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

*Uwah, Asukwo; Iwalokun, Bamidele[2]; Badaru, Gbaruwaju[1]; Gbadegesin, Yemi[1];

Oyanuga, Bunji[1], Ariyo, Fatai[1].

[1]Central Public Health Laboratory P.M.B. 2010 Yaba – Lagos. Nigeria., &
[2]Department of Biochemistry Lagos State University P.M.B. 1087, Apapa – Lagos Nigeria.

*Correspondence to Uwah, AU Federal Ministry of Health National AIDS & STIs Control Programme Edo House (2nd Flood) 75 Ralph Shotende Street Central BusinessDistrict Abuja - Nigeria. (Phone-+234-8023639146). E-mail: uwah659@yahoo.com 4 – 08 – 2005

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. meningitidis* A = 8; B = 2; C = 3; W135 = 3) were of serotype 2a, while 4 distinct serosubtypes: P1.5, 2 (37.6%), 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
Anogenital 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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Benz Adegboye, MD
Editor

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, colistin 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).

Electrophoretic 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), ampicillin (2μg) tetracycline (10μg), rifampin (2μg), penicillin G (2U) and trimethoprim sulfamethoxazole (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-mm 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^{0}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 gonorrhoeae* (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	$1.9 x 10^{-3}$
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%).

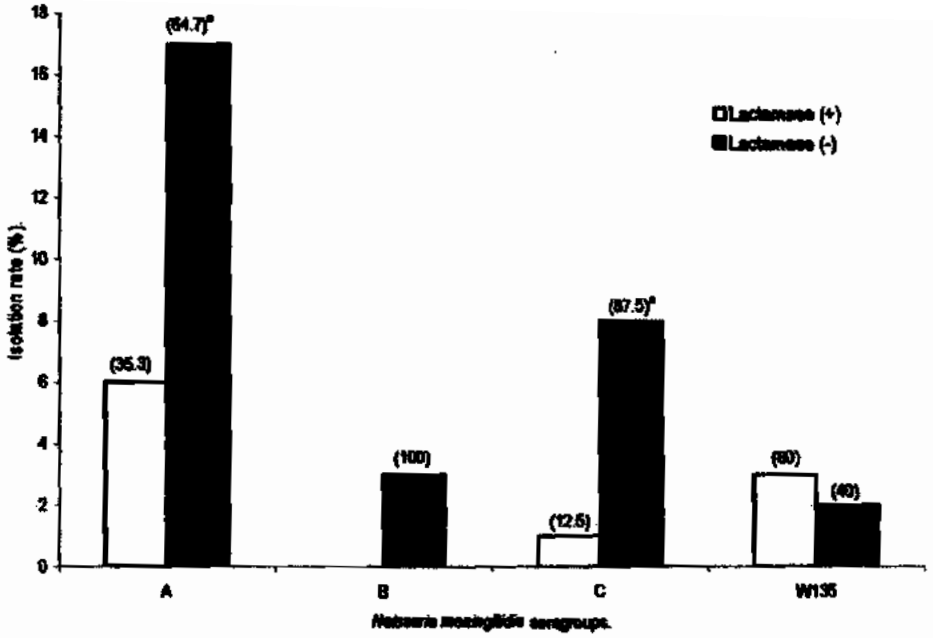

Figure 1. β-lactamase expression by *Neisseria meningitidis* isolates recovered from CSF samples of patients during a 2003 meningococcal outbreak in Jigawa State, Nigeria. Figures in parentheses indicate percentage of cases. *P < 0.05 (Lactamase (+) vs. (-), $\chi 2$ - test.

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

Rosenstein *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 patients 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; Gwagwani and Ugwabo, 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 vaccines 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 immunogenicity 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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EVALUATION STUDIES OF SOME MEDICINAL PLANT EXTRACTS AND FUNGICIDES AGAINST *ALTERNARIA SOLANI*.

Phalisteen S*, Ishaq S, Amardeep K, Arif J and Sami S

Department of Microbiology, IIIM, (CSIR)-Sanatnagar, Srinagar-190005. (Formerly, RRL)

*Division of Microbiology, Indian Institute of Integrative Medicine (CSIR)– Sanatnagar Srinagar – 190005
Email: *shakir21@rediffmail.com* Tel.: + 91-94-2431253

ABSTRACT

Alternaria is a polyphagus fungus that occurs frequently on dead and decaying organic material and is responsible for causing leaf spot disease. In Indian subcontinent, there are different varieties of plants showing antimicrobial and other medicinal properties which can be employed in plant disease management to reduce the chemical load from the environment. The present investigation has been taken to evaluate the effect of medicinal plant (leaf) extracts and their combination with fungicides (Carbendazim) against radial growth of *Alternaria solani*.. The fungus was collected from infected leaf spots of potato plant and grown in PDA (potato dextrose agar) media. The water extracts of medicinal plants viz. *Azadirachata indica* (3%), *Calotropis procera* (3%), *Nerium oleander* (3%), *Ocimum sanctum* (3%) fungicide, carbendazim (1%) and their combinations were prepared. On the basis of results, the medicinal plant extracts Neem (3%), Nerium (3%) added with carbendazim (1%) showed maximum inhibition of fungus (*Alternaria solani*).

Key words: Polyphagus, radial growth, *Alternaria solani*, medicinal plant extracts and potato dextrose agar.

INTRODUCTION

Agriculture is the inseparable and integral part of Indian economy. As largest private enterprise, Indian agriculture contributes nearly quarter of the national GDP (Bureau of Statistics), sustain livelihood of more than 60% of population and is the backbone of agro-based industry (1). Since 1950, the productivity gained by nearly 3.3 times in vegetables(2). Vegetables constitute an important item of human diet, vegetables are richest source of protein and vitamins. A large number of vegetables are grown in India. A strong vegetable sector will lead to economic growth throughout the country. India is the largest producer of vegetables in the world (3), Surpassed only by China. In 2002, India has produced 78.2 million tons of vegetables from 5.73 million hectares of land (4). Indian farmers grow about 175 different types of vegetables. Among these, potato, tomato, onion, cabbage and cauliflower account for 60% of total production. It is projected that the domestic vegetable requirement rises from current 83-91 million tons to 151-193 million tons by 2030 (5).

India is the major exporter of vegetables; exporting worth approx. $245 million of vegetables annually (10th in the world) (6). Indian vegetables were restricted to potatoes and fresh onions. But now the government of India sees great opportunities for extending export of several other vegetables including dettuce, fresh peppers, tomatoes, squash and gherkins. Agriculture and its

allied sciences aim to maximize the production of plants for food fibers, building material, fuel and some essential drugs etc. Various groups of pathogens (bacteria, fungi, viruses and insects) are known to cause losses to agricultural yield allover the world including India. In India total loss due to pest, diseases and weed is approximately 18% of our total production or Rs 5000 crores annualy (7).There are many methods being presently used for pest control as cultural, biological, physical and chemical etc.(8). Among these the chemical control has been most useful and widely adopted strategy and has been yielding phenomenal results in reducing the disease severity. Excess use of these chemicals resulted in degradation of environment and cause ecological imbalances viz. accumulation of high amount of residues in food material and natural resources, depeletion of nutrients in the soil etc. Hence, there is a need of judicious use of such chemicals with suitable alternatives which are eco-friendly and leave no residue and are thus safe for mankind. Among the several methods available to control pests, plant based alternative strategy has been found to offer promising results. Hence, botanical pest control is gaining importance and recognition as a possible alternative and practical method to control diseases of many crop plants.

Potato, a vegetable crop is widely adopted for cultivation in India. The potato has been cultivated in India since its introduction in early part of the seventeenth century by English or portguese. It is grown from October to February. At the inception of CPRI in 1999, India used to produce 1.54 million tons of potato out of 0.234 million hectares with an average yield of 6.58 tons/hac from 1999-2001(9). India has produced 23.63 million tones of potatoes from 1.29 million hectares of land with an average of 18.23/ hac. In north Indian plains, potatoes can be grown successfully almost around the year. According to the projections of international food policy Research Institute (IFPRI) and International Potato Centre (IPC), world demand for potatoes shall increase by 40% upto 2020 (8). In potato cultivation several diseases infects the crop, black wart disease of potato, leaf spot of potato, early blight of potato caused by *Alternaria solani*. *A. solani* is an important constraint in cultivation of vegetables (10). Use of plant products or extracts was explored to control various diseases and mycoplasma etc, antifungal effects of Neem and other plant products or extracts are used in management of plant diseases caused by *Alternaria sp.* Earlier it has been reported that volatile component of crude aqueous extracts of garlic bulbs inhibits the germination of micro conidia and hyphal extension of *Fusarium oxysporum* and *Alternaria zinniae* in culture(11).

The present study was conducted to evaluate the antifungal effect of extracts of common weed *Calotropis procera* (Madar), *Azadirachta indica* (Neem) *Nerium oleander* (Kanaer) and *Ocimum tenuiplorum* (Tulsi).

Azadirachta indica is found throughout India. The leaves, bark, seed and flower are bitter, astringent, acrid, refrigerant, depurative and toxic. It is also found effective against soil borne pathogens such as *Fusarium oxysporum* and some other fungi(12). *Calatropis procera* Linn belongs to the family Asclepiadaceae and found throughout dry waste places in India. *Nerium oleander* belongs to the family Apocynaceae, cultivated throughout India. Having leaves in a whorl, shortly. stalked linear, down green and shiny above. Leaves are powerful repellants and are used for scabies and hemorrhoids. *Ocimum tenuiflorium* ,(*Ocimum sanctum*) also known as

holy tulsi. It is distributed all over India. An erect branched softly pubescent under shrub, 30-60cm in height with red or purple sub-quadrangular branches. Leaves are simple, opposite elliptic oblong or acute entire, serrated or dentate, pubescent on both sides minutely gland dotted, petiole slender & hairy. The plant is bitter, acrid, aromatic, stomachic, demulcent, diaphoretic, digestive, vermifuge and alexetonic. Bavistin is a product name of carbendazim. Carbandazim is the common name of Methyl-2-bendzimidazole carbamate. Bavistin have been applied for seed treatment, soil drench, soil mix and foliar spray. It is also used to control powdry mildew of apple-scab, brown rot of stone fruit by foliar spray. The present study was undertaken with the objectives, To study the effect of medicinal plant (leaf) extracts against growth of *Alternaria solani and* to evaluate effect of combined ability of medicinal plant extracts with carbendazim on *Alternaria solani*.

Materials & Methods

The glasswares, conical flasks, Petri dishes, pipettes and test tubes were thoroughly washed and dried. Petri dishes and pipettes were sterilized in hot air oven at 160°c for 4 hours. For isolation of fungus, potato dextrose agar (PDA) media was used. The procedure adopted for the preparation of media is based on the method of Rickian, 1936. Peel and boil the potato with 500ml water in a pan. After boiling, filter through cloth in another vessel and add agar at the rate of 1.5 – 2%. After boiling for sometime, add dextrose, boil again for some more time and make volume upto 1000ml and adjust pH to 6.5. The media was sterilized at 121° C for 20 mins in the autoclave.

Isolation of fungus

Infected plant material (leaves) having typical leaf spot symptoms of *A. salani* were collected from the nearby field. Leaves were examined under the microscope to confirm the presence of pathogen *Alternaria*. The infected parts of the leaves (Fig.1) were cut into small pieces, surface sterilized with 0.1% mercuric chloride solution for 30 seconds, washed three times by distilled H_2o and transferred onto Petri plates containing solid PDA (Potato dextrose agar) media. The inoculated plates were incubated at 25 °c for 4-6 days. The characteristic features of *Alternaria* are production of beaked, pigmented, conidia with relatively thin transverse and longitudinal septa. The pathogen *Alternaria* has septate, dark colored mycelia and produces short erect conidiophores that bear single or branched chains of conidia (Fig.2). The *Alternaria spp.* was purified from isolated dishes and maintained by periodic sub-culturing on PDA slants after every 15 days. The fungus was treated with T1, (Neem leaf extracts 3%), T2 (Madar leaf extracts 3%), T3 (Kaner leaf extract 3%), T4 (Tulsi leaf extract 3%), T5 (Carbendazim 1%), T6 (Neem 3% + Carbendazim 1%), T7 (Madar 3% + Carbendazim 1%), T8 (Kaner 3%+Carbendazim 1%), T9 (Tulsi 3%+Carbendazim 1%) and T_0 (control).

