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SERO-EPIDEMIOLOGICAL EVALUATION OF CLONAL DIVERSITY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AMONG *NEISSERIA MENINGITIDIS* ISOLATES FROM EPIDEMIC CASES IN JIGAWA STATE, NIGERIA

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ABSTRACT

A total of 33 Neisseria culture-positive cerebrospinal fluid (CSF) samples from apparently ill patients during a meningococcal outbreak in Jigawa State, Nigeria were subjected to serogroup, serotype identifications schemes using agglutination and dot blot techniques. Antibiotic susceptibility patterns of the recovered *Neisseria meningitidis* isolates were also determined.
Seventeen (51.5%), 8 (24.2%), 3 (9.1%) and 5 (15.2%) of the Neisseria isolates belonged to A, B, C and W135 serogroups. Fifteen (*N. meningitides* A = 8; B = 2; C = 3; W135 = 3) were of serotype 2a, while 4 distinct serosubtypes: P1.5, 2 (57.6%), P1.9 (6.0%), P1.14 (6.0%) and P1.7, 1(15.2%) were found among 28 clones.
The proportions of serogroup A – associated cases, serotyppe 2a and serosubtype P.15, 2 were significant ($P < 0.05$) compared to other related parameters. While acquisition of meningococcal disease was neither age nor sex dependent ($P > 0.05$).
Multilocus enzyme electrophoretic typing further stratified the W135 isolates as members of the ET-37 complex. Five (15.2%), 6 (18.2%) and 7 (21.2%) of the 33 culture-positive isolates displayed resistance to ampicillin and chloramphenicol and intermediate resistance to penicillin.
Resistance pattern characterization further revealed monoresistance to trimethoprim-sulphamethazole (TMP) by 20 isolates and multiresistance with equal predominance (2 each) of patterns: Pen AmpChlTMP, PenAmpTMP, AmpTMP covering all the serogroups.
Three of the five W135, 6 of 17 A and 1 of 8 C serogroups were β–lactamase positive, while enzyme expression was not observed among the B isolates.
Key words: ***Neisseria meningitidis*** **serogroup, serotype, serosubtype, antibiotic susceptibility, Nigeria.**

INTRODUCTION

Meningococcal disease caused by *Neisseria meningitidis* serogroups A, B, C, Y and lately W135 remains a public health burden and significant cause of morbidity and mortality in sporadic, endemic and epidemic cases worldwide (1, 2). World Health Organization estimates indicate that there are 300,000 cases and 30,000 deaths per year worldwide excluding epidemics (3). Populations within the Sahel region south of Sahara bear the heavier burden of meningococcal disease as they experience periodic epidemics, higher hospital admissions, carriage and case-fatality rates compared to non-Sahel settings (4). The northern Nigeria is within the Africa meningitis belt and has been experiencing epidemics since 1960s (5). The subsequent control measures, which included mass or selective vaccination with serogroup A and later bivalent AC vaccines as well as chemotherapy with chloramphenicol, crystal penicillin with or

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EDITORIAL

VIRAL VACCINES AND CANCER

As more facts unfold about the role viruses play in the actiology of human cancer, it will become clear that viral vaccines may play a major role in their prevention. Cancer is now believed to be an accidental side effect of viral replication strategies (1). Fifteen percent (15%) of all human cancers are so far established to be caused by viruses. Table 1 shows the list of human neoplasia and the associated viruses.

Table 1

Virus – associated cancers

Human Neoplasia	Viral actiology
Hepatocellular carcinoma	Hepatitis B virus (HBV) Hapatitis C Virus (HCV)
Burkitt's Lymphoma	Ebstein-Barr virus (EBV)
Kaposi's Sarcoma	EBV, HIV is an enhancing factor
Post-transplant lymphoma	EBV
Cervical Cancer	Human papilloma virus (HPV) Herpes Simplex Virus (HSV-2)
Squarmous cell carcinomas especially epidermodysplasia verruciformis	HPV tupes 16 and 18
Anagenital cancers	HPV
Adult T-cell leukaemia	Human T-Lymphocytic virus type 1 (HTLV-1)
Primary effusion lymphoma	Human Herpes Virus 8 (HHV-8)
Brain tumors	Simian virus 40 (SV40)
Osteosarcoma	SV40
Mesothelimas	SV40

It is therefore becoming important that diagnostic methods for viral-associated cancers be improved upon. Histologic and immunohistologic techniques and detection of viral-specific proteins, transcription factors and oncogenes by molecular biology techniques should become essential to more and more laboratories in developing countries (2,3).

Just as antibiotics are now used in the treatment of peptic ulcers, antiviral agents and viral vaccines such as HBV, Polio and HPV vaccines may become useful in the prevention and control of certain human cancers (4).

