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DIVERSE GENETIC SUBTYPES OF HIV-1 AMONG FEMALE SEX WORKERS IN IBADAN, NIGERIA

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Running title: Genetic subtypes of HIV-1 among female sex workers in Nigeria.

Keywords: Diverse, HIV, subtypes, Female Sex workers and Vaccine

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ABSTRACT
Background:

Genetic diversity is the hallmark of HIV-1 infection. It differs among geographical regions throughout the world. This study was undertaken to identify the predominant HIV-1 subtypes among infected female sex workers (FSWs) in Nigeria. Methods: Two hundred and fifty FSWs from brothels in Ibadan Nigeria were screened for HIV antibody using ELISA. All reactive samples were further tested by the Western Blot Techniques. Peripheral Blood Mononuclear Cells (PBMCs) were separated from the blood samples of each subject. Fragments of HIV Proviral DNA was amplified and genetic subtypes of HIV-1 was determined by direct sequencing of the *env* and *gag* genes of the viral genome followed by phylogenetic analysis . Results: The age of the FSWs ranged from 15 to 55 years old (Mean = 25.8years, SD =3.74). Majority were Nigerians while others (1.6 %) were from neighboring West Africa countries. Four (1.6%) of the FSWs were active for less than one year as sex workers, and the mean length of sex work was 2.80 years (Range = 1.0 – 15.0 years). Sixty-four (25.6%) of the 250 CSWs were positive for HIV-1 while 7 (2-8%) had dual infections to HIV-1 / HIV-2. Among the 34 HIV-1 strains characterized by sequencing, 19 (55-9%) were subtype G, 9 (26.5%) CRF02_A/G, 3 (8.8%) CRF06_cpx while 1 (2.9%) each were identified as subtype C, CRF01_A/E and CRF09_cpx respectively. Nineteen (55.9%) of the FSWs with subtype G had been active in the sex work for between one to five years. The youngest of the HIV -1 infected FSWs with sexual activity of less than a year had subtype G strain. There is a significant probability that infection with this subtype occurred with a short incubation period (p< 0.05). Conclusion: This study showed a wide range of HIV- 1 subtypes among FSWs in Nigeria. The situation poses serious challenge for the design of HIV vaccine candidate for use in Nigeria.

INTRODUCTION

The Acquired Immune Deficiency Syndrome (AIDS) epidemic has exacted a terrible toll in terms of loss of life and decreased quantity of life worldwide, especially in Africa, where 70% of deaths from HIV-1 infection have occurred (1).

The first case of AIDS in Nigeria was reported in 1986. From 2 cases in 1986, it has been estimated that 4,050,000 HIV infections have occurred in Nigeria[2], and it has spread extensively to the rural areas of the Country (3, 4, 5).

In Africa, infection has been mainly by heterosexual intercourse, with Female sex workers (FSWs), long distant truck drivers and migrant labour serving as vehicles of spread (5).

Female sex workers (FSWs) have been at high-risk of infection since the beginning of the HIV epidemic all over the world. Multiple sex partners, irregular condom use, and co-infection with other sexual transmitted infections (STIs) are the principal risk factors for HIV infection among FSWs (7). Between 1995 and 2003 the prevalence rose from 6.4% in STD patients (8) to 34.3% in FSWs (9). The penetration into the general population cannot be over emphasized.

In women, the glandular epithelia have HIV in the zone of transformation between the columnar and squamous cell of the cervix (10). While in men, HIV is detectable in seminal cells, some sperm cells and their precursors (11). Because of high incidence of sexually transmitted infections (STIs) in African sex workers, immunologic stimulations to this infection may be higher than among the general African population (12).

HIV-1 and HIV-2 show heterogeneity among isolates (13). On the basis of the phylogenetic analysis of the *env and gag* genes, HIV-1 has been divided into three distinct groups (M, O, & N.), all of which cause AIDS in infected individuals. Group M ("For Major") comprises the greatest majority of HIV-1 infections worldwide and has been further subdivided into subtypes and sub subtypes termed A-D, F.H, J, K, and Circulating Recombinants Forms; CRF 01 to 34 (13,14). Whereas several HIV-1 subtypes co-circulate in many parts of the world, some regions seem to have predominant subtypes. Subtype B is the most predominant form in North America and Europe while subtype E is highly prevalent in part of South East Asia (where B is the minority form) (15). Subtypes A, C, and D are the most common subtypes in Africa. In Mali 80% of the HIV-1 infected sex workers were subtype A, 10% belong to subtype G and the minority belong to subtypes C and D (16), while in Kenya 56% of the infected subjects had subtypes A while others have subtypes C and D with other recombinants circulating forms (17).

In Nigeria, the first HIV-1 strain that was partially characterized was found to be subtype A (18). In recent time , we have co-circulation of multiple subtypes including, A, B, C, D, E and other groups in Nigeria with subtype C being the most prevalent (19).

The city of Ibadan, which is acclaimed to be the largest city in West Africa, and capital of Oyo State, is a major commercial centre with long distant transport workers, migrant labour workers, civil servants and students of higher institutions which have sex with multiple female sex workers with risk of acquiring HIV infection.

The occurrences of these multiple variants of HIV in different geographical regions raise the possibility that future vaccine strategies may have to consider polyvalent constructs. The effective evaluation of vaccine candidates will depend on thorough surveillance and characterization of viral variants (20).

Since HIV infection most often results from heterosexual intercourse, female sex workers in various brothels in Ibadan are likely to be the source of detection of new subtypes that might have been introduced into the country and likely point for rapid spread of these different subtypes. Therefore this study was aimed at providing information on the sero-prevalence of HIV among female sex workers in Ibadan and also to identify the predominant subtypes of HIV-1 among them.

MATERIALS AND METHODS

Study population and Sample collection

A cross- sectional seroprevalence study was conducted in a population at high risk for HIV infection from November 2002 and July 2003 in Ibadan, South Western Nigeria. Only those subjects who were willing to participate were provided written informed consent, enrolled, and sampled. A total of two hundred and fifty Female sex workers from various brothels in Ibadan that had been in the profession for at least one month and duly signed informed consent were recruited into the study. The study protocol was approved by the University of Ibadan/ University College Hospital (UCH) ethical review committee. Semi-structured questionnaire was administered to each of the FSW who gave informed consent, as an instrument to elicit base line information on their demographic characteristics and reproductive health history.

Ten millimeters of EDTA – anti coagulated blood samples were collected from the recruited female sex workers. These samples were transported on an ice parks in an insulated box to the Diagnostic Laboratory, Virology Department, University College Hospital, Ibadan for processing. Plasma or sera were subsequently separated and stored in aliquots and maintained at -70^{o}c until tested. Clinical examinations of the lower genito-urinary tracts were carried out for signs of infections such as vaginal discharge, endocervical discharge, genital ulcer diseases and genital mass (genital warts and bartholin's cyst).

Antibody Screening and Confirmation

All the sera or plasma separated from the samples collected were screened using Enzyme Linked Immunosorbent Assay (ELISA) techniques with commercial available kits (GENSCREEN PLUS HIV by Bio-Rad in France). All reactive samples were confirmed by Western blot assay (New LAV BLOT 1 by Bio-Rad, in France).

DNA Extraction and Polymerase Chain Reactions

Qiagen kit was used for the extraction of DNA from the Peripheral Blood Mononuclear Cells separated from the whole blood of the FSWs that had been previously stored and maintained at -70^{0}C. The Qiagen genomic DNA purification procedure is designed to isolate chromosomal DNA 20-150kb directly from the whole blood, buffy coat and cultured cells. Genomic DNA is eluted in a high salt buffer, concentrated and desalted by isopropanol

precipitation. About 5ul of the genomic DNA of the confirmed ELISA reactive blood samples were combined with primers: WTI (KK30) and WT2 (KK40), buffers, nucleotides, *taq* polymerase and distilled water in a micro centrifuge tubes. Positive and negative controls were included in each run. The total volume in each tube was made up to 50 ul in the 1st round of PCR. Each tube was placed in a thermal cycler and subjected to PCR under the following cyclic conditions: 94oc, 55oc and 72oc for 1 minute (5 cycles) : 94 0C for 50s, 57oC for 50s and 72oC for 1mim 30s (30 cycles) and 72o for 1mimute (1Cycle)

The 2nd round amplification was performed with 5ul of the 1st round PCR products, primers (KK30, KK40), buffers, nucleotides, *taq* polymerase and distilled water adding up to 100ul of total volume. These were also subjected to the same cyclic conditions except that the five cycles initially were reduced to three.

Detection of Amplified Products and Sequencing

Detection methods that rely on size separation and visualization of PCR products include agarose and polyacrylamide gel electrophoresis(21,22).

These methods allow the assessment of the presence and the size of the amplified products. Agarose gel used to separate a wide range of DNA fragment sizes. Visualization was achieved by staining the gel with ethidium bromide, a chromogen dye that binds to nucleic acid and florescence under UV light.

Genome fragments could be amplified and directly sequenced (without cloning) using a cycle-sequencing protocol adapted for the ABI automatic sequencer.

In order to determine the subtype of genome fragments or entire HIV -1 genome, the sequences in questions were aligned with reference sequences of different known subtypes. 34 of the PCR amplified DNA fragments of the reactive ELISA and western blot confirmed HIV-1 samples were run at the APIN Laboratory, Harvard University, Department of Immunology and Infectious Diseases, Boston, U.S.A. for the identification of different subtypes by direct sequencing. Phylogenetic analysis was performed by first aligning the sequences obtained with reference sequences.