Preparation of plant extracts

Fresh leaves of medicinal plants were collected from nearby research field. 200 grams of leaves of each plant species were taken in 100ml beaker crushed with the help of mixer and filtered the extract in test tubes. 1% of fungicide (carbendazim) was prepared by weighing the required amount of fungicide and dissolve in 100 ml distilled water. The stock solution prepared

was used for making the required concentration by serial dilution method.

Inoculation of test organism

Arrange the sterilized petri plates such that each treatment was replicated 3 times. The measured quantities of stock solution were added to the petri plates to make resultant dilution and the treatment without any addition was considered as control. When the media was solidified, then with the help of sterilized cork borer, dishes of 0.3cm diameter from actively growing fungal cultures were cut and placed in centre of each dish containing the poisoned food (extracts). Inoculated petri plates were incubated at 25°c and the radial growth of colonies (in cms) were measured after 4 days of inoculation. The F-test as suggested by Fischer and Yartes was used to determine differences.

Results

Effect of treatments

The radial growth of fungus was measured against control and Neem extract in combination was found to have significant inhibitory effect on the growth of fungus. The order of their effectiveness was, T1>T8>T3>T9> T5>T6>T2>T7>T4>T0. (Fig.3,4,5,6) However after the 96 hours of the treatment T1 (Neem leaf extract 3%) and T8 (Kanaer (3%) + carbendazim 1%) were recorded equally effective in inhibiting the radial colony growth of test fungus. The effectiveness was recorded in this order, T1>T8>T9>T5>T6>T3>T2>T7.T4>To (Table 1).

Effect of period

The maximum inhibition of mycelial growth was found after 96 hours in comparison to 48 hours. The mean radial growth after 96 hours of treatment was 1.843. But time periods did not significantly effect the radial growth of fungus *Alternaria solani*.

Azadirachta indica extracts inhibits the radial growth of *A. solani* after 48 hours of treatment in comparison to carbendazim (1%) in combination of extracts. However, after 96 hours the most effective combination was T1 and T8 in comparison to control.

Table 1: Mean radial growth of *A.solani* after 48 hours and 96 hours of treatment.

Treatment	Time		
	48 hours	96 hours	Mean
To	3.30	5.70	4.50±1.2
T1	1.20	1.25	1.28±0.02
T2	1.90	2.23	2.07±0.16
T3	1.28	2.02	1.68±0.37
T4	2.50	2.80	2.65±0.15
T5	1.50	1.60	1.55±0.05
T6	1.80	1.97	1.89±0.08
T7	2.40	2.50	2.45±0.05
T8	1.25	1.25	1.25±0.01
T9	1.30	1.37	1.33±0.03

Figures

in leaves.

Alternaria solani.

Calotropis 3% (T2).

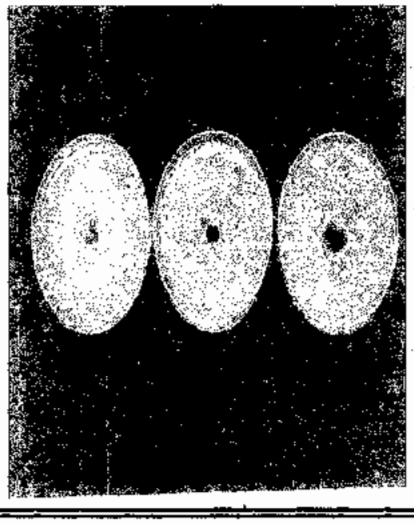

Fig.4. Control (To) compared with Nerium 3% (T3) and Ocimum 3% (T4).

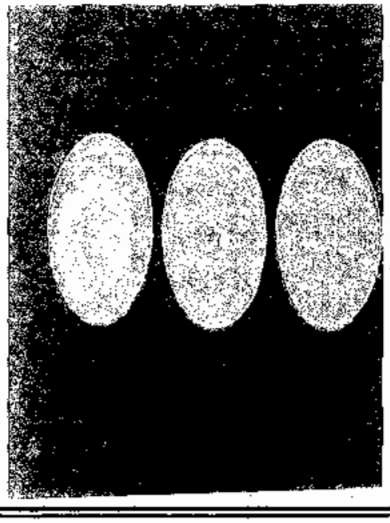

Fig.5. Control (To) compared with Neem 1% (T1) and Bavistin 1% (T5).

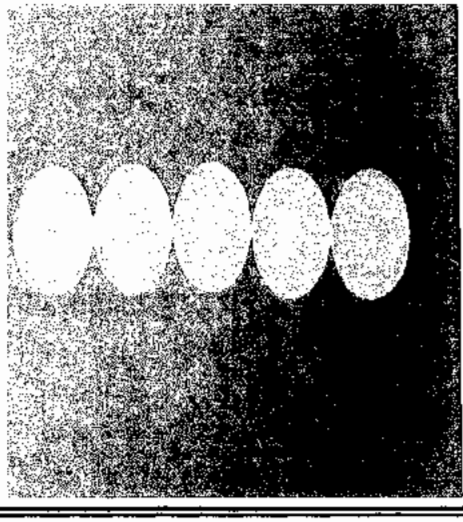

Fig.6. Control To compared with Neem 3% + Bavistin 1%, T6, Calotropis 3%+ Bavistin 1% and T7,Ocimum 3% .

DISCUSSION

The effect of leaf extracts of *Azadirachta indica, Calotropis procera, Nerium oleander, Ocimum sanctum,* fungicide, carbendazim and their combination on *Alternaria solani* were studied. The results of the present study highlighted that Neem extract in combination with carbendazim (1%) and Nerium (3%) were effective in reducing the growth of *Alternaria solani* as compared to control (13). Carbendazim alone was also effective in achieving the better results over *Alternaria solani*. The results of the experiment were in agreement with the results obtained (14), by using the fresh plant species of *Allium cepa, A. sativum, A. indica, Calotropsis procera, Datura stremonium* and *Ocimum*

sanctum, Polyalthia longifolia, Tagetus erecta, Vinca rosea and *Withania somnifera* showed antifungal properties against five pathogens *A. solani, Colletotrichum capsici, Fusarium oxysporum, Rhizoctonia solani and Sclerotina sclerotoirum* However, *A. indica, D. stramonium, Ocimum sanctum, Polyoelthia longifolia* were found to be more effective, It was also reported the seed mycoflora of chick pea was effectively controlled by plant extracts of *Azadirachta indica, Calotropis procera. Nerium oleander* compared with fungicide (Benlate, Thiram etc) against *Rhizopus nigricans, Colletotrichum dermatum* and *Alternaria solani* (15). In present study Neem extract has been found to be effective and promising botanical pesticide for further exploration and control of *A. solani*. In the present scenario there is an urgent need to develop eco-friendly, cost effective botanical pesticides as an alternative of chemical fungicides. From this study it is concluded that botanicals can be used as an alternative of fungicides in future and can be employed for sustainable agriculture. It is also evident that botanicals like Neem, Kaner and Madar leaf extracts alone and together with the fungicide can be used for the management of fungal diseases caused by *A. solani* and will reduce the chemical load, but before applied to field it needs further investigations.

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REVIEW ARTICLE

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ISOLATES FROM WOUND INFECTIONS AT FEDERAL MEDICAL CENTRE, BIDA.

[1]Odedina E.A., [2]Eletta E.A., [2]Balogun R.A., [2]Idowu O. [1]Medical Microbiology Department,

[2]Surgery Department, Federal Medical Centre, P.M.B 14, Bida, Niger State

Correspondence: Dr. E.A. Odedina, Medical Microbiology Department, Federal Medical Centre, P.M.B. 14, Bida Niger State.

ABSTRACT

A total of 589 wound swabs from 334 patients in Federal Medical Centre, Bida were studied. Samples were collected between Jan 2002 to Dec. 2003. Swabs were plated within one hour after collection unto blood, chocolate and Mac Conkey after plate, and incubated aerobically for 24hrs. The chocolate plated swabs were incubated under increased carbon dioxide for 24hrs. Organism were identified using morphological and biochemical characteristics according to Cowon and Steel's manual for identification of medical bacterial. Organism isolated were subjected to antibiotic susceptibility testing by disc diffusion using modified Kirby-Bauer method.

The number of swabs that were culture positives were 441 (74.9%). Out of these 441 samples 334 (75.7%) grew one organism each, 99 samples (22.4%) grew two organisms each while 8 samples (1.8%) grew three organisms each. The 3 commonest organisms isolated were *Staphylococcus aureus* (45.5%), *Escherichia coli* (21.8%), *Pseudomonas aeruginosa* (14.9%) which together constitute 82.2% of the isolates.

There is a high level of antibiotic resistance. Ciprofloxacin is the drug of choice for the gram positive bacteria with susceptibility of 68.3%. Ofloxacin is the drug of the choice for the gram negative bacteria with susceptibility of 76.3%

INTRODUCTION

Infection constitutes a major cause of morbidity and mortality among surgical patients especially those subjected to emergency operations for acute abdomen (1). Wound infection is an important source of concern all over the world (2). Hospitals in the United States of America have stressed the importance of continued surveillance of all hospital acquired (nocosomial) infections (3). In Nigeria a surveillance programme on nocosomial wound infections was commenced at University College Hospital Ibadan, Nigeria in 1976 by Montefiore et al (4). Medical literature reveals that there is a shift in microbial agents responsible for surgical infection predominantly from gram positive to gram-negative nocosomial pathogens (5-8). This changing pattern of pathogens is thought to be due to use of antimicrobial agents in the treatment of prevailing infections in the hospital (9). The objective of each study was to provide the hospital community with meaningful data on the prevalence and incidence of nocosomial infections in different areas of hospital (10). These would allow adequate control measures to be formulated and constantly applied to help keep the incidence of such infection to a maximum (11).

In the study conducted by Olumide et al, most common bacteria pathogen were *Escherichia coli* 30%, *Staphylococcus aureus* 17% and *Klebsiella species* 13 % respectively (12). Federal Medical Centre, Bida is a young generation tertiary health institution in Niger State. No previous study on surgical wound infection has been carried out. The objectives of this study are to identify the common aerobic pathogens wound infections and

the antimicrobial susceptibility patterns of such agent in Federal Medical Centre, Bida.

MATERIALS AND METHODS

Swabs from surgical site infections were collected using swab sticks from January, 2002 to December 2003. The specimens were submitted soon after collection from the surgical wards to the Microbiology Laboratory and processed within one hour of obtaining them by inoculation on Blood, chocolate and Mac Conkey agar plates and incubated for 24 hours. The blood and chocolate plates were incubated under increased carbon dioxide jar (13). Cultures were observed for growth if there were no growths, the cultures were re-incubated for another 24hrs before being discarded if there was no growth. Isolates from cultures were identified using standard methods according to Cowon and Steels manual for identification of medical bacteria (14).

Each organism isolated was subjected to antibiotic susceptibility testing with disc diffusion using maltodisc by Kirby-Bauer modified method (15).

RESULT

A total of five hundred and eighty-nine wound swabs were processed, out of which four hundred and forty-one (74.9%) were culture positive (Table 1). Three hundred and thirty four samples (75.7%) yielded single bacterial organisms, ninety-nine (22.4%) samples grew two organisms each and eight (1.8%) grew three organisms each.

The commonest organisms were *Staphylococcus aureus* 253 (45.5%), *Escherichia coli* 121 (21.8%) and *Pseudomonas aeruginosa* 83 (14.9%) (Table 1).