The article on Prions and Prion Diseases give a detailed review of the diseases resulting from viral persistence.

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without ampicillin were found effective in reducing cases by 55 – 80% and deaths by 40 – 100% in their first two decades of use covering over 10 northern states including Jigawa, Kano, Katsina, Bauchi, Kaduna, Sokoto, and Borno (6). Clues that these control measures have declined in efficacy in northern Nigeria and require optimization have emanated from the resurgence of epidemic *Neisseria meningitis* in 1996 with an overall case-fatality rate of 10.7% and which has remained unabated in the last 10 years (6). Instead, the epidemic is characterized by expansion, increased frequency of cases and higher fatalities (7, 8). Case-fatality rates (CFR) of 11 – 25% have been reported (9) in recent northern epidemics and data collected from our clinical surveillance during the 2003 epidemic in Jigawa state revealed 133 deaths from 230 cases, indicating a CFR of 57.8% (unpublished).

Although, capsular antigenic profile data of circulating *Neisseria meningitidis* strains in the country are lacking, the efficacy of the bivalent AC vaccine from 1979 prior to the 1996 epidemics provides a strong support for the predominance of serogroups A and C as epidemics strains in the country.

However, the fatal evolution of meningococcal disease in other susceptible populations of Africa has witnessed the involvement of new strains and serogroups as epidemic agents. For instance, serogroup W135 and its clonally divergent strains have now replaced or predominate other serogroups as actiologies of meningococcal epidemics in Cameroun (10), Niger (11), Senegal (12), Burkina Faso (13) and Ethiopia (14) causing increased frequency of meningococcaemia – associated deaths, reduced efficacy of AC vaccine and increased number of multiple drug susceptibility patterns of *Neisseria meningitidis* in some of these areas have enabled optimization of interventions with vaccines and antibiotics to achieve improved

efficacies as outcomes (12, 15). These possibilities have not been explored in Nigeria, where reports have also shown that meningococcal disease surveillance is non-continuous and vaccination is poorly timed in most parts of northern Nigeria (6). The present study evaluates serogroup diversity of epidemic cerebrospinal fluid isolates of *Neisseria meningitis* in Jigawa state, Nigeria. The expression of β-lactamase enzymes and *in vitro* responses of these isolates to various antibiotics were also determined.

MATERIALS AND METHODS

Patients and Sample Collection

Cerebrospinal fluid (CSF) samples were collected from patients presenting with clinical symptoms of meningitis (e.g fever and chills, headache, stiff neck, dizziness and confusion, rashes, convulsion etc) at primary health care facilities in 4 Local Government areas of Jigawa State during a meningococcal outbreak in February and March, 2003. Surveillance data at the time of this study indicated 230 cases and 133 deaths, producing a case fatality rate (CFR) of 57.8% that was higher than the CFRs reported from previous epidemics in northern Nigeria (3). Socio-demographic data of each patient such as age, sex, hajj travel or contact and vaccination history were also obtained using a structured questionnaire.

Neisseria meningitidis isolates

A total of thirty-three (33) culture positive *Neisseria meningitidis* isolates recovered from 45 cerebrospinal fluid (CSF) sample (0.5ml) was inoculated into Trans-Isolated medium (15ml)

supplemented with 1% VCN (vancomycin, collstin sulphate and nystain) according to Ajello *et al* (1984)(16), then transported to Central Public Health Laboratory (CPHL), Lagos for further bacteriology and serological analysis. Cultures in T-1 bottles were sub-cultured on chocolate agar plates and incubated at 37°C for 24h in 5% CO_2 atmosphere. The suspected isolates in single colonies were further subjected to oxidase reaction, o-nitrophenyl-B-D-galactopyranoside test, sugar fermentation (17) and β – lactamase test (18) for confirmation as *Neisseria meningitidis* strains. Replica isolates with confluent growth patterns were used for DNA extraction and fingerprinting.

Serology

The isolated *N. meningitis* strains were serogrouped by slide agglutination assay using *N. meningitidis* agglutination sera for A, B, C, Y, and W135 (Murex Diagnostic, Dartford, UK). The isolates were serotyped and serosubtyped by dot blot analysis of whole cell suspension immobilized on nitrocellulose paper strips and used to react with monoclonal antibodies against serotypes 1. 2a, 2b, 4 and 14 and those against the following serosubtypes: (1.2), (P1.5, 2), (P1.4), (P1.5), (P1.5), (P1.6), (P1.15), (P1.16), (P1.7,1) at Norwegian Institute of Public Health, Oslo, Norway. The monoclonal antibodies used were diluted 1:4000 to 1:32,000 with 3% bovine serum albumin in phosphate buffer saline (PBS, pH 7. 4), which served as blocking buffer. After an overnight incubation at ambient temperature, strips were washed thrice with PBS and further incubated for 2h with goat anti-mouse IgG conjugated to per-oxidase (1:4000) (Sigma, USA). The strips were subsequently developed with the substrate 3-amino-9-ethyl-carbazole and hydrogen per-oxidase (19).