Data Analysis

Data was analyzed using SPSS for widows' version 12.0. Association between groups was performed using the chi-square test for categorical variables and student-t test for continuous variables. Logistic regression was used to adjust for potential cofounders. Analysis of variance was calculated and statistical tests were carried out at 5 % significance level

RESULTS

A total of 250 Female sex workers (FSWs) from various brothels in Ibadan were enrolled and screened for HIV antibody seropositivity and predominant HIV-1/2 subtypes was determined during the period of study. As shown in Table 1, demographic characteristics of the FSWs showed that their mean age was 28.8years (Range 15-55yrs; SD = 7.35). Majority (62.8%) were in the 20-29 yrs age group. 174(69.6%) were singles 37(14.8%) were divorced, 7(2.8%) were separated while 30(12.0%) were still married.

More than two-third of the uninfected FSWs (71.5%) were single while 11.8 % were married, others were divorced, separated or widowed. Majority (98.4%) were from different parts of Nigeria while 1.6% were from Republic of Benin, Togo, Ghana and Cote d'Ivoire. Forty- one (64.1%) of the HIV – infected FSWs had their first sexual debut between the ages of 16-20 yrs and more than half (57.8%) usually attended to one to five sexual partners per day. (Table1).

Sixty-four (25.6%) of the 250 FSWs screened for antibody to HIV were confirmed HIV-1 seropositive by the Western Blot technique. Seven (2.8%) had dual infections to HIV-1/2.There was no FSW with only HIV-2 infection. The number of HIV-1 infection was significantly higher than HIV-2 ($p<0.05$). Thus the seroprevalence of HIV-1 antibody among the Female sex workers in Ibadan was 25.6%.

Among the 34 HIV-1 strains that were characterized by sequencing, 19 (55.9%) were subtype G, 9 (26.5%) belonged to CRF_02*A/G*, 3 (8.8%) belonged to CRF_06*cpx* while 1 (2.9%) each were identified as subtype C, CRF_01*A/E* and CRF_09*cpx* respectively (Table 2).

Among the 19 subtype G, 9 (47.4%) were obtained from FSWs who hailed from Edo and Delta states in the South-south zone of Nigeria while 3 (15.3%) were from Benue state in the middle belt zone. The only subtypes C identified was from FSW who hailed from Togo. (Table 3).The youngest (15years) of the HIV infected FSWs characterized as subtype G has been involved in sex trade for less than one year while 11 (57.9%) of them have been active in the business between one to five years. Also out of the 9 characterized as CRF_02A/G, 5 (55.6%) have been active in the trade between 1-5 years. This suggests that there is probability that these infections occurred very recently($P < 0.05$).

Clinical presentations that have been found to be significantly associated with HIV-1 subtype G and CRF_02A/G were weight loss , episodes of recurrent diarrheas, skin lesions recurrent vaginal discharge and puritus vulvae ($p<0.05$). The only clinical manifestation noticed with FSW with subtype C who hailed from Togo was recurrent genital herpes.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS & REPRODUCTIVE HEALTH HISTORY OF THE COMMERCIAL FEMALE SEX WORKERS

CHARACTERISTICS	FREQUENCY N=250	PERCENTAGE %	HIV POSITIVE N= 64	PERCENTAGE %
(a) Age				
< 20	32	12.8	4	6.2
20-24	101	40.4	28	43.8
25-29	56	22.4	17	26.5
30-39	45	18.0	12	18.8
40-49	13	5.2	2	3.1
> 50	3	1.2	1	1.6
(b) Marital Status				
Single	174	69.6	41	64.1
Married	30	12.0	8	12.5
Divorced	37	14.8	13	20.3
Separated	7	2.8	2	3.1
Widowed	2	0.8	0	0.0
(c) States/Zones/Nationality				
Edo/Delta	148	59.2	32	50.0
South East	41	16.4	13	20.3
South West	32	12.8	5	7.8
Middle Belt	16	6.4	8	12.5
South South	8	3.2	3	4.7
Other National	4	1.6	3	4.7
(d) Level of Education				
No formal	15	6.0	3	4.7
Primary education	87	34.8	22	34.4
Secondary	142	56.8	36	56.2
Post secondary	6	2.4	3	4.7
(e) Age of First Sexual exposure (Yrs)				
<11	3	1.6	1	1.6
11-15	66	33.2	17	26.5
16-20	109	60.0	41	64.1
>20	8	5.2	5	7.8
(f) Duration of Work as CSWs (Yrs)				
<1	2	1.1	2	3.1
1-5	112	60.2	37	57.8
6-10	54	29.0	19	29.7
11-15	15	8.1	5	7.8
16-20	3	1.6	1	1.6
(g) Number of sexual partners / day				
<5	119	64.0	41	64.1
6-10	60	32.2	22	34.4
>10	7	3.8	1	1.5
Total	186	100.0	64	100.0

TABLE 2: DISTRIBUTION OF HIV-1 SUBTYPES AMONG FSWS

Subtypes	Frequency	Percentage %
C	1	2.9
G	19	55.9
CRF_01	1	2.9
CRF_02	9	26.5
CRF_06	3	8.8
CRF_09	4	8.8
CRF_09	1	2.8
Total	34	100

TABLE 3: DISTRIBUTION OF HIV -1 SUBTYPES BY AGE AND NIGERIAN ZONES OF ORIGIN

	C	G	CRF01	CRF02	CRF06	CRF09
AGE <20		2(10.6%)		1(11.1%)		
20-29	1(100%)	14(73.5%)	1(100%)	4(44.4%)	3(100%)	
30-39		1(5.3%)		3(33.3%)		1(100%)
40-49		1(5.3%)		1(11.1%)		
50-59		1(5.3%)				
Zones of Origin	C		CRF01	CRF02	CRF06	CRF09
South East		5(26.3%)	1(100%)			
South West		1(5.3%)				1(100%)
Middle Belt		3(15.7%)		9(100.0%)	3(100%)	
South South		10(53.0%)				
Other Nationality – Togo	1(100%)					

DISCUSSION

In Africa the highest sexually transmitted infections (STIs) and HIV infection has been reported among Female sex workers (FSWs) who are at increasing risk of acquiring and transmitting STIs and Human Immunodeficiency Virus (HIV) (17). The AIDS epidemic has exacted a terrible toll in term of life and decreased quality of life worldwide (1).

Women, especially young ones, generally lack the social power to set the terms for sexual relationship. These women live in poverty and are often coerced to trade sex for support and may be forced into prostitution. A large member of unmarried, unemployed female brothel- based sex workers are all over Ibadan and they cater for the sexual needs of the male populations, mostly of the low income group asking for payment in cash in exchange for a short sexual relationship (8).

This study showed that most of the sex workers (75.6%) were below 30years of age with 62.8% in the 20-29 years age group while 1.2% were over 50years of age. This is consistent with the finding of Bakare *et al(9)* that reported 63.3% in 20-29years age group while Umar and other researchers (23-24) reported that younger women in the age group of 20-29 years were the ones mostly engaged in sex work. This is not a surprise as there is usually higher demand for younger women by men who patronize sex workers.

The prevalence rate of HIV- 1 was 25.6%. There was no sex worker with only HIV-2 infection while 2.8% had dual infections of HIV-1 and HIV-2. This prevalence was however lower than 76.0% reported in studies carried out by Egar *et al* in Jos (25) and 34.5% by Bakare et al(9,26) in Ibadan. This finding might not be unconnected with the fact that FSWs in their mid- to late thirties of age have died of undisclosed illness suspected to be AIDS. Majority of

the younger women who are now being introduced were having protected sexual intercourse with condoms.
Human Immunodeficiency virus type-2 (HIV-2) is less pathogenic than HIV-1. Lower HIV prevalence

The genomic diversity of HIV-1 has been a continuous challenge for the development of diagnostic test, anti retroviral therapies and the development of a preventive medicine.

Among 34 HIV-1 strains characterized, more than half (55.9%) were subtype G, 1(2.9%) belonged to subtype C. CRF02 which is the most predominant recombinant form constitutes about 26.5% of the HIV strains of the infected FSWs. 3(8.8%) were identified as CRF.06 while others CRF.01 and CRF.09 constitutes 2.9% each respectively. This finding is similar to earlier studies of Abimiku *et al (*28) that reported the presence of subtype G from Jos, Nigeria. It is however different with other studies where subtype G infections have been described in diverse locations from East to West Africa, though rarely as a major proportion of the infection (29)

In different locations in Africa like Mali, Republic of Benin, Nigeria and Democratic Republic of Congo, subtype G rarely account for more than 20% all HIV-1 infections. This is the first time that the prevalence of HIV-1 subtype G is exceeding 50% of the HIV strains identified. Contrary to the study conducted in Nigeria by Odaibo et al (30), a heterogeneous distribution of at least 5 subtypes were observed with subtype C being the most predominant in the south-western part of Nigeria.
In a similar study conducted in Bamako, Mali among the female sex workers (FCSWs) (16), it was reported that subtype A was the predominant HIV strain identified among them while 15.1% were subtype G . Subtype A was not detected in this study.
HIV subtype B that is the most prevalent in Western Europe and North America has not been linked with any major epidemics in Africa (31-32). Though the prevalence was 2.5% in the study conducted by Odaibo in 1999(30), it was not detected in this study. The prevalence of subtype C in this study was low (2.9%) and the only infected FSW with this subtype migrated from Togo to Nigeria. This is however contrary to other reports which indicated that subtype C was the most prevalent subtype in HIV-1 pandemic and it has been found to account for the majority of the intense epidemic observed in South Africa and Ethiopia (33-34). Subtypes D & F were not detected in this study but they had been found to be the most prevalent subtype in Uganda and Kenya (35-36).

recorded in this study was consistent with other studies reported in selected populations in other African countries (23, 24).