The gram positive organisms were most sensitive to Ciprofloxacin (68.3%) followed by Ofloxacin (66.8%) Table IV, while gram negative organism were most sensitive to Ofloxaxin (76.3%) followed by Pefloxacin (65.8%) Table V

Table I: Organisms Isolated

Organism	No of Isolates	Percentage
Staphylococcus aureus	253	45.5
Escherichia coli	121	21.8
Pseudomonas aeruginosa	83	14.9
Klebsiella aerogenes	44	7.9
Proteus species	44	7.9
Streptococcus pyogenes	7	1.3
Corynebacterium species	2	0.4
CONS	2	0.4
Haemophilus influenzae	1	0.2
	Total 556	**100**

CONS: Coagulase Negative Staphylococci

Table II: Antibiotic Susceptibility of Gram Positive Bacteria

Antibiotic	S. aureus				Str. pyogenes				Corynebacterium			
	T	S	R	%	T	S	R	%	T	S	R	%
Augmentin	49	1	48	2.0	2	1	1	50.0	1	1	0	100
Amoxycillin	38	3	35	7.9	0							
Tetracycline	130	30	100	23.1	2	1	1	50.0	1	1	0	100
Gentamicin	248	92	156	37.1	5	1	4	20.0	2	0	2	0
Ofloxacin	227	151	76	66.5	4	4	0	100.0	1	0	1	0
Ciprofloxacin	132	88	44	66.7	5	5	0	100.0	2	2	0	100
Cotrimoxazole	209	53	156	25.4	5	1	4	20.0	1	0	2	0
Ampicillin	185	1	184	0.5	5	0	5		2	0	2	0
Cloxacillin	127	1	126	0.8	4	0	4		2	0	2	0
Penicillin	144	0	144	0.0	4	0	4		2	0	2	0
Erythromycin	194	64	130	33.0	5	5	0		2	0	2	50
Chloramphenicol	82	11	71	13.4	2	0	2					
Pefloxacin	131	87	44	66.4	2	1	1					
Cefuroxime	174	47	127	27.0	2	2	0					
Ceftazidime	120	21	99	17.5	1	1	0					
Azithromycin	104	47	57	45.2	1	1	0					
Clindamycin	105	58	47	55.2	1	1	0					
Ceftriaxone	132	75	57	56.8	2	2	0					
Doxycycline	23	7	16	30.4								
Cefalexin	20	11	9	55.0								

Antibiotic	E.coli				Aeruginosa				Klebsiellaeraegenes				Proteus spp				H. influenzae			
	T	S	R	%	T	S	R	%	T	S	R	%	T	S	R	%	T	S	R	%
Augmentin	38	8	30	21.1	21	0	21	0	20	2	18	10	8	1	7	12.5				
Amoxycillin	36	0	36	0.0	24	0	24	0	20	1	19	5	11	0	11	0				
Tetracycline	117	25	92	21.4	23	0	23	0	25	4	21	16	25	0	25	0				
Gentamicin	122	42	80	34.4	77	25	52	32.5	50	19	31	38	39	18	21	46.2				
Ofloxacin	120	91	29	75.8	80	61	19	76.3	32	25	7	78.1	33	30	3	90.9	1	1	0	100
Ciprofloxacin	57	36	21	63.2	45	23	22	51.1	26	20	6	76.9	21	16	5	76.2	1	1	0	100
Cotrimoxazole	115	13	102	11.3	43	0	43	0	27	7	20	25.9	31	4	27	12.9	1	1	0	100
Chloramphenicol	28	3	25	10.7	13	0	13	0	10	2	28	20	6	2	4	33.3	1	0	1	0
Colistin	52	10	42	19.2	15	2	13	13.3	11	5	45.5	11	0	11	0					
Pefloxacin	4	3	1	75.0	57	38	13	74.5	12	7	5	58.3								
Cefuroxime	59	11	48	18.6	36	11	25	30.6	14	2	12	14.3	14	3	11	21.4				
Ceftazidime	101	24	77	23.8	35	11	24	31.4	13	4	9	30.7	19	8	11	42.1				
Azithromycin	61	21	40	34.4	42	5	37	11.9	16	7	9	43.8	19	2	17	10.5				
Clindamycin	85	7	78	8.2	46	3	43	6.5	8	0	8	0	17	1	16	5.9				
Ceftriaxone	67	36	31	53.7	54	21	33	38.9	16	10	6	62.5	22	13	9	59.1				
Doxycycline	11	56	6	45.5					4	2	2	50.0	4	0	4	0				

Key:

T = number of isolates tested

S = number of isolates sensitive to antibiotic

R = number of isolates resistant to the antibiotic

% = percentage sensitivity

Table III: Antibiotic Susceptibility of Gram Negative Bacteria

Key:

T = number of isolates tested

S = number of isolates sensitive to antibiotic

R = number of isolates resistant to the antibiotic

% = percentage sensitivity

Table IV: Antimicrobial Susceptibility Patterns of Gram Positive Organisms

Antimicrobial Agents	No Tested	No Sensitive	Percentage sensitivity
Ciprofloxacin	139	95	68.3
Ofloxacin	232	155	66.8
Pefloxacin	133	88	66.2
Ceftriaxone	134	77	57.2
Ceftazidime	121	22	18.2
Clindamycin	106	59	55.7
Augmentin	52	3	5.8
Cloxacillin	133	1	0.8
Erythromycin	201	70	34.8
Tetracycline	133	32	24.1
Gentamicin	255	93	36.5
Chloramphenicol	84	11	13.1
Cotrimoxazole	216	54	25

Key:

No tested = Total number of isolate tested

No Sensitive = Total Number of isolate sensitive to antimicrobial agent

Percentage sensitive = Percentage of Isolate sensitive to the antimicrobial agent

Table V: Antimicrobial Susceptibility Pattern of Gram Negative Organism

Antimicrobial agents	No Tested	No Sensitive	Percentage sensitivity
Ciprofloxacin	150	96	64.0
Ofloxacine	266	203	76.3
Pefloxacine	73	48	65.8
Ceftriazone	159	80	50.3
Ceftazidine	168	47	28.0
Cefuroxime	123	27	22.0
Clindamycin	156	11	7.1
Tetracycline	190	29	15.3
Cotrimoxazole	217	25	11.5
Gentamicin	288	104	26.1
Chloramphenicol	58	5	8.6
Colistin	89	17	19.1
Azithromycin	138	35	25.4

Key:

No tested = Total number of isolate tested

No Sensitive = Total Number of isolate sensitive to antimicrobial agent

Percentage sensitive = Percentage of Isolate sensitive to the antimicrobial agent

DISCUSSION

Out of the 556 isolates obtained from wound swab cultures 264 (47.5%) were gram positive organisms while 292 (52.2%) were gram negative organisms. This result agrees with other studies that gram negative organisms are the predominant agents of surgical wound infection. The study also agree with other studies that *S. aureus* (45.5%) is the most important aerobic agent of wound infection (4-16) This was followed by *E. coli* (21.8%), *Ps. aeruginosa* (14.9%), *Kl. aerogenes* (7.9%), and *Proteus species* (7.9%). Similar reports for *S.aureus* has a higher incidence in our report. *S. aureus* 31.60%, *E. coli* 25.97%, *Ps. aeroginosa* 21.21%. Johnson (15+) reported a similar incidence for *S. aureus* as obtained in this study: 42% and 45% respectively. *S. aureus* is an important nosocomial pathogen as well as body flora in the anterior nares pubis, axilla from where it may contaminate wounds by autogenous infection or from contaminated formites especially when personal hygiene is poor.

E. coli is the second most important agent of wounds infection in this study, constituting 21.8% of the isolates. This figure is higher than 26% obtained by Njoku Obi in Enugu (9) but slightly lower than 25.97% obtained by Wariso in Port Harcourt (2) . The Enterobacteri *E. coli, Proteus species* and *Klebsiella, species* in this study constitute 37.6% obtained by other (2) in Port Harcourt. The relatively high incidence of *E. coli* and the Enterobacteria may be indicative of faccal contamination and a reflection of poor hygiene (2). A good measure of control of wound infection can be established by reducing traffic in the wards, detection and isolation of patient with wound infection, more stringent measures at the aseptic procedures during wound dressing and establishment of an adequate hospital infection control unit.

There is need for continual surveillance for bacteria agents of wound infection and their antimicrobial susceptibility testing. This is basic to the control of hospital acquired wound infections as this is the method by which the changing patterns of wound pathogens and their antimicrobial susceptibility patterns may be detected and controlled.

The bacterial isolates from the present study show a high level of antibiotic resistance. The unrestricted use of antimicrobial agents by the

populace and activities of quacks as health care givers may be responsible for the development of more resistant strain of the pathogens to most of the available study, only the quinolones may be used empirically for treatment of wound infection in Bida community.

These are ciprofloxacin for gram positive organisms and ofloxacin for the gram negative organism. Pefloxacin with susceptibility of 66.2% for gram positive organisms and 65.8% for gram negative organisms is much cheaper than ofloxacin and ciprofloxacin and may be preferred when considering cost.

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PATTERN OF HIV/AIDS INFECTION IN IRRUA, A RURAL COMMUNITY IN NIGERIA.

T. At. [1]salami, S.O [2]Samuel, [1]Ojeh-Onigbe, And K.C. [3]Eze. Departments Of Medicine[1], Medical Microbiology[2] And Radiology[3], College Of Medicine, Ambrose Alli University, Ekpoma, Edo State

Correspondence: Email-tatsalami@yahoo.com

ABSTRACT
This study aims to observe the pattern of HIV infection in Irrua Hospital records of patients diagnosed and managed with HIV/AIDS between January 2001 and December 2004 were retrieved and subjected to statistical analysis. The results show that females have statistically significant lower age; more incidence of double infection with HIV1&2; and more clinical anaemia(low PCV values) compared to their male counterparts.
Women are more likely to be HIV infected than men of similar ages for biological and cultural reasons.
HIV also passes more easily from men to women than vice versa hence double infection is common.
Anaemia is commoner in females because of incidence of marrow failure due to longer survival after HIV infection in women. Efforts should therefore be made to check the spread of this dreaded infection.
Key words. Hiv. Rural.Irrua. Male/female.

INTRODUCTION

The seroprevalence of HIV in Nigeria varies according to the population groups studied and figures differs from place to place(1) The average national HIV prevalence rates in Nigeria based on sentinel surveillance study using antenatal records to represent the general population shows a steady increase from 1.8% in 1990, to 3.8% in 1993, to 4.5% in 1995 and 5.4% in 1999(2) These indicates that approximately 2.6 million Nigerians are HIV positive and may subsequently go on to develop full blown AIDS. Young people are most vulnerable, and in the 1999 study people aged 20-24years had the highest rates of infection (3)

The pattern of HIV infection in the rural area and its impact on the community is different from that in the urban setting (4) Its epidemiology and clinical manifestation also varies between the male and female sexes (5)

This study aims to see the pattern that exists in this rural area where our centre is located and the reasons for any such observed trend. This becomes important since the overwhelming majority of people with HIV infection live in the developing world and these number continues to rise in countries like Nigeria where poverty, poor health systems ignorance, nut-headness, and limited resources for prevention and care fuel the spread of the virus.

METHODOLOGY

This is an hospital based study conducted at the Irrua Specialist teaching hospital, Irrua in Esan central LGA of Edo state, Nigeria.

The hospital records of all patients managed on the medical wards with clinical features of immunosuppression and that subsequently tested positive to HIV ELISA antibody between January 2001 and December 2004 were retrieved and information was collected on a previously

designed questionnaire to ease data collection. Demographic characteristic of the patients such as age, sex, occupation were considered. Types of virus involved and complications were also considered.

The information obtained was subjected to statistical analysis using Epi-info 6 and a p-value <0.05 was taken as significant. A comparison of the features in the males and females was done.

RESULTS

A total of 134 patients fulfilled the above criteria. There were 68males and 66 females. The mean age of the patients was 38.53±5.3years. The males were found to have a higher mean age of 44.19±9.19years compared to the females with a mean age of 32.87 ±3.24years(P-value <0.005). Most of the females were in the twenties while most of the males were in the late thirties and forties.

Table 1. Demonstrates the age distribution of patients in years.

Age group(in years)	Males	Females	Total
20-29	0	33(50%)	33(25%)
30-39	17(25%)	21(32%)	38(28%)
40-49	38(56%)	0	38(28%)
50-59	9(13%)	8(12%)	17(13%)
60-69	4(6%)	4(6%)	8(6%)
Total	68(100%)	66(100%)	134(100%)

Table2. HIV Status and the prevalence in both sexes. It is worthy of note that HIV2 seropositivity was zero in males.

HIV STATUS	MALES	FEMALES	Total
HIV1	36(53%)	8(12%)	44(33%)
HIV2	0	4(6%)	4(3%)
HIV1&2	32(47%)	54(82%)	86(64%)
TOTAL	68(100%)	66(100%)	134(100%)

The mean packed cell volume(PCV) in both males and females was 26.3±8.49but there was a statistically significant lower value in the females with a mean value of 22.19±2.12 compared with that of the males of 30.50±3.43.Three of the female patients actually presented with features of anaemic heart failure.