Electropheretic typing of *Neisseria meningitidis* W135 isolates

Isolates identified as *Neisseria meningitidis* W135 and clonally confirmed by DNA fingerprinting (21, 22) were subjected to Multilocus enzymes electrophoretic mobility and defined as an electrophoretic type (ET). Standard *N. meningitidis* W135 strains from Saudi-Arabia and Norway were used as controls.

Antimicrobial susceptibility testing

The response of the *N. meningitidis* isolates to nine antibiotic discs: chloramphenicol (10μg), ciprofloxacin (1μg), cefotaxime (5μg) ceftriaxone (30μg), amplicillin (2μg) tetracycline (10μg), rifampin (2μg), penicillin G (2U) and trimethoprim sulfamenthoxazole (30μg) from Oxoid, Basingstoke, UK was investigated *in vitro* by disk diffusion assay on Iso-Sentitest agar (Oxoid, Basingstoke, UK). Minimum inhibitory concentrations (MICs) were determined using E-test strips on Mueller-Hinton agar (Oxoid, Basingstoke, Uk). Both media were supplemented with 5% whole horse blood and poured at 25ml per 90-mn plates.

In both methods, suspensions of 4 colonies from overnight plated cultures of each of the isolates in normal saline (0.85% Na_2Cl) standardized to 108 CFU/ml (0.5 McFarland standard) bacterial suspensions were prepared and inoculated onto the plates using a sterile cotton swab to achieve a confluent growth. The standard antibiotic disc (4 per plate) and E-Test strips (AB Biodisk, Sweden) (2 per plate) were then mounted on the plates with sterile forceps within 15 min of inoculation. The E-test strips contain antibiotics in multiples of

serial two-fold dilutions with concentrations ranging from 0.007 to 2μg/ml for penicillin G, ampicillin and rifampin; 0.0003 to 0.03 μg/ml for cefotaxime; 0.0007 to 0.06 μg/ml for ceftriaxone and 0.0003 to 0.012 μg/ml for ciprofloxacin. The inoculated plates were incubated for 18h in 5% CO_2 at 37°C.

Minimum inhibitory concentration (MIC) was defined as the lowest antibiotic concentration at which no visible growth of an isolate occurred. Antibiotic susceptibility level classification of the isolated *N. meningitides* strains based on inhibitory zone diameters and MIC values determined according to British Society Antimicrobial Chemotherapy guidelines (24) and breakpoints defined by the National committee on Clinical Laboratory Standards for *Neisseria gonorrhoneae* (25). Standard strains of *E. coli* ATCC 25922 *and N. meningitidis* A ATCC 13077 were respectively used as controls.

Statistical analysis

Data on isolate and case rates of the isolated *N. meningitidis* serogroups were expressed in percentages and disparity between data based on age and serogroup was analyzed using the STATISTICAL program of EPI-INFO version 2002 to obtain chil-squares (χ^2) values with Mantel-Haenszel modification. A probability (P) values less than 0.05 was considered to be significant.

RESULTS

A total of forty-five CSF samples from clinical cases of epidemic meningitis (male 57.8% versus female, 42.2%; P > 0.05) were cultured and 33 yielded positive bacterioscopic results, which was neither sex nor age dependent (57.6 vs. 42.4%, P> 0.05). Thirty-seven (82.2%) of these patients were not hajj pilgrims, while 35.6% had contact history with hajj pilgrims. The percentage of patients with a single dose vaccination history was found to be 13 (28.9%) (Table1). The isolated *N meningitidis* strains belonged to serogroups A (n=17), B (n=3), C (n=8), and W135 (n=5) with proportion of A (51.5%) significantly higher than those of other serogroups (P<0.05) (Table 2).

Distribution of these isolates by age, showed their occurrence in all age but with incidence highest among the 0-5 year olds (12, 36.4%) and lowest in patients aged ≥ 30 years (4, 12.1%) (Table 3).

The results presented in Table 4 shows that *Neisseria meningitidis* serptype 2a occurred most frequently with an incidence rate of 48.5% compared to 18.2% due to serptype 4 (P=0.09) 33.3% non-typable cases (P=0.13).

Serosubtype profiling of the isolates further revealed 19 (57.6%) P1.5,2 strains, 5 (15.2%) each of P1.7,1 and NT strains and 2 (6.0%) each of P1.14 and P1.9 strains with P1.5, 2 significantly (P<0.05) occurring mostly compared to other serosubtypes (Table 5).