Among HIV-1 circulating recombinants forms, CRF. 02 was the predominant and accounted for 26.5% of the HIV-1 strains identified in this study. This CRF often designated as "1bNG", the name given to the first sample described from Ibadan, Nigeria (37). This is contrary to the earlier study(30) where 18.9% of the strains belonged to subtype A. Many of the genomes within the 1bNG CRF cluster may have been erroneously classified as subtype A. This subtype has been responsible for regional epidemics along the coast of West and East Africa (38).
Detection of the CRF 06 cpx-a complex recombinants between Subtypes A, G, J, and K which constituted 8.8% in this study was in agreement with the findings of Montavon *et al (*39). However, this was the first time this complex recombinant form has been isolated in Nigeria.
CRF01-AE which has been responsible for a large number of infections in Thailand(40) and other South-east Asian countries (40) was detected in this study, though the prevalence was low (2.9%).
Information about recombinant CRF.09 cpx is very limited (13). Its detection in this study would however form a baseline data for further studies in this part of the country. The youngest of the HIV infected FSWs with sexual activity of less than a year had HIV-1 Subtype G while 55.9% of the FSWs with subtype G have been active in the commercial sex work between 1-5years. These findings gave a significant probability that infections with this subtype occurred very recently and it has very short incubation period (P<O.05). The report was also similar with that of Peeters *et al* (16), which stated that among the 10 HIV-1 subtype G strains obtained, 8 were from younger FSWs with an activity of less than a year .
All the HIV-1 infected FSWs who had subtypes G, CRF01, CRF02, CRF06 and CRF09 usually engaged in heterosexual mode of intercourse while FSW with subtype C usually practiced bisexual mode of intercourse. There is significant statistical relationship between bisexual mode of intercourse and HIV infection.

CONCLUSION

Until the problem of economic development in Nigeria is tackled, convincing young people to adapt their sexual behaviours to secure their future remains difficult. The recent renewed international attention on HIV/AIDS epidemics in sub-Saharan African should translate to commitment to reconstruct social services and to tackle economical underdevelopment.

Additional efforts are needed to provide alternative economic choices for young women.It is important to continue the surveillance of subtypes on a systematic basis in order to see to what extent the proportions of the different subtypes will change over time. Since there are diverse circulating strains of HIV in the country, a polyvalent vaccine will be required for effective preventive prophylaxis against HIV in Nigeria. Given this diverse nature of HIV-1 subtypes , this epidemic may well be more challenging for HIV vaccine candidate design than previously anticipated.

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MOLLUSCUM CONTAGIOSUM VIRUS INFECTION AMONGST PLWHA IN IBADAN, NIGERIA

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RUNNING TITLE: *MOLLUSCUM CONTAGIOSUM* VIRUS INFECTION IN IBADAN, NIGERIA.

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ABSTRACT

Background: *Molluscum contagiosum* (MC) infection is caused by a pox virus and the virus is probably passed on by direct skin-to-skin contact which may affect any part of the body. There is anecdotal evidence associating facial lesions with HIV-related immunodeficiency. This study was aimed to determine the prevalence and associated risk factors of *Molluscum contagiosum* infection among PLWHAs attending ART clinic at the University College Hospital, Ibadan, Nigeria. Methods: This is a descriptive cross-sectional survey of 5,207 patients (3519 female and 1688 males) attending ART clinic between January 2006 and December 2007. Physicians performed complete physical and pelvic examinations. Diagnosis of *Molluscum Contagiosum* infection was based on the clinical findings of typical lesions on the external genitalia, perianal, trunk, abdominal and facial regions. Results: The mean age of the patients was 34.67 yrs. ± 9.16). About 10% (542) had various sexually transmitted infections (STIs). The male to female ratio was 1: 4.2. One hundred and twenty seven subjects (23.4%) had no formal or primary education with 247 (45.6 %) beingtreatment naïve while 295 (54.4 %) were treatment experienced. Of the 542 PLWHAs with STIs, 3.3 % had undetectable viral load (< 200 copies/ ml) while 272 (50.1 %) had low CD_4 count (< 200 cells / mm^3.) and The Mean log_{10} viral load was 5.02 ± 0.94. *Molluscum Contagiosum* infection was diagnosed in 13 patients (0.024%; 8 females and 5 males). Vaginal Candidiasis was the commonest genital infection diagnosed in 223 (41.1%) of the patients with STIs. MC patients had higher viral load, lower CD4 count and more likely to be treatment experienced".Conclusions: *Molluscum Contagiosum* infection is not uncommon among the HIV-infected patients, but underreported. Awareness of this cutaneous manifestation should be known to Physicians in AIDS care.

Key Words:*Molluscum contagiosum*, HIV, Sexually Transmitted Infection

INTRODUCTION

Molluscum contagiosum virus (MCV) infection is a disorder of the skin and mucous membranes, characterized by discrete, single or multiple, flesh-colored papules. It was first described by Bateman in 1817 (1). MCV infection is caused by up to four closely-related types of pox virus, MCV-1 to MCV-4 and their variants (2). In small children, virtually all infections are caused by MCV-1; whereas in patients infected with HIV, MCV-2 causes the majority (60 %) of infections, suggesting that HIV infection-associated Molluscum does not represent recrudescence of childhood Molluscum (3). Epidemiologic studies also suggest that transmission may be related to factors such as warmth and humidity of the climate and poor hygiene (4).

Although, transmission of Mollusci poxvirus in children is thought to occur by intimate skin-to-skin contact or through fomites, in adults, MC is most often sexually transmitted and is being increasingly diagnosed in the sexually active population.*Molluscum contagiosum* virus infection occurs worldwide but is more prevalent in tropical areas (5). The infection is most common in children, sexually active adults, and persons with impaired cellular immunity, particularly HIV-positive patients. MC has a worldwide incidence of between 2-8%. Between 5- 20% of HIV patients have MC infection. The incubation period for MC has been reported to be

between 14 and 50 days, although there are reports of newborns having lesions as early as 7 days post-partum (6).An individual infected with *Molluscum contagiosum* virus can also spread the infection via autoinoculation. The virus infects epidermal keratinocytes and viral replication occurs in the cytoplasm of these cells(7).

The typical lesion is a smooth-surfaced, firm, spherical papule; with an average diameter of 3-5 mm. Giant lesions of up to 1.5 cm have been described and are seen more often in immunocompromised patients. Lesions may be flesh colored or translucent white or light yellow in color. The number of lesions is usually less than 30, but as many as several hundred may be seen. Up to 100 lesions may coalesce to form a plaque.The most distinctive feature of MCV infection is the central umbilication.

It has been recognized for sometime that MC is a common cutaneous disorder seen in patients with HIV infection. The association was first reported in 1983 when it was noted in an autopsy study that two of ten patients with Acquired Immune Deficiency Syndrome (AIDS) had lesions of MCV. In contrast to HIV seronegative adults, in whom MCV lesions are usually genital, lesions in HIV individuals most often involve the face, neck, and trunk. Because this distribution is similar to that seen in children, in whom the spread is thought to occur through fomites or casual contact, transmission in HIV infected patients does not appear to be solely by sexual contact(8).

Patients with advanced HIV disease often have persistent Molluscum lesions that gradually increase in size, with some patients developing giant, tumor-like, nodular lesions that can exceed 1 cm in diameter and become very deforming(8-10). The number of lesions generally varies inversely with CD4 cell count, with some patients with very advanced immune suppression developing several hundred lesions in a disseminated pattern(10).

There is paucity of data on the burden of *Molluscum contagiosum* viral infection among adult PLWHA in Nigeria. This study was aimed at providing information on prevalence of *Molluscum contagiosum* infection among PLWHAs attending ART clinic, University College Hospital, Ibadan, Nigeria.

METHODS AND PATIENTS

This was a descriptive cross-sectional survey of 5,207 patients attending ART clinic between January 2006 and December 2007.

For each patient, the demographic data, brief complaints, site of lesion, morphology, progression of the disease, family history, sexual history, and risk factors for HIV infection were noted. Physicians performed complete physical and pelvic examinations for signs of sexually transmitted infections (STIs).

Initial diagnosis of *Molluscum Contagiosum* viral infection was based on the clinical findings of typical multiple, painless dome-shaped lesions with characteristic central umbilication on any of the following areas: the external genitalia, perianal, trunk, abdominal and facial regions(Figure 1-3) .This wasfollowed by the expression of the thick white core from the center of the lesions which was then smeared and stained with Giemsa stain on a slide to reveal Molluscum bodies. The diagnosis was confirmed with hematoxylin –eosin staining of the skin biopsy from the subjects.Histology of the skin biopsies showed the presence of large, eosinophilic, intracytoplasmic Henderson-Patterson inclusion bodies (Figure 4).(11) The urethral, high vaginal and endocervical swabs were taken and processed by standard microbiological methods(12)from each of the subjects to establish diagnosis of other STIs.

Data Analysis

Data was analyzed using SPSS (Inc, Chicago, IL) version 15 software for widows'. Association between groups was performed using the chi-square test for categorical variables and student-t test for continuous variables and statistical tests were carried out at 5 % significance level.

RESULTS

A total of 5,207 patients, 3519 female and 1688 males attended the ARV clinic during the period. The mean age of the patients was 34.67 yrs±9.16). About 10% (542) had various STIs.