The commonest presenting pathology or illness in both groups was pulmonary Tuberculosis (diagnosed clinically and radiologically).

The Erythrocyte Sedimentation Rate was consistently elevated in both groups especially with co-morbid pulmonary tuberculosis.

DISCUSSION

There is an equal prevalence of HIV infection in both sexes based on the result of this study in this centre however the age at presentation and the clinical manifestations are different.

The females are considerably younger than their male counterparts with an average age difference of about a decade. This is not totally unexpected since worldwide, about half of all HIV infections are estimated to occur in young people under 25years(6). In developing countries, 60% of all HIV infections occur in 15-24year olds, with a female to male ratio of 2:1 in this age group(7). Girls and young women are especially vulnerable to HIV infection. They marry, or have sexual intercourse with older more sexually experienced men. Older men may approach school girls for sex, since they believe that these girls are still virgins and therefore less likely to be already infected with HIV. Girls are more likely than boys to have been sexually abused at home. This may lead them to run away and eventually to find themselves in commercial sex work. Other girls maybe tempted to leave home by promises of work in another country or region, only to find that the work is actually prostitution. Even those employed legitimately in factories or offices may be targeted for sex by more powerful male bosses.

Globally (8) women have less access to education, have less power to refuse unwanted sex, and to negotiate safer sex, and maybe unaware of having a sexually transmitted disease(STD). In Africa, women aged 15-24years are more likely to be HIV infected than men of similar ages for biological and cultural reasons (9). Other factors include poverty that makes high risk behaviour necessary as a means of survival by selling sex or street hawking of wares which exposes them to sexual abuse or commercial sex; loss or disintegration of their family system (often due to migration or forced displacement) leading to loss of control by parents, schools and the adult community; migration into urban areas where standards of living, role models and peer groups are in conflict with traditional norms and values; unavailability of or access to accurate information; and absence of health and social services to help young people due to restrictive policies and legislation.

The result of our study also shows that females have a higher rate of double infection with HIV 1 & 2 (82%) to males (47%). A combination of factors appears to be at work here, including the fact that HIV passes more easily from men to women through sex than from women to men(10). Nicolosi et al found male to female transmission of HIV to be 2.3 times greater than vice versa(11).

Furthermore, male to female transmission is more effective than female to male. Women are slightly more vulnerable physiologically to HIV infection than men. The area of mucous membrane exposed during intercourse is larger in the woman than in the man, and the mucous membrane surface of the vagina (compared to the penis) can more easily be penetrated by virus. Young girls are more vulnerable than women in the 18-45year age group (12). their immature cervix and relatively low vaginal mucous production present less of a barrier to HIV. A woman who is menstruating is likely to be at higher risk to HIV through sexual intercourse . Another possible factor for a more effective male to female transmission is the active carriage of HIV in the ejaculate.

Anaemia is a prominent feature among the females in this study. The PCV value was

significantly lower in females than in their male counterparts and as shown in the study, three of them actually presented in anaemic heart failure. This is also not totally unexpected as HIV infection is dominated by peripheral blood cytopaenias (13) Anaemia occurs in approximately 60-70% of AIDS patients(14) and it is a common manifestation of HIV disease. The pathophysiology of haematologic complications involves multiple defects in haematopoiesis such as decreased numbers of bone marrow progenitors; HIV infection of marrow progenitors with resulting abnormal maturation and proliferation; deficient production of haematopoietic growth factors by the bone marrow and HIV induced factor that inhibit normal heamatopoiesis (15). The reason why women seems to be affected more than men is not fully understood. A combination of factors are clearly involved such as the difference in age patterns of HIV infection in men and women (16) Women tend to become infected far younger than men for both biological and cultural reasons (9,16). The second factor involves survival time from infection to death. The older the patient before HIV infection, the shorter the time between infection and death. Therefore, African women , who as a rule become infected younger than their male counterparts can expect to live longer with HIV on average than men and therefore experience more organ failure such as bone marrow failure leading to anaemia.

The finding of pulmonary tuberculosis(PTB) as the main presenting illness is not surprising as PTB is the most common AIDS defining illness in Africa(17) and this has threatened to overwhelm the tuberculosis control programme in some centres in this country(18) This devastating effect on TB programmes has resulted in over 100% increase in TB cases in some setting(17-18) which thus creates an increased demand for diagnostic services, anti-tuberculous drugs, hospital beds and other supplies and services in areas such as ours where they are already in short supply(18)

In conclusion the pattern of HIV infection found in this centre is comparable to that found in other centers in sub-Saharan Africa and all efforts to check the continued spread of this most dreaded viral infection must be vigorously pursued.

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PRIONS AND PRION DISEASES

Robert Kelechi OBI[1]* and Ferdinand Chidi Nwanebu[1]
Dept. of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

ABSTRACT
A prion is a small infectious particle, which resist inactivation by procedures that modify nucleic acids. Transmissible spongiform encephalopathies (TSEs also known as prion diseases) are a group of progressive conditions that affects the brain and nervous system of humans and animals and are transmitted by prions. Unlike other kinds of infectious diseases that are spread by microbes, the infectious agent in TSEs is a specific protein called prion protein (PrP). TSEs are unique diseases in that they can be inherited, occur spontaneously (sporadic TSE) or can be spread through infection. The clinical signs of the disease in humans vary, but commonly include personality changes, psychiatric problems such as depression, lack of coordination and/or an unsteady gait (ataxia). Patients also may experience involuntary jerking movements called myoclonus, unusual sensation, insomnia, and confusion or memory problems. In the later stages of the disease, patients may have severe mental impairment (dementia) and may lose the ability to move or speak. Well known prion diseases include scrapie (in sheep and goat), bovine spongiform encephapathy (BSE or mad cow disease) and Creutzfeldt- Jakob disease (CJD). Less well known prion diseases include the transmissible mink encephalopathy (TME) (in mink), chronic wasting disease (CWD) (in mule, deer and elk), feline spongiform encephalopathy (FSE) (in cats), Gerstmann-Straussler-Scheinker syndrome (GSS), Alpers syndrome, and fatal familial insomnia (FFI). Six of these affect humans: CJD, GSS, FFI, mad cow disease known as (new) variant CJD, (nvCJD), Alpers syndrome and kuru. These conditions form a spectrum of diseases with overlapping signs and symptoms. There is currently no treatment that can cure or control TSEs.
Key words: prions, transmissible spongiform encephalopathy
**Corresponding Author's E-mail:* robertobi_2003@yahoo.ca

INTRODUCTION

Several slowly progressive neurological diseases are caused by a group of infectious agents called prions designated PrP, a protease resistant protein of molecular mass 27-30KDa(1,2).

A prion has been defined as a small proteinaceous infectious particles (PrP) which resist inactivation by procedures that modify nucleic acids(3). A Prion is a molecule of a normal body protein that have changed its three dimensional configuration. The normal protein is called PrP^C (for cellular). This is a transmembrane glycoprotein normally found at the surface of certain cells (e.g neural and hematopoietic stem cells). Its secondary structure is dominated by alpha helices (probably 3 of them). It is soluble and therefore easily digested by proteases. The protein is encoded by a gene designated in humans as PRNP and located on chromosome 20. On the other hand, the abnormal, disease producing protein is called a PrP^{Sc} (for scrapie). This protein has the same amino acid sequence as the normal protein, that is, their primary structures are identical but its secondary structure is dominated by beta conformation. It is insoluble in all but the strongest solvents and is also highly resistant to digestion by protease. When PrP^{Sc} comes in contact with PrP^C, it converts the PrP^C into more copies of itself. These molecules bind to each other forming aggregates. However it is not yet clear if these aggregates are themselves the cause of the cell damage or are simply a side effect of the underlying disease process(4).

Prion proteins occur in the brains of all mammals so far studied. However their normal function is

not well understood, but recent research on mice that lack the PrP gene, which encodes the prion protein suggest that it protects the brain against dementia and other degenerative problems associated with old age(5).

Extensive studies on this agent have so far failed to identify any nucleic acid associated with the infectious materials, yet biochemical studies to date cannot conclusively rule out the requirement for nucleic acid for infectivity. Whether or not prions contain RNA or DNA, their known biological and physical properties can best be described and appreciated in comparison with those of other small infectious agents (e.g. RNA species)(6).

Prion diseases are often called transmissible spongiform encephalopathies (TSE) because of the postmortem appearance of the brain with large vacuoles in the cortex and cerebellum (3). These large vacuoles cause mental and physical abilities to deteriorate and myriad tiny holes appear in the cortex causing it to appear like a sponge (hence spongiform), which becomes visible when brain tissue obtained at autopsy is examined under a microscope. The disorders cause impairment of the brain including memory changes, personality change and problems with movement that worsen with time (7).

TSEs are a group of progressive conditions that affects the brain and nervous system of humans and animals. They are unique diseases in that they can be inherited, occur spontaneously ("sporadic" TSE) or can spread through infection(8). Most TSEs are sporadic and occur in an animal with no prion protein mutation. Inherited TSE occurs in an animal carrying a rare mutant prion allele, which expresses prion proteins that contort by themselves into the disease causing conformation. Transmission occurs when healthy animals consume tainted tissues from others with the disease(8). The transmission of disease depends on the abnormal prion being similar enough to the host prion to be able to 'lock in' to its structure and convert it. Transmission works best between animals of the same species(5). Prions cannot be transmitted through the air or through touching or most other forms of casual contact. However they can be transmitted through contact with infected tissues, body fluids or contaminated medical instruments. Normal sterilization procedures such as boiling or irradiating materials fail to render prions non infective(9).

Inherited prion diseases include Creutzfeldt-Jakob (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and Alpers syndrome. Infectious prion diseases include kuru, scrapie, bovine spongiform encephalopathy (BSE) or "mad cow disease", CJD and variant Creutzfeldt- Jakob disease (vCJD). Sporadic prion diseases include CJD and FFI(4).

Examples of prion diseases of humans include: CJD, GSS, FFI,vCJD, kuru and Alpers syndrome. Prion diseases of animals include: scrapie (sheep and goat), transmissible mink encephalopathy (TME) (mink), chronic wasting disease (CWD) (elk, mule, deer), bovine spongiform encephalopathy (BSE) (cow) and feline spongiform encephalopathy (FSE) (cats)(3).

In view of the rising cases of neuropathological syndromes worldwide and their concomitant fatal consequencies, this study was designed to draw attention of the Nigerian health care providers and indeed, Nigerian public, to the existence of these rare neurodegenerative disorders that are hitherto without any known treatment, and which may have been responsible for the mental disability

(commonly refered to as old age sickness), shortly before death, of many old people.

PATHOGENESIS

Ingested prions may be absorbed across the gut all at Peyers patches. These are a part of the mucosal associated lymphoid tissue (MALT). It is thought that the MALT presents microorganisms to the immune system in a contained and ideal fashion, facilitating a protective immune response. Prions could be taken up in the same way. Lymphoid cells then phagocytose the particle and travel to other lymphoid sites such as lymph nodes, the spleen and tonsils. The prion can replicate in these sites. Many of these sites are innervated and eventually the prion gains access to a nerve and then propagates back up the axon to the spinal cord and eventually the brain (3).

GENETIC BASIS OF PRION DISEASES

Familial forms of prion diseases are caused by inherited mutations in the PRNP gene. Only a small percentage of all cases run in families, however. Most cases are sporadic, which means they occur in people without any known risk factor or gene mutations.

The PRNP gene provides the instruction to make a protein called the prion protein (PrP). Normally this protein maybe involved in transporting copper into cells. It may also be involved in protecting brain cells and helping them communicate. 24 point mutations in this gene cause cells to produce an abnormal form of the prion protein, known as PrP^{Sc}. This abnormal protein builds up in the brain and destroys nerve cells, resulting in the signs and symptoms of the diseases [10]. Familial forms of prion disease are inherited in an autosomal dominant pattern, which means that one copy of the altered gene in each cell is sufficient to cause the disorder. In many cases, an infected person inherits the altered gene from one infected parent (11).