Multilocus enzymes electrophoresis and DNA fingerprinting further indicated that the W135 isolates are members of the electrophoretic type 37 clonal complex (ET-37) with two of the strains eliciting two bands not found in the Norwegian and Saudi-Arabia isolates (Table 6). Five (152%), 6 (18.2%) and 7 (21.2%) of the 33 culture - positive isolates displayed resistance to ampicilliin and chloramphenicol and intermediate resistance to penicillin (Table 7). Resistance pattern characterization further revealed mono-resistance to trimethroprim-sulphamethazole (TMP) by 20 isolates and multi-resistance with equal predominance (2 each) of patterns:

Pen^{i}AmpChlTMP, Pen^{i}AmTMP, AmpTMP covering all the serogroups (Table 8).

Stratification of the isolated *Neisseria meningitidis* serogroups based on β-Lactamase expression revealed significance (P<0.05) for A (35.3 vs. 64.7%) and C (12.5 vs. 87.5%) respectively.

Three of five W135 strains were β-lactamase positive, while enzyme expression was not observed among the B isolates (Figure 1).

Table 1. Socio-Demographic characteristics of clinical cases with meningococal infections during 2003 epidemic in Jigawa State, Nigeria.

Parameters	Outcome
Sample size n (%)	45(100)
Sex	
Male	26(57.8)[b]
Female	9(42.2)
Age rage (mean age) in years	
Male	0.5 – 34 (14. 0 ± 8.6)
Female	.8 - 38 (14.3 ± 9.5)[b]
Cultural positive males n (%)	
Males	19 (57.6)[b]
Females	4 (42.2)
Total	33 (100)
Hajji Travel n (%)	
Yes	8 (17.8)
No	37 (82.2)[a]
Pilgrims contact history n (%)	
Yes	16 (35.6)
Unknown	29 (64.4)[a]
Vaccination History	
One	13 (28.9)
None	32 (71.1)[a]

Data are presented as number (%) and mean + SD. Differences between percentages were analyzed by Chi-square (χ^2) statistics with Mantel-Haenszel modification.
Disparity between the mean ages was analyzed by student's t-test. P< 0.05 was considered significant. P< 0.05; [b]P>0.05.

Table 2. Sero-group analysis of *Neisseria meningitidis* isolates recovered from CSF cultures during a 2003 meningococcal outbreak in Jigawa state, Nigeria.

Sero-group	N (%)	χ^2 (Mantel-Haenszel)	P
A	17 (51.5)	-	-
B	3 (9.1)	3.9	$2.0 X10^{-4}$
C	8 (24.2)	5.1	0.02*
W135	5 (15.2)	9.7	1.9×10^{-3}
Total	33 (100)	-	-

χ^2 (Mantel-Haeszel) =0.06; P = 0.8 (sero-group A vs other serogroups combined RR = 1.06 (0.66< RR > 1.72)

N (%) = Number (percentage) of cases.

The number of *N. meningitidis* serogroup A cases compared to other serogroups individually or in combination was analyzed using χ^2 statistics with Mantel-Haenszel modification. RR = Relative risk at 95% confidence limits. *P < 0.05.

Table 3. Age Distribution of *Neisseria meningitidis* serogroups recovered from CSF cultures during a 2003 meningococcal outbreak in Jigawa state, Nigeria.

	Serogroups (N = 33)				
Age group (y)	A	B	C	W135	Incidence (%)
0 – 5	8	0	3	1	12 (36.4)
6 – 12	3	1	2	1	7 (21.2)
13 - 19	2	1	1	1	5 (15.15)
20 – 29	2	0	1	2	5 (15.15)
≥ 30	2	1	1	0	4 (12.1)

NOTE: Figures in parentheses are percentage of the total number of culture positive cases. (N = 33).

Table 4. Serotyping of the recovered epidemic *Neisseria meningitidis* isolates in Jigawa state, Nigeria in 2003.

Serogroup / Serotype	A	`B	C	W135	Total	P
2a	8	2	3	3	16 (48.5)	-
4	3	1	2	0	6 (18.2)	0.09
NT	6	0	3	2	11 (33.3)	0.13

Data are presented as number of cases with percentages in parentheses. Disparity between percentages was analyzed by χ^2 – test with Mantel-Haeszel modofication.

P = Exact probability value (Serotype 2a vs Serotype 4 or NT). NT = Nontypable.

Table 5. Serosubtyping of the recovered epidemic *Neisseria meningitidis* isolates in Jigawa state, Nigeria in 2003.