The male to female ratio was 1: 4.2. 127 had no formal or primary education, 8.9 % of them were widowed while 9.0 % separated from their spouses. Two hundred and forty-seven of them (45.6 %) were treatment naïve while 295 (54.4 %) were treatment experienced. 27.4% of the 536 PLWHA reported willingness and consistent usage of condom. 3.3 % out of the 542 PLWHAs with STIs, had undetectable viral load (< 200 copies/ ml) while 272 (50.1 %) had low CD_4 count (< 200 cells / mm^3.) and The Mean log viral load was 5.02 $\pm$ 0.94.

The prevalence of *Molluscum Contagiosum* infection was 0.024 %(8 female and 5male). 10-20 Molluscum contagiosum lesions were seen in 6 cases, followed by

less than 10 in 3 cases, 20-30 lesions in 2 cases, and more than 40 lesions in 2 cases. Vulvo-vaginal Candidiasis was the commonest genital infection diagnosed in 223 (41.1%) of those screened.

Higher risky sexual behavior was found to be significantly associated with low level of education (P<0. 0001) while treatment- experienced patients were more likely to use of condom (Table 1).Other inflammatory genital infections diagnosed were; genital warts-(35.0%), bacterial vaginosis (27.1%), trichomoniasis (10.0 %) and tinea cruris (0.5%).

MC patients had higher viral load, low CD4count (mean-85 cells/mm^3) and more likely to be treatment experienced.

TABLE 1: ODD RATIO FOR TREATMENT STATUS AND CONDOM USE

Treatment Status	CONDOM USE : Yes	CONDOM USE : No	TOTAL	OR (95% CI)
Naïve	37 (15.4%)	204 (84.6%)	**241(45.0%)**	**3.28 (2.15-5.00)**
Experienced	110 (37.3%)	185 (62.7%)	**295 (55.0%)**	
TOTAL	**147 (27.4%)**	**389 (73.6%)**	**536**	

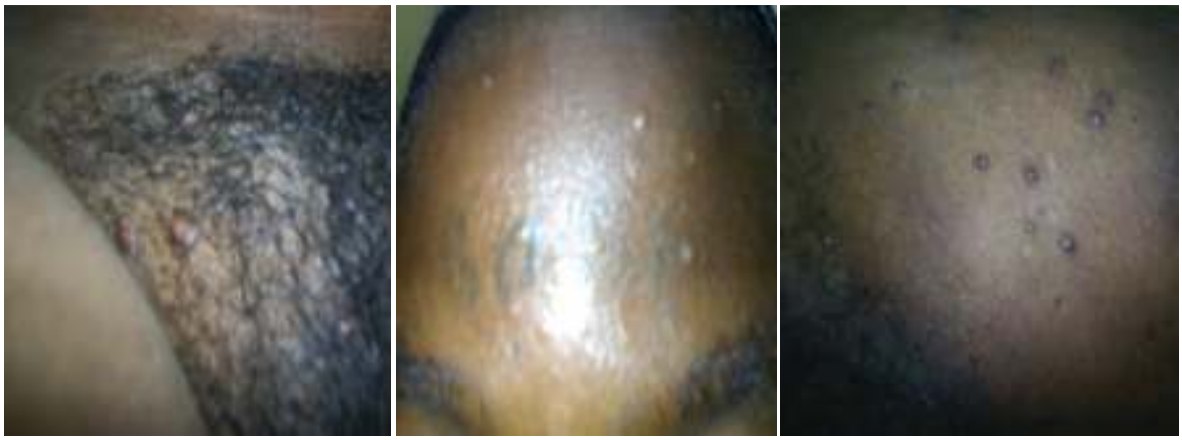

Figure 1: Genital Molluscum contagiosumContagiosum Lesion **Figure 2 : Facial Molluscum Contagious lesion** **Figure 3: Abdominal Molluscum**

The prevalence of *Molluscum contagiosum* was 2.0 times higher in patients with higher Viral Load (Mean Log – 1.97), lower CD4 Count (Mean – 85 Cells/mm^3), treatment experienced compared to treatment naïve patients.

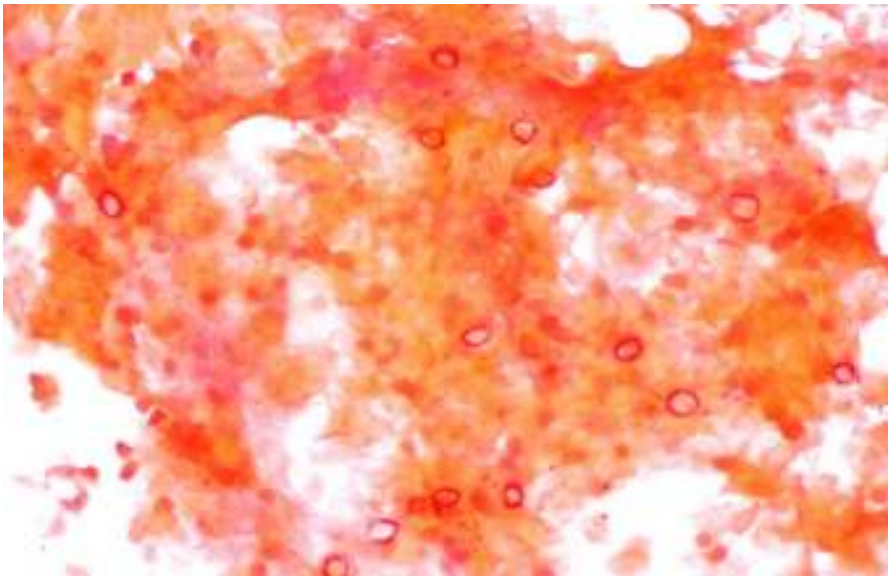

Fig 4: Photomicrograph of skin scrapings showing

molluscum bodies

DISCUSSION

Although the exact incidence of *Molluscum contagiosum* (MC) infection in HIV-infected persons remains unknown, studies have estimated that 5 to 18% of untreated HIV-infected patients develop the Lesions during their clinical course(13). In our study, the prevalence of MC lesions was 0.024%. This was found to be quite low compared to what was obtained in other studies with similar group of patients(14-16).

MC is a self-limiting disease which if left untreated, will eventually resolve in immunocompetent hosts, though it may be protracted in atopic and immunocompromised individuals. Among HIV-infected patients who develop *Molluscum contagiosum,* most have advanced immune suppression. This was confirmed in our study that showed a mean CD4 count of approximately 85 cells/mm ^{3} among the patients with MC infection. Our finding was also supported by other studies that established that MC tends to affect more advanced stage HIV-positive patients and with low CD4+ levels(10, 12-14).Overall, compared with immune competent persons, Molluscum lesions in HIV-infected persons with advance immune suppression are characterized by greater number, larger size, more rapid growth, and atypical locations (8,10).

In adult patients with HIV infection, facial and multiple-site presentations are common in many patients. There was no significant difference in the appearance or location of lesions in immunosuppressed versus healthy patients. It was also found that clinical presentation of aggressive, disseminated and atypical MC lesions (giant or verrucous) are more common in HIV-positive patients(17,18). In our study, the most common sites of involvement are the genitals, head and neck. Widespread facial lesions, and those persisting with a poor response to treatment, are highly characteristic of late HIV disease. Eyelid Molluscum lesions may also be the initial manifestation of AIDS.

One of the most common, quick, and efficient methods of treatment is Cryotherapy. Liquid nitrogen, dry ice, or Frigiderm are applied to each individual lesion for a few seconds. Repeat treatments at 2-3 week intervals may be required.

An easy method to remove the lesions is by eviscerating the core with an instrument such as a scalpel, sharp tooth pick, the edge of a glass slide, or any other instrument capable of removing the umbilicated core. Because of its simplicity, patients, parents, and caregivers may be taught this method so that new lesions can be treated at home.

Conclusion

Low CD4 cell counts have been linked to widespread facial Mollusca and therefore have become a marker for severe HIV disease. MC associated infection is morecommon among the HIV-infected patients, but

underreported. Awareness of this cutaneous manifestation should be known to Physicians in AIDS care.

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SEROPREVALENCE OF PARVOVIRUS BI9 ANTIBODY IN BLOOD DONORS AND SICKLE CELL DISEASE PATIENTS AT LAGOS UNIVERSITY TEACHING HOSPITAL (LUTH): A COMPARATIVE STUDY.

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ABSTRACT

INTRODUCTION: Parvovirus B19 (PVB19) is a DNA virus transmissible by blood transfusion. It is a major cause of aplastic crisis especially in chronic haemolytic anaemic patients such as sickle cell disease patients.

OBJECTIVE: The study was aimed to determine the seroprevalence of PVB19 in blood donors and sickle cell anaemia (SCA) patients and to evaluate its association with blood transfusion in SCA patients.

METHODS: This is a cross sectional study conducted at the Lagos University Teaching Hospital, Lagos Nigeria. Three hundred participants, consisting of 150 voluntary blood donors and 150 sickle cell anaemia subjects were enrolled into the study. Seroprevalence of parvovirus was determined using ELISA kits for IgG and IgM anti-PVB19 antibodies by Immuno-Biological Laboratories, (IBL) inc. Minneapolis, USA. Results was analyzed with SPSS 11 software and presented in tables. Fishers Exact test, Chi-square and student T-test were used as appropriate to compare variables between both groups. P-values <0.05 were considered significant.

RESULTS: Ninety nine (66%) blood donors were positive for anti-PVB19 IgG antibody while ninety two (61.3%) sickle cell patients were positive. Two (1.3%) blood donors were positive anti-PVB19 IgM antibodies while 8 (5.3%) SCD patients were positive for anti-PVB19 IgM antibodies. There was no significant difference in the seroprevalence of IgG and IgM antiPVB19 virus in both groups. There was no association of parvovirus seroprevalence with blood transfusion.