CHARACTERISTICS OF DISEASES

The degenerative tissue damage caused by human prion diseases (CJD, GSS, Alpers syndrome and Kuru) are characterised by four features: spongiform change, neuronal loss, astrocytosis and amyloid plaque formation. These features are also shared with prion diseases in animals(12). The clinical signs in humans vary, but commonly include personality changes, psychiatric problems such as depression, lack of co-ordination and/or an unsteady gait (ataxia). Patients also may experience involuntary jerking movements called myoclonus, unusual sensations, insomnia, confusion, or memory problems. In the later stages of the disease, patients may have severe mental impairment (dementia) and may lose the ability to move or speak (13).

DIAGNOSIS

Neuropathological features have formed the basis of the histological diagnosis of human prion disease for many years, although it was recognised that these changes are enormously variable both from case and within the central nervous system in individual cases [11]. However not all encephalopathies are caused by prions as in the cases of PM1 (caused by the JC virus), CADASIL (caused by abnormal NOTCH3 protein activity), and Krabb disease (caused by deficiency of enzyme galactosylceramidase). PSL-, which is a spongiform encephalopathy, is also probably not caused by a prion, although the adulterant, which causes it among heroin smokers, has not yet been identified (7,8,10). This, combined with the highly variable nature of prion disease pathology,

is why a prion disease cannot be diagnosed based solely on a patient's symptoms (9).

Another hindrance to a successful diagnosis of the disease is the fact that although their infectious natures and small size suggest similarities to conventional viruses, no prion has been observed, even by electron microscopy (1). However an approach towards successful diagnosis has been made by Montagna *et al.*, (12According to the research the abnormally folded proteins that cause prion disease have been found to expose a side chain of amino acids which the properly folded protein does not expose. Antibodies specifically coded to this side chain amino acid sequence were also found to stimulate an immune response to the abnormal prion and leave the normal protein intact. The research concluded that while assisting in diagnosis, that the discovery could also be helpful in formulating a vaccine that could be used to control the disease.

APPROACHES TO TREATMENT

PrP over expression facilitates the development of prion diseases. For treatment therefore it follows that knowledge of the agents which reduce PrP expression will delay the onset of the diseases. In this case agents, which bind and stabilize the PrP^{C} conformation may be beneficial. Similarly agents destabilizing the PrP^{Sc} may also be effective. In addition agents, such as Congo red, which interfere with the putative PrP^{C} - PrP^{Sc} interaction might similarly be effective. Chemicals affecting the endocytosis, exocytosis, intracellular trafficking and degradation of proteins and in particular PrP may also be effective (3).

Another approach towards treatment concern gene therapy, where by the gene for encoding protease-resistant protein is considered to be an error in several species, and therefore something to be inhibited (12). As our knowledge of the structure of PrP however increases, the chances of rationally deducing effective therapeutics based on these ideas also increases.

PRION DISEASES

Creutzfeldt-Jakob disease (CJD) was first described by two German neurologists, Hans Gerhard Creutzfeldt and Alfons Maria Jakob. Some of the clinical findings described in their first papers do not match current criteria for Creutzfeldt-Jakob disease, and it is considered highly likely that at least two of the patients in their initial studies were suffering from a different disorder (14). Many Americans first learned about the disease when the famed choreographer, George Balanchine died of it in 1983(15).

Creutzfeldt-Jakob disease is a very rare and incurable degenerative neurological disorder (brain disease) that is ultimately fatal. It is the most common of the transmissible spongiform encephalopathies (TSEs)(16). Typically, onset of symptoms occurs at about age 60. Three major categories of the disease exist. These are the sporadic, hereditary and acquired CJDs (17).

The prion that is believed to cause CJD exhibits at least two stable conformations. One, the native state, is water-soluble and present in healthy cells. As at 2006, its biological function was unknown. The other conformational state is very poorly water-soluble and readily forms protein aggregates (18). The CJD prion is dangerous because it promotes refolding of native proteins into the diseased state. Subsequently the number of misfolded protein molecules will increase exponentially and the process will lead to a large quantity of insoluble prions in affected cells. This mass of misfolded proteins disrupts cell function and cause cell death. Once the prion is transmitted, the defective proteins invade the

brain and get produced in a self-sustaining feedback loop, causing exponential spread of the prion, and the patient usually dies within a few months although a few patients have been known to live as long as two years (19).

Although CJD is the most common human prion disease, it is still rare and only occurs about one out of every one million people. It usually affects people aged 45-75, most commonly appearing in people between the ages of 60-65. The exception to this is the more recently recognized " variant" CJD (vCJD), which occurs in younger people (20). Some cases of CJD are clustered in certain families, and the fact that some of these families also have an apparently higher incidence of Alzheimer's disease has lead to the supposition that the two diseases may be related (6).

The first symptoms of CJD is rapidly progressive dementia leading to memory loss, personality changes and hallucinations. This is accompanied by physical problems such as speech impairment, jerky movements (myoclonus), balance and co-ordination dysfunction (ataxia), and changes in gait, rigid posture, and seizures. The duration of the disease varies greatly but sporadic CJD can be fatal, killing its victims within months, or even weeks (21). In most patients, these symptoms are followed by involuntary movements and the appearance of a typical diagnostic electroencephalograph tracing (22).

The symptoms of CJD are caused by the progressive death of the brain nerve cells, which are associated with the build-up of abnormal prion proteins. When brain tissue from a CJD patient is examined under a microscope, many tiny holes can be seen where whole areas of nerve cells have died. The word "Spongiform" in transmissible spongiform encephalopathies refers to the spongy appearance of the brain tissue (19).

There is currently no single diagnostic test for CJD. The first concern is to rule out treatable forms of dementia such as encephalitis or chronic meningitis. The only way to confirm a diagnosis of CJD is by brain biopsy. Because a correct diagnosis of CJD does not help the patient, a brain biopsy is discouraged unless it is needed to rule out a treatable disorder (17).

There is no treatment that can cure or control CJD. Currently treatment is aimed at alleviating symptoms and making the patient as comfortable as possible. Opiate drugs can help relieve pain, and the drugs clonazepam and sodium valproate may help relieve involuntary muscle jerks (17). However search for viable treatment has continued (21).

The defective CJD protein can be transmitted by human growth hormone (HGH) products, corneal grafts, dural grafts or electrode implants (acquired or iatrogenic form: iCJD). Less than 5% of CJD cases are iatrogenic; it can be inherited (hereditary or familial form: fCJD). Familial cases are associated with a gene mutation and make up about 10-15% of all CJD cases; or it may appear for the first time in the patient (sporadic form: sCJD). Sporadic cases have an unknown cause and occur through out the world at the rate of about one per million which account for 85-90% of CJD cases (4,23).

Humans can contract the disease by consuming material from animals infected with the bovine form of the disease. The only cases to arise thus far have been vCJD, although there are fears - based on animal studies- that consuming beef or beef products containing prion particles can also cause the development of classic CJD.There is no evidence of CJD transmission through blood or blood products (21).

INHERITANCE AND GENETICS OF CJD

As already pointed out, 10-15% of the cases of CJD are inherited; that is the patient comes from a family in which the disease has appeared before. The disease is inherited as an autosomal dominant. This means that the patients have inherited at least one copy of a mutated PRNP gene. Some of the most common mutations are: a change in Codon 200 converting glutamic acid (E) at that position to lysine (K) (thus designated "E200K"), a change from aspartic acid (D) at position 178 in the protein to asparagine (D178N) when it is accompanied by a polymorphism in the gene encoding valine at position 129(4).

A new variant form of CJD (usually called variant Creutzfeldt-Jakob Disease (vCJD)) or new variant Creutzfeldt -Jakob Disease (nvCJD) is a rare and fatal human neurodegenerative condition. Like Creutzfeldt-Jakob disease, vCJD is classified as a Transmissible Spongyform Encephalopathy (TSE) because of characteristic spongy degeneration of the brain and its ability to be transmitted. However unlike the traditional forms of CJD, vCJD has affected younger patients (average age of 29 years as opposed to 65 years), has a relatively longer duration of illness (median of 14 months as opposed to 4-5 months) and is strongly linked to exposure, probably through food, to a TSE of cattle called Bovine Spongyform Encephalopathy (BSE) or mad cow disease

VCJD is a new disease that was first described in March 1996(15,23).

Early in the infection, patients usually experience psychiatric symptoms, which most commonly result in a form of depression or less often a schizophrenia-like psychosis. Unusual sensory symptoms, followed by ataxia and myoclonus usually occur with dementia appearing in the final stages of the illness (14).

There is no available, completely reliable diagnostic test for use before the onset of symptoms. However magnetic resonance scans, tonsillar biopsy and cerebrospinal fluid analysis are all useful diagnostic tests. Currently the diagnosis of vCJD can only be confirmed following pathological examination of the brain (18). vCJD is strongly linked to exposure to the BSE agent. BSE is a TSE affecting cattle and was first reported in the U.K in 1986. From October 1996 to November 2002, 129 cases of vCJD were reported in the U.K, six in France and one each, in Canada, Ireland, Italy and the USA [14]. In 2005 five people died from vCJD in the U.K (16).

In 2004, a possible transmission of vCJD through blood transfusion was reported, though to date no case of vCJD has ever developed in recipients of any blood. However in reaction to the report some countries prohibited donations of blood from persons who have resided in countries with higher risk of BSE (23,24).

Gerstmann-Straussler syndrome (GSS) is a very rare, usually familial, fatal neurodegenerative disease that affects patients from 20 to 60 years of age (25). This prion disease is caused by the inheritance of a PRNP gene with a mutation encoding most commonly, leucine instead of proline at position 102 (P102L) or valine instead of alanine at position 117 (A117V). The disease is strongly associated with homozygosity for a polymorphism at position 129 (both residues being methionine). Brain extracts from patients with GSS can transmit the disease to Monkeys, apes and transgenic mice containing a portion of

the human PRNP gene. Transgenic mice expressing the P102L gene develop the disease spontaneously[4]. GSS occurs typically in the 4th-5th decade, characterised by cerebella ataxia and concomitant motor problems, dementia less common and disease course lasts several years to death. It was originally thought to be familial, but it is now known to occur sporadically as well (3).

Kuru (also known as laughing sickness due to the outbursts of laughter that marks its second phase), the first slow infectious disease of humans to be identified, was first noted in Papua New Guinea in the early 1900s. By the 1950s most of the women and many of the children were being attacked by a fatal neurological disorder that began by causing its victims to giggle uncontrollably. The syndrome baffled American epidemiologist Carleton Gajdusek, who analysed soil, drinking water, food, and even ashes in the fires in search of the etiologic agent and its mode of transmission. After months of inquiry, Gajdusek discovered that the tribe was cannibalistic. As an expression of respect for their dead relatives, the survivors would consume portions of the corpses, including the brain. Years after preparing the brains for cooking, the women and children would begin the fatal giggles. Kuru (shaking death in the language of the Fore) was subsequently shown by Gajdusek to be caused by a previously undiscovered type of pathogen, originally called a slow virus because of its 2-to-20-years incubation period. The agent now recognised as a prion is transmitted by eating the infected neurological tissue of someone who has died from the laughing death or by cutaneous inoculation of the virus while preparing the brain. The tribe's extinction was avoided when they were persuaded to abandon their cannibalistic tribute to the dead (26,1,6).

The kuru epidemic reached its height in the 1960s. Between 1957 and 1968, over 1,100 of the South Fore died from disease. The vast majority of the victims were women. Infact, eight times more women than men, contracted the disease. It later affected small children and the elderly at a high rate as well. This disproportion was later traced to the distribution of the corpse's remains between the sexes. The males got the "good" parts of the corpse, which usually consisted of the muscles and fatty organs. The females and children got the "bad" parts, which included the brain and other less desirable parts. Thus, the women and children directly ingested the prion, leading to a much higher occurrence rate of the disease[27].

SYMPTOMS OF KURU:

1. The ambulant stage, which is accompanied by unsteadiness of stance, gait, voice, hands, and eyes; deterioration of speech; tremor; shivering; loss of coordination in lower extremities that moves slowly upward, and dysarthria.
2. The sedentary stage: at this stage the patient can no longer walk without support, more severe tremors and ataxia (loss of coordination of the muscles), shock-like muscle jerks, emotional liability, outbursts of laughter, depression, and mental slowing. It is important to note that muscle degeneration does not occur at this stage, and tendon reflexes are usually still normal.
3. The terminal stage, which is marked by inability to sit up without support, more severe ataxia, tremor and dysarthria (slurring of speech), urinary and faecal incontinence, difficulty in swallowing (dysphasia), and deep ulceration appear.