Serogroup / Serosubtype	A	B	C	W135	Total	P
PI. 5,2	9	1	4	5	19 (57.6)	-
PI. 7,1	3	0	2	0	5 (15.2)	3.8×10^{-4}
PI. 14	1	1	0	0	2 (6.0)	8×10^{-6}
PI. 9	1	1	0	0	2 (6.0)	8×10^{-6}
NT	3	0	2	0	5 (15.2)	3×10^{-4}

Data are presented as number of cases with percentages in parentheses. Disparity between percentages was analyzed by χ^2 – test with Mantel-Haeszel modofication.

P = Exact probability value (PI. 5,2 vs other serosubtypes or NT). NT = Nontypable.

Table 6. Serotyping, subtyping and comparative analysis of *Neisseria meningitidis* W135 isolates from Nigeria.

Code	Serotype	Subtype	Clonal complex		
			Saudi- Arabia	Norway	
Jigawa – 005	2a	PI. 2, 5	ET-37	+	?
Jigawa – 013	2a	PI. 2, 5	ET-37	-	-
Jigawa – 016		PI. 2, 5	ET-37	+	-
Jigawa – 019		PI. 2, 5	ET-37	+	-
Jigawa – 021		PI. 2, 5	ET-37	-	-

NT = non-typable, PFGE@ = Pule –field gel electrophoresis banding pattern interpretation and comparison with Saudi Arabia and Norwegian *N. meningitidis* W135 isolates.

+ = Indistinguishable

- = Distinguishable

? = Unclear

Table 7. Antibiotic resistance patterns of the recovered *Neisseria meningitidis* serogroups during a 2003 meningococcal outbreak in Jigawa state, Nigeria.

Neisseria meningitidis	Resistance patterns^	Frequency#
Serogroup A	Pen^{i} Amp Chl TMP	2
	Pen^{i} Amp TMP	2
	Amp TMP	1
	TMP	12
Serogroup B	Pen^{i} AmpTMP	1
	TMP	2
Serogroup C	Chl TMP	1
	Am TMP	2
	TMP	5
Serogroup W135	en^{i} AmpTMP	1
	Pen^{i} Amp Chl	1
	Amp TMP	2
	TMP	1

^{i}Intermediate; Pen = Penicillin; Amp = Ampicillin; TMP = Trimethoprim sulmethoxazole; Chl = Chloramphenicol; RR = Relative risk; $P < 0.05$ = significant; ^Antibiotic resistance pattern was based on in vitro response of the isolates to the antibiotics tested by disk diffusion assay.

#Number (%) of isolates with multiple antibiotic resistance phenotype = 13 (39.4); P = 0.09. [RR = 0.65 (0.39 <RR> 1.08)] at 95% confidence limit.

Table 8. Minimum Inhibitory Concentrations (MIC) of antibiotics against the recovered *Neisseria meningitidis* serogroups during a 2003 meningococcal outbreak in Jigawa State, Nigeria.

Antibiotics	MIC (μg/ml) Range	50%	90%	S	I	R
A Serogroup (n=17)						
[a]Penicillin G	0.06 – 0.75	0.25	0.50	13 (76.5)	4 (23.5)	0
[b]Ampicillin	0.06 > 1	0.25	0.75	12 (70.6)	0	5 (29.4)
Cefotaxime	≤0.003 – 0.015	0.003	0.007	17 (100)	0	0
Ceftiaxone	0.003 – 0.007	0.003	0.007	17 (100)	0	0
[c]Chloramphenicol	0.25 - 2	0.25	0.512	15 (88.2)	0	2 (11.8)
TMP	128 ≥ 256	256	>256	0	0	17 (100)
Ciprofloxain	< 0.002 – 0.004	0.002	0.004	17 (100)	0	0
Serogroup B (n= 3)						
[a]Penicillin G	0.06 – 0.5	0.12	0.25	2 (66.7)	1 (33.3)	0
[b]Ampicillin	0.06 > 1	0.25	0.50	2 (66.7)	0	1 (33.3)
Cefotaxime	≤0.003 – 0.007	0.003	0.007	3 (100)	0	0
Ceftiaxone	0.003 – 0.007	0.003	0.007	3 (100)	0	0
[c]Chloramphenicol	0.25 – 0.512	0.25	0.512	3 (100)	0	0
TMP	64 – 256	128	256	0	0	3 (100)
Ciprofloxain	< 0.002 – 0.004	<0.002	0.004	3 (100)	0	0
Serogroup C (n=8)						
[a]Penicillin G	≤ 0.06	< 0.06	0.06	8 (100)	0	0
[b]Ampicillin	0.06 – 0.25	0.06	0.12	6 (75)	2 (25)	0
Cefotaxime	≤0.003 – 0.015	0.003	0.007	8 (100)	0	0
Ceftiaxone	0.003 – 0.007	0.003	0.007	8 (100)	0	0
[c]Chloramphenicol	0.12 - 2	0.25	0.512	7 (87.5)	0	12 (12.5)
TMP	128 ≥ 256	256	>256	0	0	17 (100)
Ciprofloxain	< 0.002 – 0.004	0.002	0.004	8 (100)	0	0
Serogroup W135 (n=5)						
[a]Penicillin G	0.06 – 0.75	0.25	0.50	3 (60)	2 (40)	0
[b]Ampicillin	0.06 > 1	0.5	> 1	1 (20)	2 (40)	2 (40)
Cefotaxime	≤0.003 – 0.015	0.003	0.007	5 (100)	0	0
Ceftiaxone	0.007 – 0.015	0.003	0.007	5 (100)	0	0
[c]Chloramphenicol	0.25 - 4	0.25	2	3 (60)	0	2 (40)
TMP	128 ≥ 256	256	>256	0	0	5 (100)
Ciprofloxain	< 0.002 – 0.004	0.002	0.004	5 (100)	0	0