CONCLUSION: The study has shown a high seroprevalence of IgG anti-PVB19 antibodies in both blood donors and SCA patients. Therefore routine screening for parvovirus infection for donor blood is not justified. However seronegative SCA patients who require blood transfusion should have the blood screened for parvovirus to reduce the risk of associated aplastic crisis.

Key words: Seroprevalence, parvovirus B19, blood donors, sickle cell anaemia

INTRODUCTION

Parvovirus B19 (PVB19) a DNA virus belonging to the parvoviridae family and erythrovirus genus (1). It is one of the transfusion transmissible viruses. Others include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T cell leukaemia virus I (HTLV1), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (1).

It is non-enveloped, with icosahedral nucleocapsid symmetry, and measures 22-24 nm in diameter. The virus has a linear nucleic acid (DNA) of ~5.6 kb in length. There are 3 genotypes 1, 2 and 3. Genotype 1 is responsible for the majority of human infections worldwide; genotypes 2 and 3 appear to have some geographic and temporal variation in distribution. The virus is resistant to dry heat, freezing and lipid solvents. It is inactivated by formalin, β-propiolactone and gamma irradiation (1).

The virus is transmitted mainly as droplet infections, vertically through placental to fetus and through blood transfusion. At risk population include children, pregnant women, immunocompromised and those with chronic haemolytic anemia such sickle cell and Thalassemia patients (1, 2).

Clinical manifestations range from asymptomatic disease in immunocompetent to symptomatic disease in the immunocompromised. Clinical features include erythema infectiosum (fifth disease) in children, arthropathy, aplastic anemia/crisis and fetal hydrops (1).

Acute infection is associated with a viremic phase shortly followed by IgM antibody production (10 – 14 days post-infection). This is followed by IgG antibody production against the viral capsid. Viraemia declines with IgM production; IgM declines after a few months but IgG persists longer to convey immunity against reinfection. Infrequently, low level PVB19 nucleic acid may persist with IgG for months or years.

Parvovirus B19 infection has been reported globally. Recent infection is associated with the secretion of immunoglobulin M (IgM) antibodies in plasma while IgG signify previous exposure.

Anti-parvovirus IgG antibody seroprevalence rates are similar in the United States, Europe, and Asia (3 – 5).

The virus has a direct cytopathic effect on erythroid progenitors in bone marrow leading to an arrest in the maturation and subsequent anaemia. The clinical manifestation varies widely depending on the immunological and haematological status of the host. In individuals with underlying haemolytic disorders, as in patients with sickle cell anaemia (SCA), PVB19 infection may cause transient erythroblastopenia (TEB), characterized by a fall in haemoglobin level with reticulocytopaenia (6). Other manifestations of parvovirus infection include arthritis, vasculitis, myocarditis, liver failure and fetal loss.

Sickle cell anaemia is one of the most prevalent genetic diseases in Nigeria (7). SCA patients have accelerated premature haemolysis with significant reduction in red cell half life. Generally, the chronic haemolysis and resulting anaemia is well tolerated however, a reduction in the level of haemoglobin below the steady state may be detrimental to the patient; necessitating blood transfusion (8, 9). Transfusion may predispose them to increased risk of immunological and infectious complications.

Accurate epidemiologic data on the frequency of Parvovirus B19 infection in patient with sickle cell anaemia are essential for assessing the potential effect of viral prevention programs in this patient population (10). However there are limited data on the seroprevalence of PVB19 infection in our environment hence the justification for this study.

The findings from this work may justify need for routine parvovirus B19 screening of blood products before transfusing sickle cell patients or justify the need for institution of vaccination program SCA patients.

OBJECTIVES

The study was aimed at determining the seroprevalence of parvovirus B19 antibodies (IgG and IgM) among SCA patients and blood donors; to compare the seroprevalence rates between both groups and to determine its association with blood transfusion.

METHODOLOGY

Study design: This was a cross sectional study conducted at the Lagos University Teaching Hospital, Idi-Araba.

Sample size: This was calculated using the Kirkwood formula for cross sectional study. An estimated prevalence rate of 50% was used. A total of 300 subjects were recruited for the study.

Study participants: There are 2 study groups. Group 1 comprises 150 voluntary blood donors recruited from the donor clinic. Group 2 comprises 150 SCA patients attending clinic at the Sickle Cell Centre in LUTH. A structured questionnaire was used to obtain personal and medical data from the subjects and venous blood was collected for serological and haematological analysis.

Ethical Considerations: The study was approved by the ethical committee of the hospital. An informed consent was given by all participants above 18 years while consent was obtained from guardians for patients below 18 years in the language best understood by them.

Inclusion and Exclusion Criteria

Inclusion criteria for blood donors: Age between 18-60 years; Weight >50 kg; Hb >12.5g/dl; Normal blood pressure, pulse, and temperature.

Exclusion criteria for blood donors: History of chronic illness e.g. Hypertension, Diabetes, Asthma; commercial sex workers and Intravenous drug users.

Inclusion criteria for sickle cell patients include SCA aged 5 years and above.

Sample Collection: Ten milliliters (mls) of venous blood was collected from the antecubital fossa using aseptic technique. Five mls was dispensed into a sterile plain bottle and allowed to stand at room

temperature until clotted. The samples was centrifuged, serum separated into another sterile bottle stored at -20^{o}C until the required sample size were obtained.

The other 5mls was dispensed into EDTA bottle for full blood count, reticulocyte count and red cell indices. Samples were analyzed within 2 hours of collection using automated haematology analyzer. The reticulocyte count was performed manually as described in Decie and Lewis (11).

Parvovirus B19 IgG and IgM Assays

IgG and IgM assay were determined using a solid phase enzyme – linked immunosorbent assay (ELISA) kits for IgG and IgM anti-PVB19 antibodies by Immuno-Biological Laboratories, (IBL) inc. Minneapolis, USA.

Assay Procedure

The required number of microtitre strips or wells were selected and inserted into the holder. Wells were filled with 300µL of diluted wash solution and allowed to soak for 5 minutes and aspirated off. Sample was dispensed into each properly identified well using the work sheet as a guide. 100 µL each of negative control, cut-off control, positive control, diluted samples were dispensed into appropriate wells and a microwell was left for substrate blank. Wells were covered with foil and incubated for 60 minutes at room temperature.

The contents of the wells were briskly shaked out and rinsed five times with diluted wash solution and then strike on absorbent paper to remove residual droplets.

100µL of enzyme conjugate was dispensed into each well except the blank. All the wells were covered with foil and incubated for 30 minutes at room temperature. The contents of the wells were briskly shake out and rinsed five times with diluted wash solution and then strike on absorbent paper to remove residual droplets.

100 µL of substrate solution was added into all wells. Wells were covered with foil and incubated for exactly 15 minutes at room temperature. Enzymatic reaction was stopped by adding 100µL of stopped solution to each well. There is a colour change from blue to yellow. The intensity of the colour is proportional to the antibody titre.

Optical density was read at 450nm blanking the instrument with the blank microwell.

Results were interpreted according to manufacturer's instructions.

Internal Quality Control Measures

Negative, cut-off, positive controls and blanks were used in each run.

For IgM assay, patient serum samples are diluted and simultaneously absorbed with sample diluents containing hyper immune anti-IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factor.

Statistical analysis

The results were analyzed using statistical package for social science (SPSS 11.0), and Epi-info 6.0. The results were presented with in frequency tables. Comparism of the seroprevalence of IgG and IgM anti-PBV19 antibodies in both groups was made using chi-square and Fishers Exact test as appropriate. Other numerical parameters were compared using the student T-test. The association of anti- PVB19 antibody with blood transfusion was determined using odd ratio. Significance level was set at $p< 0.05$**.**

RESULTS

Demographic parameters of the subjects

A total of 300 subjects were studied consisting of 150 sickle cell patients and 150 blood donors. Their mean ages (in years) were 20.29 ± 11.27 and 30.43 ± 9.58 for the sickle cell subjects and blood donors respectively. The SCA subjects included 70 (46.7%) females and 80 (53.3%) males while blood donors included 6 (4%) females and 144 (94%) males. Tables 1and 2 show the age and sex distribution of the participants.

TABLE 1: SHOWS THE MEAN AGE, SEX, SEROPREVALENCE AND HAEMATOLOGICAL PARAMETERS OF THE STUDY SUBJECTS

Variables	Blood Donors (N = 150)	SCA (N = 150)	P values
Age (Mean ± SD)	30.43 ± 9.58	20.29 ± 11.27	<0.01
Sex			
Males	80	144	
Females	70	6	
Positive history of Blood Transfusion	13 (8.67%)	76 (50.67%)	
Seroprevalence of PVB19			
IgG	99 (66.0%)	92 (61.3%)	0.47
IgM	2 (1.3%)	8 (5.3%)	0.11
IgM and IgG	1 (0.67%)	4 (2.67%)	
Haematological Parameters			
WBC/mm³	9.85 ± 3.87	4.28 ± 1.01	<0.01
Neut/mm³	5.16 ± 2.48	2.13 ± 0.80	<0.01
Lymph/mm³	3.84 ±1.68	1.81 ± 0.51	<0.01
Platelet x 10⁹/l	355 ± 150	227 ± 168	<0.01
Hb (g/dl)	7.51 ± 1.62	13.72 ± 1.54	<0.01
Reticulocyte (%)	5.30 ± 2.43	1.22 ± 0.47	<0.01
MCV (fl)	81.53 ± 5.54	84.24 ± 10.35	<0.01
MCHC (g/dl)	32.51 ± 13.66	32.15 ± 12.40	0.02
MCH (pg)	26.88 ± 3.22	27.22 ± 2.77	0.33

TABLE2: AGE AND SEX DISTRIBUTION OF THE STUDY SUBJECTS

Age (Yrs)	SCA (N = 150)		Blood donors (N = 150)	
	Female	Male	Female	Male
< 11	9	24	0	0
11 – 17	19	15	0	0
18 – 24	19	22	2	47
25 – 31	8	6	3	46
32 – 38	8	6	0	20
39 – 45	4	6	0	21
46 – 52	3	1	0	6
>52	0	0	1	4
Total	70	80	6	144

History of blood transfusion
Seventy six (50.67%) of the Sickle cell patients had a history of previous blood transfusion while 13 (8.67%) of the blood donors had been transfused in the past.

