *Current Science*, 89 (1): 113-123.

[23] Spratt, B. G., & Maiden, M. C. J. (1999). Bacterial population genetics, evolution, and epidemiology. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 354: 701–710. doi: 10.1098/rstb.1999.0423.

[24] Brik, A., Terrade, A., Hong, E., Deghmane, A., Taha, M. K., Bouafsoun, A., et al. (2020). Phenotypic and genotypic characterization of meningococcal isolates in Tunis, Tunisia: High diversity and impact on vaccination strategies. International Journal of Infectious Diseases 91: 73–78. doi.org/10.1016/j.ijid.2019.11.013.

[25] De Filippis, I., de Lemos, A. P. S., Hostetler, J. B., Wollenberg, K., Sacchi, C. T., Harrison, L. H., et al. (2012). Molecular Epidemiology of Neisseria meningitidis Serogroup B in Brazil. *PLoS One* 7 (7): 10.1371. doi: 10.1371/journal.pone.0033016.

[26] Zarantonelli, M. L., Lancellotti, M., Deghmane, A. E., Giorgini, D., Hong, E., Ruckly, C, et al. (2008). Hyperinvasive genotypes of *Neisseria meningitidis* in France. *Clincal Microbiology and Infection* 14 (5): 467–472. doi: 10.1111/j.1469-0691.2008.01955.x.

[27] Martin, D. R., Walker, S. J., Baker, M. G., & Lennon, D. R. (1998). New Zealand epidemic of meningococcal disease identified by a strain with phenotype B:4:P1.4. *Journal of Infectious Diseases* 177: 497–500. doi: 10.1086/517385.

[28] Whitney, A. M., Mayer, L. W., Mothershed, E., Sacchi, C., Schmink, S., Theodore, J., et al. (2006). Distribution of multi-locus sequence types of *Neisseria meningitidis* serogroup B in the US – 2000 – 2005. Poster presented at International Pathogenic Neisseria Conference 2006, P4.3.01. Cairns, North Queensland, Australia, Cambridge Publiahing, West Leederville, WA, Australia.

[29] Kwambana-Adams, B. A., Amaza, R. C., Okoi, C., Murtala, R., Worwui, A., Foster-Nyanrko, E., et al. (2018). Meningococcus serogroup C clonal complex ST-10217 outbreak in Zamfara State, Northern Nigeria. *Scientific Reports*, 8: 14194. doi: 10.1038/s41598-018-32475-2.

[30] Bambini, S., Piet, J., Muzzi, A., Keijzers, W., Comandi, S., De Tora, L., et al. (2013). An Analysis of the Sequence Variability of Meningococcal fHbp, NadA and NHBA over a 50-Year Period in the Netherlands. *PLoS One* 8 (5): e65043. doi: 10.1371/journal.pone.0065043.