MIC = Minimum Inhibitory Concentrations; **S, I, R** = Sensitive, Intermediate and Resistant cases. Figures in parentheses represent percentage of cases per serogroup. [a] Total penicillin intermediate cases = 7/33 (21.2%); [b]Total ampicillin resistant cases = 6/33 (18.2%); [c]Total chloramphenicol resistant cases = 5/33 (18.2%).

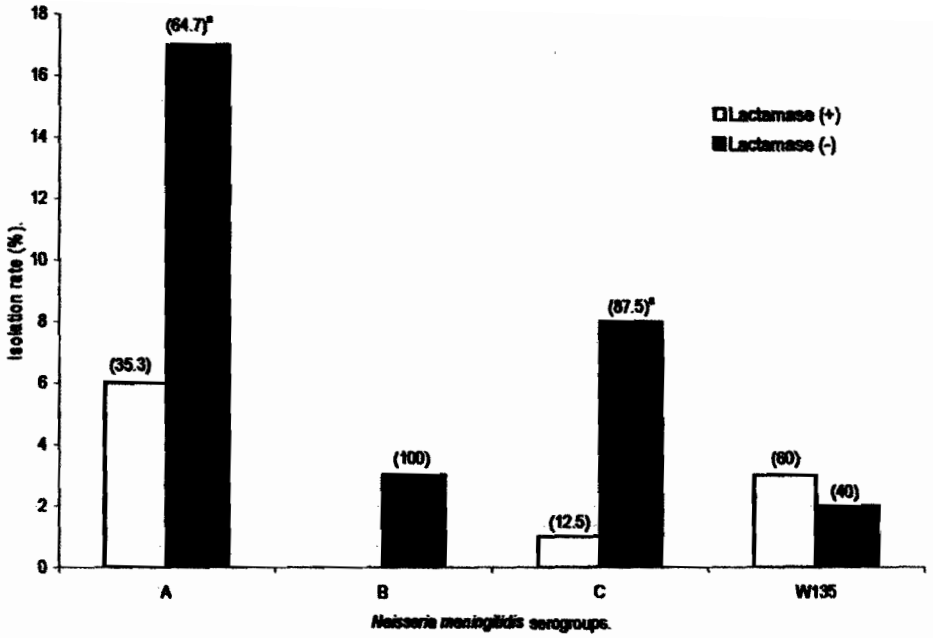

Figure 1. β-lactamase expression by *Neisseria meningitidis* isolates recovered from CSF samples of patients during a 2003 meningococcal outbreak in Jigawa State, Nigeria. Figures in parentheses indicate percentage of cases. *P < 0.05 (Lactamase (+) vs. (-), $\chi 2$ - test.

DISCUSSION

Neisseria meningitis outbreaks have substantially become a cause for hospitalization with increasing fatalities in northern Nigeria where 15 – 20% of 26 million people are affected (9.14). The present study has revealed the contribution of *N. meningitidis* serogroups B and W135 to epidemics in Jigawa state with a population close to 3 million people (9). Previous epidemiologic reports in northern Nigeria have mostly implicated serogroups A and C as aetiologic agents (6, 25). While serogroups B strains are mostly recovered from nasopharyngeal, vaginal and anal swab cultures among carriers in eastern, western and northern Nigeria (26). Odugbemi *et al* (27) only reported serogroup W135 as a nasopharyngeal isolate among school children carriers in Ijede, Lagos.

Meanwhile, *N. meningitidis* serogroups B and W135 as epidemic pathogens have been reported in several African countries within the meningitis belt. Taha *et al* (11) reported equal prevalence of serogroups W135 and A among meningitis cases occurring at the end of 2001 epidemics in Burkina Faso and Niger where 1,813 deaths from 13,039 cases and 595 deaths from 7906 cases were respectively recorded. Similarly in Gambia, a study conducted between 1990 – 1996 showed that 6 of the 14 *N. meningitidis* isolates recovered were of serogroup W135 (12). The serogroup B isolates have also been identified as epidemic strains during epidemics in Cameroun (10), Senegal (13), Tunisia (28) and recently in Egypt (29).