Cerebella dysfunction is the cause of these conditions (28).

Knowledge of the dynamics of kuru has continued to grow even though the disease all but disappeared with the termination of cannibalism in Papua New Guinea (29).

Fatal familial insomnia (FFI) is a very rare autosomal dominant inherited disease of the brain. The dominant gene responsible has been found in just 28 families worldwide; if only one parent has the gene, the offspring have a 50% chance of inheriting it and developing the disease. The disease's genesis and the patients' progression into complete sleeplessness is untreatable, and ultimately fatal (12). People with this rare disorder have inherited a PRNP gene with asparagines instead of aspartic acid encoded at position 178 (D178N); and the susceptibility polymorphism of methionine at position 129 of the PRNP gene. The mutation changes the shape of the protein so that it becomes a prion and makes other normal protein molecules change to the abnormal shape. This causes plaques to develop in the thalamus, the region of the brain responsible for regulation of sleep. This first results in insomnia, and then progresses to more serious problems over time (4).

The Italian doctor, Ignazio Rioter in 1979, who discovered two women from one family who apparently died of insomnia, first detected FFI. Family records showed a history of seemingly related deaths. When another member of the family fell ill in 1984, his deterioration was studied and after his death, his brain was flown to the U.S for further investigation (30).

The age of onsets is variable, ranging from 30 to 60, with an average of 60. Death usually occurs between 7 to 36 months from onset. The presentation of the disease varies from within the same family (27).

The disease has four stages, taking 7 to 18 months to run its course:

1. The patient suffers increasing insomnia, resulting in panic attacks and phobias. This stage lasts for about four months.
2. Hallucinations and panic attacks become noticeable, continuing about five months.
3. Complete inability to sleep is followed by rapid loss of weight. This lasts about three months.
4. Dementia, turning unresponsive or mute over the course of six months. This is the final progression of the disease, and the patient will subsequently die.

There is no cure or treatment for FFI; hopes rest on the so far unsuccessful gene therapy. Sleeping pills have no effect (12).

Alpers syndrome, first described more than 70 years ago, is a rare, progressive neurodegenerative disorder that occurs in infants and children. It is an autosomal recessive, developmental mitochondrial DNA depletion disorder characterized by deficiency in mitochondrial DNA polymerase gamma (POLG) catalytic activity, refractory seizures, neurodegeneration and liver disease (31). The birth incidence is believed to be between 1/100 000 and 1/250 000. Most patients with Alpers syndrome are asymptomatic at birth and develop normally for weeks to years before the onset of symptoms. About 80% present in the first two years, and 20% present between 2 and 25 years of age (32).

First signs of the disease, which include intractable seizures and failure to meet meaningful developmental milestones, usually

occur in infancy. Primary symptoms of the disease are developmental delay, progressive mental retardation, hypotonia (low muscle tone), spasticity (stiffness of the limbs), and dementia. Seizures may include epilepsia partialis continua, a type of seizure that consists of repeated myoclonic (muscle) jerks. Optic atrophy may also occur, often leading to blindness. And, although physical signs of chronic liver dysfunction may not be present, many patients suffer liver impairment leading to liver failure (33). The prognosis for individuals with liver disease is poor. Those with the disease usually die within their first decade of life. Liver failure is usually the cause of death, although cardio respiratory failure may also occur (34).

Prenatal diagnosis is now available by POLG DNA testing in couples with a previously affected child and known genotype (32).

There is no cure for Alpers' disease and, currently, no way to show its progression. Treatment is symptomatic and supportive. Anticonvulsants may be used to treat the seizures (35). Physiotherapy, avoidance of group settings that promote the spread of common seasonal, childhood respiratory infections and attention to good nutrition can help to ease symptoms and reduce the frequency of neurodegenerative episodes, but are not proven to improve the overall severe prognosis (32).

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of deer, elk, (wapiti), and moose. First recognized as a clinical "wasting" syndrome in 1967 in mule deer in a wildlife research facility in Northern Colorado, it was identified as a TSE in 1978. CWD is typified by chronic weight loss leading to death. There is no known relationship between CWD and other TSE of animals or people. Although there have been reports in the popular press of humans being affected by CWD a study by the CDC failed to find any relationship (36).

Most cases of CWD occur in adult animals. The disease is progressive and always fatal. The most obvious and consistent clinical sign of CWD is weight loss over time. Behavioural changes also occur in the majority of cases, including decreased interaction with other animals, listlessness, lowering of the head, blank facial expression, and repetitive walking in set patterns. In elk, behavioural changes may also include hyper excitability and nervousness. Affected animals continue to eat grain but may show decreased interest in hay. Excessive salivation and grinding of teeth also are observed. Most deer show increased drinking and urination (37).

The agent responsible for CWD is a prion, an abnormal form of a normal protein, known as prion protein (PrP), most commonly found in the central nervous system (CNS), and is capable of spreading to the peripheral nervous system (PNS), thus infecting meat, or muscle, of deer and elk. The abnormal prion protein infects the host animal by promoting conversion of normal cellular prion protein (PrP^{C}) to the abnormal prion form (PrP^{Sc}). The build up of PrP^{Sc} in the brain is associated with widespread neurodegeneration (14).

Research is being conducted to develop live animal diagnostic tests for CWD. Currently, definitive diagnosis is based on postmortem examination (necropsy) and testing. Gross lesions seen at necropsy reflect the clinical signs of CWD, primarily emaciation. Aspiration pneumonia, which may be the actual cause of death also, is a common finding in animals

affected with CWD. On microscopic examination lesions of CWD in the central nervous system resemble those of other TSEs. In addition, a technique called immunohistochemistry has been developed to test brain tissue for presence of the abnormal prion protein to diagnose the disease (38).

The origin and mode of transmission of the prions causing CWD is unknown, but recent research indicates that prions can be excreted by deer and elk and is transmitted by eating grass growing in contaminated soil[39,40]. Animals born in captivity and those born in the wild have been affected with the disease. Based on epidemiology, transmission of CWD is thought to be lateral or from animal to animal; although maternal transmission may occur, it appears to be relatively unimportant in maintaining epidemics. Research has recently shown that an infected deer's saliva is also able to spread the CWD prions (37).

Scrapie, the first TSE to be studied, was described in sheep and goats in the 18th century, precisely in 1732. However it is still found in most parts of the world despite attempts to eradicate the agent by destroying infected flock [4,6].

Scrapie is a fatal, degenerative disease that affects the nervous system of sheep and goats. It is one of several transmissible spongiform encephalopathies (TSEs), which are related to bovine spongiform encephalopathy (BSE or "mad cow disease") and chronic wasting disease of deer. Like other spongiform encephalopathies, scrapie is believed to be caused by a prion (5).

The name scrapie was derived from one of the symptoms of the condition, wherein affected animals will compulsively scrape off their fleece against rocks, trees or fences. The disease apparently causes an itching sensation in the animals. Other symptoms include excessive lip smacking, strange gait and convulsive collapse (6,13).

Scrapie is infectious and transmissible among similar animals in feed contaminated with nerve tissue and so one of the most common ways to the disease (since it is incurable) is to quarantine and destroy those affected. However it tends to persist in flocks and can also arise apparently spontaneously in flocks that have not previously had cases of the disease. The mechanism of transmission between animals and other aspects of the biology of the disease are only poorly understood. Recent studies suggest that scrapie agents may be spread through urine and persist in the environment for decades[41]. Scrapie agent, in the form of extracts from infected brains, has been passed experimentally to mice, hamsters, ferrets, mink, and monkeys, but apparently is not infectious for humans, Chimpanzees or rabbits (6).

Feline spongiform encephalopathy (FSE) affects felines. It is a prion disease thought to be related to bovine spongiform encephalopathy (BSE). It is known to affect domestic and captive felines [25]. Lezmi *et al.*, (2003), suggested that this infectious agent might spread by both haematogenous and nervous pathways. Like BSE, this disease can take several years to develop. It is probable, but not proven, that the affected animals contract the disease by eating contaminated bovine meat[27].

The clinical signs include ataxia that was observed to last for about 8 weeks in the affected animals. The ultimate result is death of the infected animals (42).

The disease was first reported in the United Kingdom in 1990. Uptil about 5 years ago, there were reports of 87 FSE cases (only domestic cats) in the UK, one in Norway, one in Northern Ireland and one in Switzerland. However in 1990, other feline species in zoos were reported to have contracted the disease (43).

FSE can only be confirmed at the postmortem, which includes identification of bilaterally symmetrical vacuolation of the neutrophil and neurons. Lesions are likely to be found in basal ganglia, cerebral cortex and thalamus of the brain (18).
FSE unfortunately is a terminal condition and currently there is no specific treatment for the disease (38).

Bovine spongiform encephalopathy (BSE) commonly known as mad cow disease is a fatal, neurodegenerative disease of cattle, which infects by mechanism that surprised biologists on its discovery in the late 20th century. While having never killed cattle on a scale comparable to other livestock diseases, such as foot and mouth disease and rinderpest, BSE has attracted wide attention because it seems possible to transmit the disease to humans; it is thought to be the cause of variant Creutzfedt-Jakob disease (vCJD), sometimes called new variant Creutzfeldt-Jakob disease (nvCJD), a human brain wasting disease.

An epidemic of BSE began in Great Britain in 1985 and before it was controlled, over 170,000 cattle were sickened by it. Its origin appears to have been cattle feed that contained brain tissue from sheep infected with scrapie; and feed that had been treated in a new way that no longer destroyed the infectiousness of the scrapie prions (25). The use of such food was banned in 1998 and after peaking in 1992, the epidemic declined quickly (8).

Cattle, like most other animals, are herbivores. In nature, cattle eat grass or grains. In modern industrial cattle farming, various commercial feeds are used, which may contain ingredients including antibiotics, hormones, pesticides, fertilizers and protein supplements. BSE began when meat and bone meal were used as protein supplements in cattle feed in Europe shortly before 1986(5).

Following an outbreak of BSE in Britain, 155 people (up till 2004) acquired and died of a disease with similar neurological symptoms subsequently called vCJD or (new) variant Creutzfedt-Jakob disease. This is a separate disease from "classical" Creutzfedt-jakob disease, which is not related to BSE and has been known since the early 1900s. Of the 155 cases of vCJD in humans so far, 148 occurred in the United Kingdom, 6 in France, and one in Italy (25).

For many of the vCJD patients, direct evidence exists that they had consumed tainted beef, and this is assumed to be the mechanism by which all affected individuals contracted it. Disease incidence also appears to correlate with slaughtering practices that lead to the mixture of nervous system tissue with hamburger and other beef. It is estimated that 400,000 cattle infected with BSE entered the human food chain in the 1980s. Although the BSE outbreak was eventually brought under control by killing all suspected cattle populations, people are still being diagnosed with vCJD each year (though the number of new cases currently seems to be dropping). This is attributed to the long incubation period of prion diseases, which are typically measured in years or

decades. As a result the full extent of the human vCJD outbreak is still not fully known (11). The scientific consensus is that infectious BSE prion material is not destroyed through normal cooking procedures, meaning that contaminated beef foodstuffs prepared "well done" may remain infectious (10,13).

In 2004 researchers reported evidence of a second contorted shape of prions in a rare minority of diseased cattle. In other words, this implies a second strain of BSE prion. The finding of a second strain of BSE prion raises the possibility that transmission of BSE to humans has been under estimated, because some of the individuals diagnosed with spontaneous or "sporadic" CJD may have actually contracted the disease from tainted beef. So far nothing is known about the relative transmissibility of the two disease strains of BSE prion (15).

The tests used for detecting BSE vary considerably as do the regulations in various jurisdictions for e.g. when and which cattle, must be tested. For instance, in the EU the cattle tested are older (30 months +), while many are slaughtered earlier than that. At the opposite end of the scale, Japan tests all cattle at the time of slaughter.

Testing animals before slaughtering is difficult as the altered prion protein has very small level in blood or urine, and no other signal is found. Currently the only reliable test is examination of tissues during autopsy (44). No particular medication has been found to control BSE, but to contain the disease, all suspected cattle are killed and cremated and the carcass buried (3).