Seroprevalence of parvovirus antibodies in the participants
A total of 92 (61.3%) sickle cell patients were anti-PVB19 positive and 99 (66.0%) of the blood donors were also positive for IgG. For IgM anti-PVB19, 8 (5.3%) sickle cell anaemia patients were positive while 2 (1.3%) of the blood donors were positive. There was no significant difference in the prevalence of IgG and IgM anti-PVB19 antibodies between both group (P = 0.471 and 0.1078 respectively). Four of the sickle cell anaemic patients were positive for both IgG and IGM anti-PVB19 antibodies while only one of the healthy donors was positive for both antibodies as shown in Table 1.

The seroprevalence by age and sex are presented in Tables 3 and 4. Tables 5 and 6 represent its association with blood transfusion in the study subjects.

Haematological parameters
The haematological parameters of the participants are as presented in Table 1. Table 7 compares haematological parameters in SCA subjects with IgG and IgM antibodies. Reticulocyte counts were significantly reduced in SCA subjects with IgM antibodies.

DISCUSSION
Parvovirus B19 is one of the emerging transfusion transmissible infections. It has been widely studied in various countries among healthy blood donors and

sickle cell patients with results indicating a high seroprevalence of the virus in the study areas however there are limited publications on parvovirus in SCA patients and blood donors in our environment. In this study, we found a seroprevalence rate of 66% for IgG antibody in healthy blood donors.

Abraham et al and Munoz et al reported seroprevalence of 65% each for IgG antibody in blood donors in India (12) and Salamanca, Spain (13) respectively. In various studies in developed nations, rates between 55 – 77% were reported (3 – 5). The seroprevalence rates from these studies were comparably the same with our findings. However, Mata et al reported a low seroprevalence of 9.8% (anti-IgG) in a cross sectional study conducted among 92 blood donors in Galicia in Spain (14). This is significantly lower than what we found in our study. This shows that there are geographical variations in the seroprevalence of parvovirus infection.

TABLE3: SEROPREVALENCE OF IGG AND IGM ANTIBODIES BY AGE IN SCA PATIENTS AND BLOOD DONORS

Age (Yrs)	SCA (IgG Positive)	Blood donors (IgG Positive)	SCA (IgM Positive)	Blood donors (IgM Positive
< 11	18	0	0	0
11 – 17	20	0	0	0
18 – 24	28	33	2	47
25 – 31	10	31	3	46
32 – 38	6	14	0	20
39 – 45	7	16	0	21
46 – 52	3	1	0	6
>52	0	4	1	4
Total	92	99	6	144

TABLE 4: ASSOCIATION OF IGG PARVOVIRUS ANTIBODIES WITH SEX IN SCA PATIENTS AND BLOOD DONORS

Sex	SCA IgG status			Blood donors IgG status		
	Negative	Positive	Total	Negative	Positive	Total
Female	22	48	70	1	5	6
Male	36	44	80	50	94	144
Total	58	92	150	51	99	150
P-value	0.089			0.360		

TABLE 5: ASSOCIATION OF IGG PARVOVIRUS ANTIBODIES WITH BLOOD TRANSFUSION IN SCA PATIENTS AND IN BLOOD DONORS

Transfusion Status	SCA IgG status			Blood donors IgG status		
	Positive	Negative	Total	Positive	Negative	Total
Transfused	44	32	76	6	7	13
Not transfused	48	26	74	93	44	137
Total	92	58	150	99	51	150
	P-value = 0.4785			P-value 0.2026		

TABLE 6: ASSOCIATION OF IGM PARVOVIRUS ANTIBODIES WITH BLOOD TRANSFUSION IN SCA PATIENTS AND IN BLOOD DONORS

Transfusion Status	SCA IgM status			Blood donors IgM status		
	Positive	Negative	Total	Positive	Negative	Total
Transfused	1	75	76	0	13	13
Not transfused	7	67	74	2	135	137
Total	8	142	150	2	148	150
	P-value = 0.0635			P-value 0.4085		

The seroprevalence of Parvovirus B19 IgG antibody in SCA patients was found to be 61.3% in this study. Ujo et al (15) in a cross sectional study in paediatric SCA patients in Zaria found a seroprevalence rate of 85.4%.

The study also found that there is no sex association in the seroprevalence of the virus in both SCA patients and healthy blood donors. This is in agreement with the findings of Ujo et al (15) and Teuscher et al (16) in their separate studies.

This study also found that though blood donors have a slightly higher seroprevalence than SCA patients, this was not statistically significant. Teuscher et al (16) and Serjeant et al (6) in their separate studies also found no significant difference in the seroprevalence of the virus in both study groups. This suggest that SCA patients are not at increased risk when compared to the general populace however due to the fact that they have a lower stable haemoglobin value, parvovirus infection in them may result in symptomatic anaemia necessitating transfusion.

TABLE 7: COMPARISM OF HAEMATOLOGICAL PARAMETERS IN SCA PATIENTS WITH IGG AND IGM PARVOVIRUS ANTIBODIES

Haematological Parameters	IgG Mean ± SD N = 92	IgM Mean ± SD N = 8	P value
WBC x 10^9	9.93 ± 4.29	9.81 ± 3.80	0.94
Neutrophils x 10^9	5.25 ± 2.72	4.58 ± 1.80	0.49
Lymphocytes x 10^9	3.90 ± 1.93	4.40 ± 2.11	0.49
Platelets x 10^9	348.66 ± 149.69	293.63 ± 176.96	0.33
Hb (g/dl)	9.46 ± 1.57	7.15 ± 1.97	0.60
Reticulocyte (%)	5.48 ± 2.42	1.19 ± 0.59	<0.01
MCV (fl)	82.30 ± 8.33	80.18 ± 9.35	0.50
MCH (pg)	27.11 ± 3.37	26.29 ± 3.36	0.51
MCHC (g/dl)	32.64 ± 1.31	32.74 ± 0.89	0.83

Studies have shown that by 15 years of age, about 50% of some populace are positive for PVB19 IgG antibodies (1, 17, 18). Some studies have noted an increase in seroprevalence of the virus with age.[18] Kim et al in their study on the epidemiology of parvovirus in sickle cell disease patients reported an increase in seroprevalence with age (19).In this study, we found a peak age prevalence to be 18 – 24 years in SCA patients and blood donors. This is higher than that reported by Ujo et al (15).

The seroprevalence of parvovirus B19 IgM antibody in sickle cell anaemia subjects and blood donors were found to be 5.3% and 1.3% respectively. Doyle and his coworker found seroprevalence of 1% prevalence among American blood donors (4) while Munoza reported 0% in Spanish blood donors (20). The seroprevalence of PVB 19 may vary with the sensitivity of the test used in addition to geographical and seasonal variations. The possibility of transmission of PVB19 by blood and blood products raises several blood safety questions still unanswered (21 – 23). Human blood and its components are widely used as life saving therapy in hospital practices. However, there is an associated risk of transmission of infections such as HIV, hepatitis and parvovirus inclusive due to infected donor blood (23). Parvovirus lacking a lipid envelope is not susceptible to the solvent-detergent treatment which can inactivate pathogens with envelopes such as HIV, hepatitis B and C among others transmissible by blood. The virus is stable in heat and remains infective even after treatment with dry heat at 80°C for 72 hours, which was used for treating some blood products. Hence the reports on a high prevalence of PVB19 in haemophilic patients receiving pooled blood products (24, 25). In non-immune sickle cell anaemia patients, the clinical manifestation of the virus upon infection may include transient aplastic crisis, which is indicated by a fall in haemoglobin with reticulocytopaenia. Serjeant et al in their study of epidemiology of human parvovirus B19 infection in Jamaica in homozygous sickle cell disease found that PVB19 infection account for most if not all aplastic crisis in SS disease (6).

The lack of a statistically significant difference between the seroprevalence of PVB19 antibodies between blood donors and SCA patients will suggest that blood transfusion may not the major means of transmission of parvovirus SCA patients. This finding highlights the importance of investigating other means of transmission other than blood transfusion as recommended in the report of the Committee on Infectious Diseases (17). The reticulocyte percentages of IgG antibody positive SCA patients are generally higher than that of IgM positive SCA subjects. This affirms the risk of transient aplasia associated with acute or persistent parvovirus infection documented in some previous studies (19). This is understandable

in the light of the cytopathic effect of PVB19 on haemopoietic precursor cells in the bone marrow. In conclusion, this study has shown a high seroprevalence of IgG anti-PVB19 antibodies in sickle cell patients and voluntary blood donors in our environment. This suggests that it may not be cost effective to recommend routine donor screening for PVB19 antibodies. However, because of the increased risk of aplastic crisis in SCA patients, SCA patients who require transfusion and are seronegative for PVB19 should have the blood screened for PBV virus.