Although the B and W135 serogroups identified in this study did not occur equally as serogroup A, their emergence has tremendous public health implications. Firstly, we found our W135 strains

similar to clones responsible for 2000 and 2001 epidemics in Saudi Arabia, Niger and the Gambia (30, 11, 12) since they belong to the clonal ET-37 complex. They also show diversity by serotyping similar to previous findings (31). Secondly, serogroup B-associated deaths have been found to occur frequently in sporadic, endemic and epidemic cases in the USA and Brazil (32,33). Furthermore, there is also a strong possibility the meningococcal diseases caused by serogroups A and C has undergone significant evolution in the study area. This is due to the observed display of antigenic heterogeneity by our isolates, which elicited 2 – 4 distinct subtypes comprising the predominant P1.5, 2 and other antigenic determinants that have not been reported in northern Nigeria. The latter, 4:P1.9 have also been found to contribute to meningococcal outbreaks in the neighouring West African countries such as Cameroon, Senegal and Niger (10,34, 11).

The isolation of *N. meningitidis* strains in all the age groups but with highest and lowest incidence rates in age groups 0-5 years and > 30 years respectively-year age group implies that children carry the greatest risk, while other age groups are also susceptible to of meningococcal meningitis in the study area. Our observations are in consonance with previous epidemiologic reports in northern Nigeria and other countries within the African meningitis belt (5,6,8). Our results have also provided a strong indication that the age pattern of meningococcal meningitis in northern Nigeria has not changed since the recurrence of epidemics in 1996. An epidemiologic survey by Blakebrough *et al* (3) had previously reported children as frequently the first carrier of meningococci in northern Nigeria households and Hassan-King and Greenwood (35) revealed structural and phenotypic similarities between carriage and invasive strains of *Neisseria meningitidis* serogroups A. The latter indicates the possible use of meningococcal carriage as a predictor of an epidemic in a northern Nigerian Community. A further support to this possibility is the observed disparity in carriage rate between Bornu, an epidemic prone area and Anambra, a non-meningococcal epidemic zone in Nigeria reported by Gugnani and Uganabo (26). The high number of cases in ≤ 5 year old may be related to the well-documented roles played by pre-school children in the establishment of endemicity of *N. meningitidis* in disease susceptible populations (36). Loss of transpacement antibodies from 8 weeks of postnatal life, school attendance, smoking, poverty, malnutrition and enormous body contacts in camps, prisons and among people with low socio-economic status have several been implicated as factors of susceptibility to meningococcal infections in most affected populations including northern Nigeria (32, 33,37, 38).

Contacts with clinical cases of W135 meningococcal disease and asymptomatic carriers have been found to play a significant in the spread and intensification of epidemics (39). This possibility may also exist or now emerging in northern Nigeria, since in this 35.6% of cases from patients with pilgrims' contacts history were found. Meanwhile, symptomatic meningococcal infections a medical emergency and thus requires prompt diagnosis and treatment with antibiotics. Diagnosis, which is primarily based on bacterioscopy may fail due to low bacterial count in culture, limited sample sources and initial antibiotic use. In the work of Antignac *et al* (40) 10 out of 29 samples cultured and subjected to bacterioscopy yielded positive results. While

Rosentein *et al* (32) observed disparity in culture positivity by CSF, pleural fluid, blood and nasopharynx when used as sample sources. It is therefore not surprising that 33 of the 45 CSF samples analyzed in this study were culture positive. The non-reliance on one sample source and the use of molecular methods of diagnosis based on species and serotype DNA and capsular antigens identification have been found highly useful in optimizing detection of meningococci in clinical specimens from suspected patents and instituting treatment with better prognosis (40, 41). That some of our isolates were multidrug resistant displaying non-susceptibility to penicillin, cotrimoxazole, rifampin, penicillin and chloramphenicol may not be unconnected with indiscriminate use of these antibiotics in the study area. In most Nigerian hospitals and community medicine stores, ampicillin, penicillin, cotrimoxazole and chloramphenicol are among the commonly dispensed and sold antibiotics (42). The empirical use and abuse of these drugs have been found as the driving force for the spread and endemicity of multidrug resistant meningococcal diseases (43). In a few of the neighbouring states where surveillance of meningitis have been conducted, 30 – 60% resistant cases to cotrimoxazole, 20 – 40% resistant cases to ampicillin and chloramphenicol and 10 – 32% of *N. meningitidis* showing reduced susceptibility to penicillin were reported (Angyo and Okpeh, 1998; Emele *et al*, 1999; Guagnani and Uganabo, 1989). Whether the *in vitro* susceptibility result would correlate to the clinical efficacy of these antibiotics remains grossly unclear. Akpede *et al* (46) reported only 2 cases of treatment failure in patients whose isolates displayed in vitro resistance to chloramphenicol. Nevertheless, the drug resistant patterns found in our isolates connote an increased trend of antibiotics resistance by *Neisseria meningitidis* strains in Jigawa and informs the need to modify anti-meningococcal drug policy in the area. In areas where drug switch to third – generation cephalosporins for meningococcal disease management has been effected owing to endemicity of strains with pen and ampicillin resistance phenotypes, improve treatment outcomes have been observed (47). Therefore, there is a strong possibility of obtaining similar clinical outcome in Jigawa since none of the isolates tested showed resistance to cefotaxime and ceftriaxone. Furthermore, all the isolates were susceptible to ciprofloxacin, which agrees in toto with previous reports on the response of African *Neisseria meningitidis* to fluoroquinolones in vitro and in vivo (5, 26, 27, 44, 45) contrary to recent observations in Spain (48).