CONCLUSION

Unconventional transmissible agents cause a group of human and animal fatal neurodegenative disorders. They result in spongiform change, neuronal loss, reactive gliosis and amyloid plaque formation in the affected brain. Extensive investigations on the nature of the infectious agent in these disorders have so far failed to reach any conclusion. The prion hypothesis that states that the transmissible agent is composed entirely of PrPSc has gained much favour at present since it appears to explain many of the transmissible and genetic aspects of these remarkable diseases. PrP^{Sc} is derived from a large precursor host glycoprotein (PrP^{C}), which is normally expressed in neurones and is encoded by gene on chromosome 20 in humans. Human prion disease occur as sporadic, familial and acquired diseases. The commonest of these is sporadic CJD, the underlying cause for which is not known. Familial prion diseases occur as inherited disorders, which are invariably associated with mutations or insertions in the human prion protein gene. A large body of evidence exists to support the claim that susceptibility to both iatrogenic and sporadic human disease is controlled by a naturally occurring polymorphism at Codon 129 in the PrP gene. Individuals who are homozygous at this Locus accounts for the large majority of CJD patients. A new variant form of CJD has been identified in the UK, which is causally linked to exposure to the BSE agent.

Since prions cause fatal diseases with no known forms of remission or recoveries, preventive measures must be taken to safeguard human and animal lives. To do this effectively no part or product of any animal that has shown signs of a TSE should enter any human or animal food chain.

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REVIEW ARTICLE

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LYMPHOGRANULOMA VENEREUM: A REVIEW OF LITERATURE.

[1]* Fadeyi, Abayomi , [2] Adigun, Ismaila Abiona and [1] Adegboro, Boaz

[1] Department of Medical Microbiology, and [2] Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Ilorin Teaching Hospital, Ilorin, Nigeria.

*** Correspondence** : abayomifadeyi @yahoo.com Tel: 08033597835

ABSTRACT

Lymphogranuloma venereum (LGV) is a systemic STD caused by *Chlamydia trachomatis* serotypes L1, L2 and L3. The disease is endemic in parts of Africa, Asia, South America and the Caribbean but rare in Western countries where the disease occurs mainly in sporadic form. Large outbreaks occurred recently in Europe and America mostly among men who have sex with men (MSM). The clinical course of the disease is stratified into primary, secondary and late stages but the presentation may be atypical particularly when coexisting with HIV and may result in diagnostic confusion. We present here a review of literature on LGV.

KEY WOREDS: Lymphogranuloma, Venereum, Literature, Review

INTRODUCTION AND EPIDEMIOLOGY

Lymphogranuloma venereum (LGV) is a systemic STD caused by *Chlamydia trachomatis* serotypes L1, L2 and L3. LGV is endemic in parts of Africa, Asia, South America and the Caribbean but rare in Western countries where the disease occurs mainly in sporadic form (1). Large outbreaks of LGV occurred recently in Europe and America mostly among men who have sex with men (MSM) (2, 3, 4, 5). This has prompted the US Centers for Disease Control and Prevention to alert health care providers in industrialized countries about the possibility and implications of such an outbreak (2). Preliminary evaluation of the outbreak determined that all of the patients were white, concurrent STDs were prevalent, and the majority of patients were HIV-positive and had participated in casual sex gatherings and had had unprotected anal sex within the 12 months before onset of symptoms (5). Only 1 patient presented with the classic symptoms of LGV (inguinal adenopathy and genital ulcer). Most of the infected men presented with gastrointestinal symptoms (bloody proctitis with anal discharge and constipation). In all cases, LGV was associated with the presence of high-titre *C. trachomatis* antibodies in sera. LGV is believed to predispose to HIV infection (2). Whether HIV predisposes to LGV is still being debated. HIV infected persons with LGV have more extensive disease and require longer treatment duration with the same drugs used for HIV negative individuals (2). We present here a review of literature on LGV.

The causative agent

Chlamydiae as a group are obligate intracellular parasites (1, 6). Three biovars are recognized until recently when an additional biovar was described

(6). *C. trachomatis,* one of the biovars consist of at least 15 serovars: A, B, Ba, C-k, L_1, L_2 and L_3. This classification is based on antigenic composition, intracellular inclusion types, disease production and susceptibility to sulfonamides (1, 6). Major outer membrane proteins (MOMP) and LPS are the antigenic components used in typing chlamydiae. Serovars Da, Ia and L2a have also been described (1, 6).

Chlamydiae are prokaryotes. They exhibits morphologic and structural similarity to gram negative bacterial including a tri-laminar outer membrane which contains lipopolysaccharides and several membrane proteins that are functionally and structurally analogous to proteins found in *Escherichia coli* (7, 8,). They lack the classic peptidoglycan layer although their genome contains all the genes necessary for its synthesis.

Chlamydiae exhibit a biphasic life cycle consisting of alternating phases known as elementary body (EB) and reticulate body (RB). EB is the infective particle and upon entry into host cell develop into RB from which many EBs will be formed and eventually released from the host cell. Factors governing this transformation are not known but relative concentration of cyclic nucleotides (cAMP and cGMP) is important (1).

The EB and RB differ in metabolic activity, their size, structure of their nucleus and cell membrane. EB is metabolically inert, smaller in size, has electron denser nucleus and extensive disulfide cross-linking between cystein residues both within and between outer membrane proteins compared with RB (1, 6).

Pathogenesis

Following exposure to infective particle, the EB enters squamocolumnar epithelial cells by a receptor mediated endocytosis via a clathrin coated pits (1, 9). Other mechanisms like pinocytosis via non-coated pits can also be used (1, 6). Lysosomal fusion is inhibited by undefined mechanism with EB residing within intracytoplasmic membrane bond vesicle called inclusion body where it transforms to RB from which more EB are subsequently released with host cell rupture (1).

Unlike the more common *C. trachomatis* serotypes A–K, which tend to be confined to columnar epithelial cells in the genital tract and eye, LGV serotypes predominantly infect monocytes and macrophages and pass through the epithelial surface to regional lymph nodes getting disseminated to other body parts (2). *Chlamydia trachomatis* inhibits apoptosis in the infected cells until developmental cycle is completed- a crucial determinant of survival (4, 10).

The mechanism by which *C. trachomatis* induces inflammatory tissue destruction are poorly understood. They are known to produce effectors like cytokines, phosphatases and kinases that influence eukaryotic cell signaling (1). The characteristic histology is that of granuloma formation with development of small abscesses that may become necrotic or coalesce into suppurative foci (1).

CLINICAL FEATURES

The clinical course of LGV can be divided into 3 stages: the primary, secondary and the late stages.

The primary stage

The primary stage presents with a small, painless papule or herpeteiform ulcer, typically seen on the penis, vulva or rectum, after an incubation period of 3–30 days. *C. trachomatis* cannot infect squamous epithelial cells and thus, when primary

lesion occurs on external genitalia or vagina, the organism probably gained entry through minor laceration or abrasions. The lesions may not be noticed by infected people (1, 7).

The secondary stage

The secondary stage usually presents some day to weeks later with painful, unilateral lymph nodes in the inguinal or ano-rectal region. The lymphadenopathy is bilateral in 1/3 of cases (11). When unilateral femoral and inguinal lymphadeopathy occurs, the "groove sign" which is characteristic of LGV is seen being a groove separating the two groups of lymph nodes (1, 6). Proctitis is a common presentation of LGV in women and since lymph vessels from the rectum, cervix and vagina tend to drain to the retroperitoneal area, there is often retroperitoneal lymphadenopathy in women whereas men present more often with unilateral, enlarged, painful inguinal nodes (1, 2). MSM more commonly present with proctitis, protocolitis or enteritis that results from direct inoculation in the anal region and these forms the disease may be associated with retroperitoneal lymphadenopathy (2, 3, 4). Other groups of lymph nodes that may be affected are the deep iliac and obturator (12).

Initially the lymph nodes are discrete and tender with overlying erythema but because of extensive peradenitis, inflammatory cells spread beyond the lymph nodes to the surrounding tissue, forming an inflammatory mass (1, 6). Abscesses develop within coalesce masses forming a bubo that may rupture spontaneously with the development of loculated abscesses, fistula or sinuses. Only about one third of the abscesses rupture (1). Rupture relieves pain and the associated fever, and the sinus may continue to drain thick yellowish pus for several weeks or months before resolving. Healing leaves scaring and relapse occurs in 20% of untreated cases (1).

Other systemic manifestations associated with this stage apart from fever include headache, myalgia, meningismus and arthritis (1, 2, 6).

The late stage

Chronic untreated LGV can lead to lymphatic obstruction, resulting in elephantitis of the genitalia in either sex. Rectal involvement can lead to the formation of strictures and fistulas, which may require surgery to correct (1, 2, 3, 4, 5, 6).

CLINICAL MANAGEMENT:

The diagnosis of LGV is made primarily on the basis of clinical findings but laboratory investigations including microscopy, serologic tests, antigen detection tests, culture or nucleic acid testing are necessary to confirm clinical assessment (1, 6).

Clinical Assessment

The differential diagnosis of sexually acquired ulceration with or without accompanying inguinal adenopathy includes chancroid, herpes, syphilis, donovanosis (granuloma inguinale) and lymphoma (13).

LGV manifesting as inguinal lymphadenopathy can be distinguished from genital herpes by the presence of multiple painful ulcers at the site of primary herpes infection in contrast to painless lesion of LGV. Also, lymphadenopathy is frequently bilateral in herpes unlike LGV.

Syphilitic infection is suggested by a primary lesion with indurated margins (chancre) and bilateral, non-tender inguinal lymphadenopathy.

Large, multiple and extremely tender ulcers with associated lymphadenopathy suggest chancroid.

The pseudo-bubo which occurs in granuloma inguinale is nodules in the skin and subcutaneous

tissue with lymph node involvement arising from secondary bacterial infection (11).

Specimens

Several specimen types could be taken depending on the site and nature of infection, and type of test to be done. Pus aspirates from buboes give high yield on culture (1). Fluctuant nodes should be aspirated through healthy skin. Incision and drainage or excision of nodes may delay healing. Biopsy particularly of uterine tube for diagnosis is considered a research tool. Other specimens include epithelial scrapings, swabs, blood, stool, urine and CSF. Swabs could be of different material types but should be on plastic or metal support, wooden materials are toxic to cell culture.

Specimen for culture must be maintained at 4°c and inoculated within 24 hours otherwise stored frozen at -70°c until inoculation. A useful transport medium has 0.2 mol/L sucrose in 0.02M phosphate buffer, pH 7.0-7.2 with 5% fetal calf serum (14).

Microscopy and Staining

Direct Microscopic examination by cytological method is not sensitive. Direct fluorescent antibody testing can be used to examine endocervical and urethral specimens. The technique is fast but needs verification by other method.

Microscopy is also useful in reading cultures for identification of intra-cytoplasmic inclusions. Giemsa, Macchiavellos and Gimenez stains or (indirect) fluorescent antibody testing (FAT) may be used. Iodine can also demonstrate glycogen granules. FAT is the most sensitive and specific (1).

Culture

The causative agent of LGV grows well in a variety of cell lines. Commonly used cell lines are McCoy cells and HeLa cells. Cell lines are monolayer and are grown on cover slips in shell vials or on the surface of multi-well cell culture dishes containing cycloheamine. Incubation is for 40-72 hours following which microscopy is used to examine culture (15).

Serology

Being non-specific, serology is used to complement other tests. CFT is available and titres grater than or equal to 1:64 is supportive of LGV. Micro-IF test is more sensitive and can detect IgM antibodies (6).

Direct Antigen Detection

This could be by EIA or FAT. Both are fast and reasonably sensitive and specific though not effective as culture. MOMP (species specific) and LPS (genus specific) antigens are targeted (14).

Nucleic acid tests

This could be by Hybridization or PCR. Hybridization identifies rRNA with sensitivity in the range of EIA. PCR is the most resent of diagnostic techniques with sensitivity, specificity and predictive value all approaching 100% (1, 14).

Histology

The characteristic histology is that of granuloma formation with development of small abscesses that may become necrotic or coalesce into suppurative foci. Similar histological features can be found in cat-scratch disease and inflammatory bowel diseases (1, 2).

Treatment

Recommended regimens of treatment of LGV include 100 mg of doxycycline taken orally twice a day for 14–21 days, or 500 mg of erythromycin taken orally 4 times a day for 14–21 days (1, 2). Sulfonamides though active in-vitro does not produce bacteriological cure (1). Other useful drugs include minocycline, chloramphenicol, and rifampicin (16).