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SERO-PREVALENCE OF HUMAN PARVOVIRUS B19 AMONG PATIENTS ATTENDING SOME HOSPITALS IN KANO METROPOLIS, NIGERIA

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ABSRACT

Background: Human Parvovirus B19 (HPVB19) belongs to the family *Parvoviridae*, causes *Erythema infectiosu*m, aplastic crises in persons with blood disorder and prolonged anaemia in immuno-compromised persons. During pregnancy the virus may be transmitted to the foetus which can result in hydrops foetalis, spontaneous abortion or intrauterine foetal death. The study determined the sero-prevalence of IgG antibodies to HPVB19 among patients in Kano metropolis in order to provide information on their immune status and the possible risk factors for acquiring the virus.

Method: The study population comprised 460 patients seen at the outpatient department of two hospitals in Kano whose mean age was 28.8 (range 0 –70) years. Questionnaire was used to obtain data on socio-demography and risk factors. Blood sample was collected from each patient, serum was obtained and analysed for IgG antibodies to HPVB19 by ELISA according to manufacturer's instruction.

Result/Discussions: Sero-prevalence of 41.5% (191/460) was obtained for HPVB19 and seropositivity increased significantly with age with the highest prevalence (51%) recorded among patients ≥51 years old while the lowest prevalence was among those < 1 year old (χ^2=13.130, df=6, P=0.04). Seropositivity was higher in female (42.6%: 98/230) than male patients (40.4%: 93/230) (p>0.05). Highest seropositivity was observed among farmers (64.2%:18/28) while the lowest was among retired persons (27.2%:3/11). There was significant association between HPVB19 and level of formal education attained (χ^2 =10.363, df=4, P=0.03) and number of persons living in a house (χ^2 =14.30, df=1, P=0.00). There was no association between HPVB19 and marital status (P=0.3) and type of residence (P=0.5). Blood transfusion (OR=2.0:1; P=0.08) and sickle cell anaemia (OR=1.7:1; P=0.08) were important risk factors for HPVB19 transmission in this study. Having blood transfusion showed a 2.0 fold risk while having sickle cell showed a 1.7 fold risk of acquiring HPVB19 infection. During the study, 48.2% of women of child bearing age had antibodies to HPVB19 IgG antibodies leaving about 52% still susceptible to the virus.

Conclusion: Seroprevalence to B19 among patients was low leaving a large proportion of the population especially women in Kano still susceptible to B19 infection.

Keywords: *Sero-prevalence, Human Parvovirus B19, Patients, Hospitals, Kano, Nigeria*

INTRODUCTION

Human parvovirus B19 (HPVB19) was accidentally discovered during screening of healthy blood donors for hepatitis B (1) and has since then been recognized as an important human pathogen causing significant morbidity and mortality in various patient population groups (2). Immature cells in the erythroid lineage are principal target for human B19 parvovirus. Hence viral replication causes cell death, interrupting red cell production (3).

Parvovirus B19 can be found in the blood and respiratory secretion of infected patients. The virus can be transmitted parentally by blood transfusions, infected blood products, packed red cells from blood collected during the short viremic phase (4) and vertically from mother to foetus (3).

The virus causes *Erythema infectiosu*m (fifth disease) in children and is responsible for various clinical manifestations whose characteristics depend on the interplay between the viral properties and the physiological and immune status of the infected individuals (5). Parvovirus B19 virus may cause transient anaemia amongst healthy adults, aplastic crises in infected persons with an underlying blood disorder and prolonged anaemia in immuno-compromised persons such as AIDS and organ transplant patients. In addition, during pregnancy the virus may be transmitted to the foetus which can result in hydrops foetalis, spontaneous abortion or intrauterine foetal death (IUFD), most commonly during the second trimester of pregnancy (6). However, a persistent infection may be observed in immunocompromised patients unable to produce neutralizing antibodies and to clear the virus leading to chronic B19 carriage with or without anaemia (7). Persistence of infection in the bone marrow has also been reported in immunocompetent individuals with or without symptoms (7).

Though the association between HPVB19 and *Erythema infectiosum* has been established since 1983, there is dearth of information on the prevalence of HPVB19 and associated risk factors in Nigeria especially among individuals in Kano state. The study therefore aimed at determining the sero-prevalence of IgG antibodies to Parvovirus B19 among patients in Kano metropolis in order to

provide information on the immune status of the general population to the virus and possible risk factors for acquiring the virus, with the hope of aiding in the control and spread of this virus.

MATERIALS AND METHODS

The cross sectional study was conducted between July and October 2009 in two hospitals in Kano state; Hasiya Bayero Paediatric Hospital and Aminu Kano Teaching Hospital. The ethical review boards in these hospitals approved the study and informed consent was obtained from parents/caregivers as well as patients before inclusion. The study group comprised 460 patients whose mean age was 28.8 (range 0–70) years. Each enrolled patient had a serum specimen tested for the presence of anti-parvovirus IgG antibodies by enzyme-linked immunosorbent assay (InstitutViron/serionGMbHWurzbury, Germany). Demographic information and data on possible risk factors were collected from the patients using structured questionnaires prior to sample collection. Data obtained from the questionnaires and the result from the sample analysis were entered into spread sheet and analysed using the SPSS version 15.0. Prevalence rates of B19 IgG antibodies were compared with the parameters by chi-square test. *P* values < 0.05 were considered significance at 95% Confidence Interval (CI).

RESULTS

The sero-prevalence of IgG antibodies to HPVB19 in the two hospitals is shown in Table 1. The sero-prevalence of B19 IgG antibodies increased from 25.0% (1/4) in children to 51% (24/47) in adults (P = 0.04). The 460 patients in the study were equally distributed between male and female. Though sero-prevalence was higher in female patients than male patients, the difference observed was not statistically significant (P=0.3). There was no significance difference in the sero-prevalence of the antibodies by marital status though prevalence was higher among married compared to unmarried patients. Level of formal education was significantly associated with presence of antibodies to the virus with the highest prevalence observed among those without formal education (44%: 64/145) while the lowest was among those with tertiary education (33%:26/78). There was a significant association between infection with parvovirus and occupation of patients (P=0.02). Farmers had the highest prevalence (64%:18/28) of antibodies to Parvovirus compared to retirees with the lowest (27.2%: 3/11).

The result was analysed according to type of house patients live in and the highest prevalence of 45% (90/200) was recorded among patients living in family compound house while the lowest (28.5%: 14/49) was recorded amongst patients who live in duplex type of residential houses. The difference observed was not statistically significant (P=0.5). Further analysis of the results showed that parvovirus infection was significantly associated with the number of persons living in a house; with patients living in houses with more than 5 persons having the highest prevalence (51.8%: 99/191) while those living in houses with less than 5 persons had the least (34.2%: 92/269).

TABLE 1: SERO-PREVALENCE OF HPVB19 IGG ANTIBODIES AMONG PATIENTS 0-70 YEARS OLD ATTENDING SOME HOSPITALS IN KANO METROPOLIS

Hospital	Number Screened	Number Positive (%)	95%CI
AKTH	**368**	**158 (42.9)**	**37-48**
HBPH	**92**	**33 (35.8)**	**26-46**
Total	**460**	**191 (41.5)**	**37-46**

χ^2 =1.513, df =1, p=0.2

Key: AKTH = Aminu Kano Teaching Hospital; HBPH =Hasiya Bayero Paediatric Hospital

Some possible risk factors that might be associated with transmission of HPVB19 infection among the population was analysed (Table 3). The results of this study showed that history of transfusion is an important factor for acquiring infection with HPVB19. There was a 2- fold risk of getting infected among those who have had transfusion (**95% CI =0.8-4.6, OR =2.0)** though the difference was not statistically significant (χ^2=2.917, df=1, P=0.08).

Others risks factors such as pregnancy, sickle cell anaemia and organ transplantation were not significantly associated with Parvovirus B19 with highest prevalences of 41.5%, 54.5% and 100% and lowest prevalences 41.5%,40.9% and 41.4% (P=0.9,P=0.2, P=0.2) respectively.

The result was further analysed according to age group of the female patients and it was observed that women within age group 31-40 years had the highest prevalence of HPVB19 IgG (55%: 27/49) and the lowest prevalence was recorded among women in age group 10-20 years (38%: 13/34) (Table 4). The results showed that 170 (73.9%) of the 230 female patients studied were of child bearing age and HPVB19 IgG antibodies was detected among 48.2% (82/170) of these women. Among the women of child bearing age, 41/170(24%**)** were pregnant and only 17/41(41.5%) of them had antibodies to HPVB19 which leaves about (24/41) 58.5% of those pregnant still susceptible to HPVB19 infection.