From the pharmacokinetic viewpoint, ceftriaxone and cefotaxime have been found to attain clinically achievable concentrations, 8 – 100 fold greater than their MICs in the CSF and elicit greater bactericidal potency than ampicillin-chloramphenicol combination when administered intravenously at 80mg/kg per day to patients with bacterial meningitis (49, 50).

Studies on the molecular basis of penicillin resistance have implicated acquisition of altered penA gene, which encodes the penicillin binding protein – (PBP2) with polymorphism within the peptidase loci (51). Expression of β- lactamase enzymes as the basis of resistant to β-lactam drugs by neisseria has been reported in a very few number of isolates (40). In the present study, we observed β-lactamase production among isolates that exhibited resistance to ampicillin and intermediate resistance to penicillin in a manner that was independent on serogroup, serotype and serosubtype characteristics. Capsular switch

mediated by mutation, transformation and genetic exchange is often the basis for serotype and serosubtype diversity within *Neisseria meningitidis* serogroups and has been found to be driven by drug resistance (40,51). This scenario is crucial to the efficacy of meningococcal vaccine as an epidemic prevention tool and a strategy for conferring 'herd' immunity in a susceptible population (52). The northern Nigeria is an epidemic prone area and multiple bouts of meningitidis outbreak have been reported after the 1996 episode despite the interventions with vaccines. To the best of our knowledge, monovalent A and bivalent AC vaccines are the two available vaccines in Nigeria. The serogroup, serotype and serosubtype profiles of our isolates provides a strong indication that either of these vaccine could halt epidemics in Jigawa as reported in the 1980s and other African countries where epidemics is solely caused by the A and C serogroups (3, 6, 8, 53).

The isolation of W135 serogroup in this study further suggests the need to employ other meningitis vacciunes for improved serogroup coverage and greater efficacy. Such vaccines include the ACYW135 quadrivalent vaccine, which is now being used in some African countries (1, 28, 29). Furthermore, the serosubtype diversity observed among the A and C serogroups supports the use of new generation bivalent AC vaccines in which the A and C capsular antigenic mixture is conjugated with non toxic mutant Diphtheria toxin or tetanus toxoid to induce immunologic memory lacked by previous vaccines (38, 52). Administration of these vaccines in some countries within and outside Africa has been found to halt epidemics, reduce carriage rate and reduce the risk of infection in non-immunized population (i.e. 'herd' immunity) (52). Although, the immunogenicity and duration of protection of these vaccines are still very low in children compared to older age groups, their administration in repeated doses every 2 – 4 years in the former has been recommended (25). The emergence of serogroup B strains as an epidemic agent in Jigawa is a cause for concern from immunization viewpoint. This is because the development of B vaccine is still ongoing and a few clinical trials conducted on the vaccine have unanimously revealed its zero and very poor immuniogenicity in children and adults respectively (54). Outer membrane protein (OMP) and vesicles (OMV) are currently been exploited as vaccine candidates and their constructs have been found eliciting IgG1 and IgG3 protective immunity among volunteers in the Netherlands (38). In summary, the results of the present study have revealed changing trend of epidemic meningitis in Jigawa with the emergence of serogroups B and W135 as agents of meningococcal outbreak in the state. Serotype and serosubtype diversity exhibited by these epidemic isolates warrant a continuous need to optimize antibody therapies against meningitis in all age groups coupled with the introduction and use of new meningitis vaccines to augment the existing ones. These approaches would go a long way in reducing morbidity and mortality associated with *Neisseria meningitis* in northern Nigeria.

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