Sex partners who had contact with the patient within 30 days of the patient's onset of symptoms should be given either 1 g of azithromycin in a single dose or 100 mg of doxycycline twice daily for 7 days (Post exposure prophylaxis) (1, 2, 3).

Surgical aspiration, through healthy skin, of buboes is important to prevent rupture and sinus formation. Strictures may also require surgery (1).

Control and Prevention

Barrier methods, such as condoms, provide good protection against transmission. The use of vaginal sponges containing the spermicide nonoxynol 9, which has antichlamydia activity, is also advocated (1). Counseling on delaying the age of first sexual intercourse is an important attitudinal change that must be induced. Central to control measure is identification and treatment of affected persons. Patients must be investigated for other STDs and treated appropriately (17).

CONCLUSION

LGV is a sexually transmitted disease with resent outbreak amongst MSM and atypical feature when coexisting with HIV infection. High index of suspicion is required for this disease with the current HIV pandemic. Proper treatment must be instituted if affected persons must escape late sequelae of the disease and not being a risk to the community at large.

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CASE REPORT
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FULMINATING LYMPHOGRANULOMA VENERUM IN AN HIV-POSITIVE MIDDLE AGED PATENT MASCURADING AS SOFT TISSUE SARCOMA.

[1]*Adigun, Ismaila Abiona, [2] Fadeyi, Abayomi and [2] Adegbora, Boaz

[1]Division of Plastic and Reconstructive Surgery, Department of Surgery, [2] Department of Medical Microbiology, University of Ilorin Teaching Hospital, Ilorin, Nigeria.

*** Correspondence author e-mail:** **Tel:** 08033464868, 08051750085

ABSTRACT

This is a case report of a 40-year old male patient presenting with a 17-month history of right original/growing swellings. Initial chemical assessment was suggestive of soft tissue sarcoma, and he was admitted in the surgical ward. Histopathology investigation suggested inflammatory process was taking place. Assessment for Chlarydia IgG with Enzyme-Linked Immunosorbent Assay (ELISA) using Immunocomb (Organics, Israel) gave a positive titre of 1 in 32. HIV serology using p24 ELISA and Western Blotting gave positive results.
He made significant progress after two weeks on antibioties and antiretroviral (HAART) therapy.
KEY WORDS: LGV, HIV, Sarcoma

INTRODUCTION

Lymphogranuloma venerum (LGV) is a systemic sexually transmitted disease (STD) caused by *Chlamydia trachomatis* serotypes L1, L2 and L3. LGV is endemic in parts of Africa, Asia, South America and the Caribbean but rare in Western countries where the disease occurs mainly in sporadic form (1).

Large outbreaks of LGV occurred recently in Europe and America mostly among men who have sex with men (MSM) (2, 3, 4). Most of the infected men were co-infected with human immunodeficiency virus (HIV) and had atypical presentation. HIV infected persons with LGV have more extensive disease, and this may cause diagnostic confusion as found in this patient being reported. We present here therefore a case of fulminating LGV in a middle aged man with HIV infection who presented with signs and symptoms suggestive of soft tissue sarcoma (STS) of the thigh.

CASE PRESNTATION

Mr. B is a 40-year-old Nigerian who had tertiary education and resident in Lagos. He has history of 17 month right inguinal/ groin ulcerating swelling, 12-month right leg multiple ulcerating swellings and 3-month progressive weight loss. Lesions had started as fluctuant swellings of the affected areas with some treatment received before presentation at the University of Ilorin Teaching Hospital including incision and drainage on right inguinal and leg swellings. This resulted in progressive increase in wound size, failure of wound to heal and production of sero-purulent discharge. No history suggestive of primary LGV. No identifiable risk factor. The only admitted sexual partner (wife) was apparently healthy at the time the patient presented. On examination, there was lymphoedema ~~of right lower~~ limb, right inguinal/ groin ulcerating mass which together measures 20cm x 18cm (Figures 1) with raised irregular edges and right leg, below the knee joint ulcer measuring 10cm x 12cm with sloppy edge. The

ulcers were all producing offensive seropurulent discharge. The anterior abdominal wall veins were distended; the patient was chronically wasted and had left inguinal lymphadenopathy. A clinical assessment of soft tissue sarcoma (STS) was made and patient admitted for investigations. Significant results included that of histopathology that was not suggestive of malignancy but revealed focus of inflammation and serological assessment for *Chlamydia* IgG with ImmunoComb (Orgenics, Israel) that gave significant titre (1:32). Patient was confirmed HIV positive by p24 ELISA and Western Blotting techniques. Treatment given included doxycycline 100mg bd for more than 21 days, twice daily dressing of wounds with honey and highly active anti-retroviral therapy (HAART) including Starvudine-400mg b.d, Nevirapine-200mg b.d and Lamivudine. 150mg b.d. Patient improved significantly as evidenced by the wounds reducing size, less discharging and subsiding lymphoedema (Figure 2).

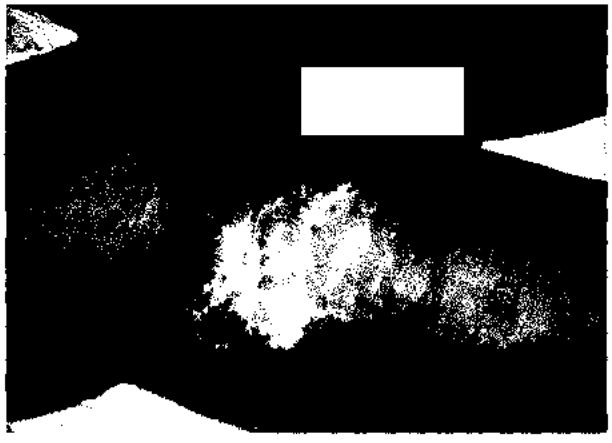

Figure 1: Fumigating left groin / inguinal mass before treatment

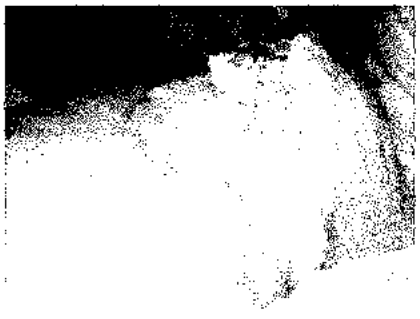

Figure 2: Fumigating left groin/ inguinal mass after two weeks treatment

DISCUSSION

The clinical course of LGV can be divided into the primary, secondary and the late stages. The prymary lesions may not be noticed by infected people (1) as in this patient. He presented earlier to a medical practitioner, during the secondary stage, who aspirated the swellings before doing incision and drainage that worsened the patient's condition. On presentation in our hospital, diagnosis of LGV was not strongly considered until histological assessment of the masses was done. Reason being that rarely do we see LGV in our hospital in recent time despite the claim that the disease is endemic in Africa. Besides, the association of LGV with HIV caused the deviation from the classical presentation pattern. This however is in line with resent reports from the Western industrialized nations (2, 3, 4) although the patient denied history of homosexualism and did not have features of colitis or procto-colitis. The only admitted sexual partner (wife) was apparently healthy at the time of presentation. What then are the other factors at play?

There were diagnostic challenges. The patient wife who was clinically healthy could not be

screened for HIV and *Chlamydia* infection due to socio-cultural barriers. The serological kit used to screen for *Chlamydia*, though certified all right by the manufacturer, would have been more useful if IgM rather than IgG was assayed for. Diagnostic challenges not withstanding, the patient responded well to the medications given. We recommend that medical practitioners should not forget to exclude LGV before incising any inguinal fluctuant swellings and screen for HIV in suspected LGV cases.

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CORRECTIONS

1. In our journal Vol8(2):107-113 we published an article titled "Intestinal Helminthiasis in Children in a suburb of Lagos, Nigeria: Evaluation of risk factors and Habits". We inadvertently omitted Table 1c in page 111. This table is published below.

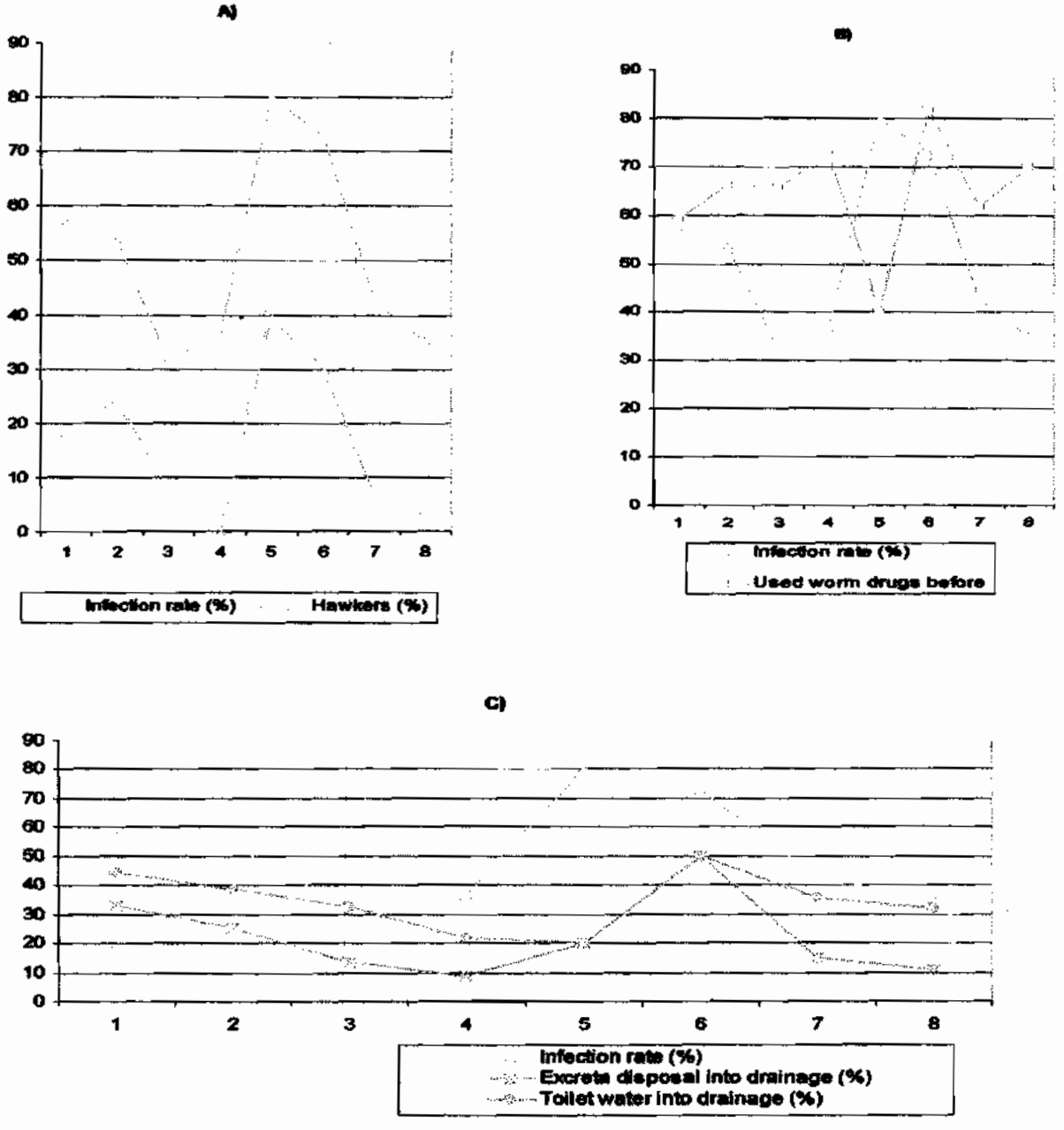

Figure 1c: Hawking (A), use of antihelmintihics (B), community hygiene (C) and prevalence across different group of parental educational status and occupation.
Y-axis = Positive values (%); X-axis = Groups:

1 = Trading Fathers	4 = Professional Mothers	7 = Educated Fathers
2 = Trading Mothers	5 = Uneducated Fathers	8 = Educated Mothers
3 = Professional Fathers	6 = Uneducated Mothers	

2. In our publication of May 2006 we gave Omotosho's initials as J.O. instead of J.A. It is corrected below.

Buhari, M. O. and Omotayo, J. A.; Viruses and Cancer – An Overview. Afr. J. Clin. Exp. Microbiol (2006) 7(2): 125-131