TABLE 2: SOME SOCIO-DEMOGRAPHIC FACTORS ASSOCIATED WITH HPVB19 IGG AMONG PATIENTS IN SELECTED HOSPITALS IN KANO METROPOLIS

Variable	No. of patient	No. positive (%)	p-value
Age (Years)			
<1	**4**	**1 (25.0)**	
1-10	**91**	**24 (26.3)**	**0.04**
11-20	**59**	**23 (38.9)**	
21-30	**103**	**47 (45.6)**	
31-40	**97**	**44 (45.4)**	
41-50	**59**	**28 (47.5)**	
≥51	**47**	**24 (51.0)**	
Gender			
Male	**230**	**93 (40.4)**	**0.3**
Female	**230**	**98 (42.6)**	
Marital Status			
Married	**253**	**115 (45.4)**	**0.06**
Single	**207**	**76 (36.7)**	
Formal educational level			
None	**145**	**64 (44.0)**	
Primary	**93**	**40 (43.0)**	**0.03**
Secondary	**144**	**61 (42.4)**	
Higher	**78**	**26 (33.0)**	
Occupation			
Civil Servant	**58**	**19 (32.8)**	
Unemployed	**94**	**32 (34.0)**	
Self employed	**50**	**27 (54.0)**	
Farmer	**28**	**18 (64.2)**	
Fulltime Housewife	**82**	**41 (50.0)**	**0.02**
Artisan	**31**	**12 (38.7)**	
Student	**63**	**22 (34.9)**	
Retired	**11**	**3 (27.2)**	
Others	**43**	**17 (39.5)**	
Type of residence			
Single room	**8**	**3 (37.5)**	
Room and parlour	**47**	**21 (44.7)**	**0.5**
1-3Bedroom	**156**	**63 (40.3)**	
Family compound	**200**	**90 (45.0)**	
Duplex	**49**	**14 (28.5)**	
No of persons living in a house			
≤5	**269**	**92 (34.2)**	**0.00**
>5	**191**	**99 (51.8)**	

Key: Mean age = 28.8; S.D = 1.6; Others = Unskilled labourers, bike riders.

TABLE 3: SOME POSSIBLE RISK FACTORS ASSOCIATED WITH HPVB19 AMONG PATIENTS ATTENDING SOME SELECTED HOSPITALS IN KANO METROPOLIS

Variable	No of patient	No.Positive (%)	p-value	OR	95%CI
History of transfusion					
No	**430**	**183 (42.5)**		**1**	**0.8-4.6**
Yes	**30**	**8 (26.6)**	**0.08**	**2.0**	
Pregnancy					
No	**419**	**174 (41.5)**	**0.9**	**1**	**0.5-1.9**
Yes	**41**	**17 (41.5)**		**0.9**	
Organ transplant					
No	**459**	**190 (41.4)**	**0.2**	**1**	**0.9-1.0**
Yes	**1**	**1 (100)**		**0.9**	
Sickle cell Disease					
No	**438**	**179 (40.9)**	**0.2**	**1**	**0.7-4.1**
Yes	**22**	**12 (54.5)**		**1.7**	

KEY: HPVB19=Human Parvovirus B19, OR=Odds ratio

TABLE 4: DISTRIBUTION OF HUMAN PARVOVIRUS B19 ANTIBODY AMONG WOMEN OF CHILDBEARING AGE ATTENDING SOME SELECTED HOSPITALS IN KANO METROPOLIS

Age group (yrs)	No. Screened	No. Positive	Percentage (%)	95% CI
10-20	34	13	38	23-54
21-30	57	28	49	36-61
31-40	49	27	55	41-68
41-50	30	14	46.7	30-63
Total	170	82	48.2	

DISCUSSION

The overall prevalence rate of HPVB19 IgG antibody determined in this study was 41.5%. This was slightly higher than the prevalence of 39.5% reported in Jos (2) and lower than the prevalence of 85.4% reported from Zaria (8) other studies reported 60-70% from developed countries such as England (9) but higher than 16.2% reported in Singapore (10). These discrepancies could be due to differences in the specificity and sensitivity of the assays used.

Several studies in different regions of the world have identified different socio-demographic variables like age, gender, socio-economic status and environmental conditions as risk factors for acquisition of parvovirus B19 infection. For example, age has been consistently shown to be a major predictor of anti-parvovirus B19 IgG seropositivity (11,12,13).

This was also demonstrated in this study where seropositivity significantly increased with age; with a gradual increase in seropositivity from patients <1 year old through ≥ 51years old. The present study, the first in Kano to assess HPV B19 IgG sero-prevalence amongst patients, shows that by age 20 about 40% of the population have been infected by HPV B19.

Parvovirus B19, seropositivity is a synonym of immunity, the increase in seroprevalence with age means that, the proportion of individuals susceptible to parvovirus B19 decreases with age (14). This increasing seropositive rate with increasing age is consistent with the work of Girei in Jos (2) and reports from other countries (11, 5, 15). However, the results contrast that of Ujo and colleagues whose study reported that seropostivity did not increase with age (8). This discrepancy could be due to the fact their study was carried out in children with sickle cell disease whereas this study was carried out among all kinds of patients in the general population aged from 0-70 years.

The prevalence of 51% among age group >51 years found in this study is similar to 50% reported in India (15) and slightly lower than 55% reported in Malaysia (11) for the same age group. This difference could be attributed to dwindling immune response in the elderly, as a result of the natural decay of antibodies with time, which probably account for the lower seropositivity seen in the age group 51 years and above (11) as the antibodies could have decreased to a level beneath the detection limit of the test used in this study.

This study however has not demonstrated any association between gender and seropositivity of parvovirus as the difference in the prevalence rates was not significantly different between male and female patients. Overall a slightly higher seropositivity was observed in female patients than in male patients, which is in agreement with the findings of Ooi *et al* in Malaysia (11), Salimi *et al* in Iran (5) and Girei *et al i*n Nigeria (2).

Marital status did not affect the prevalence of the virus in this study. No statistically significant difference in the positivity was evident among the married patients and the unmarried individuals. This was also observed in India where the prevalence in married donors was not statistically different from the unmarried as transmission of the virus is not through sexual route.

The relationship between socio-economic status and health outcome is well known (13). Higher positivity found among farmers, bike riders and unskilled labourers could be explained by their poor living conditions such as poor housing, overcrowding and low educational levels. This finding conforms to work of Kishore *et al*.(15) among blood donors in India where high positivity was observed among unskilled labourers. Higher prevalence among these occupational groups was attributed to outdoor activities which could lead to more exposure and thus contact with infectious agents (15). High positivity observed in this study among full time housewives could also be due to the fact that women are always in contact with children both at home and outside the home. This finding does not conform to the work of Ujo *et al.* (8) who did not find any association between occupation and HPVB19 among parents of children in their study.

In this study it was observed that lower educational levels and overcrowding predispose to Parvovirus infection. Studies have indicated that education, to some extent compensates the effects of poverty on health, irrespective of the availability of health facilities (15). In this study the highest prevalence was observed among those without formal education and lowest among the highly educated. The finding is consistent with findings of Kishore and colleagues on blood donors in India who also reported higher prevalence among illiterates, low educated group and lowest among the highly educated group (15).

The seroprevalence of parvovirus was significantly higher among patients who lived in houses with more than five people. This finding is in close agreement with reports of Alao and colleagues (13) who reported higher parvovirus B19 prevalence rate in Jos among children who lived in crowded dwellings which was attributed to overcrowding in urban areas. Overcrowding is very common in urban areas where most people live in highly compacted areas with little or no ventilation. This occurrence is common in a populous

country like Nigeria where there is usually an influx of people from the rural areas to the urban areas. Overcrowding leads to a higher and faster spread of infection through the respiratory route (15) and HPVB19 is transmitted primarily through respiratory secretions and saliva. Therefore, in crowded environments such as daycare centers, kindergartens and schools transmission of the virus from infected to non-infected individuals is probable (16, 17).

History of transfusion, sickle cell anaemia, being pregnant and organ transplant, showed no significant association. is acutely infected (13). The prevalence among pregnant and those without pregnancy coincides with the overall prevalence of 41.5% obtained from the study. Though the only organ transplant patient tested positive, number in the group was too small to draw any conclusion. There may be other factors apart from those studied which contribute to transmission of the virus.

In this study, almost half (48.2%) of the women of reproductive age defined as women from 10-50 years old (18) had IgG antibodies to HPVB19. This finding shows that over half (52%) of women within this age group in the study are still susceptible to parvovirus B19 infection which poses a public health problem. This result is similar to 47% obtained in a study in Brazil (19). Parvovirus infection during pregnancy can cause severe foetal anaemia as a result of foetal erythroid progenitor cell infection with a shortened half-life of erythrocytes, causing high output cardiac failure and subsequently non-immune hydrops foetalis (16, 17).

Higher prevalence observed among the non transfused group is in agreement with the work of Ujo and colleagues (8) who also found higher prevalence among this group, though a 2 fold risk was observed with respect to blood transfusion in this study. Although significant association was not established, higher prevalence was observed among those with sickle cell anaemia disease. Secondary attack rates of between 50 to 60% have been reported in families with more than one child with sickle cell anaemia when one child

In conclusion, the findings in this study confirmed the presence of human parvovirus B19 in Kano state and seroprevalence rate comparable with those found in various countries in the world. Seroprevalence to HPVB19 among patients was low leaving a large proportion of the population in Kano still susceptible to HPVB19 infection.

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CURRENT ANTIBIOTIC SUSCEPTIBILITY PROFILE OF THE BACTERIA ASSOCIATED WITH SURGICAL WOUND INFECTIONS IN THE BUEA HEALTH DISTRICT IN CAMEROON

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ABSTRACT

Background: Most surgical wounds seen in clinical practice in the Buea Health District, Cameroon are infected prior to arrival or while they are in the hospital. Sometimes the infection necessitates a combination of local wound site measures and systemic antibiotherapy to properly manage the patient.

Objective: To identify the current antibiotic susceptibility profile of the common germs that cause surgical wound infections in the Buea Health District of Cameroon.

Methods: A total of 2120 specimens comprising swabs from burns, ulcers, open or post-operative wounds were collected from hospitalized patients attending health institutions in Buea. The samples were collected from different anatomic sites of the patients. Cultures were effected from the specimens and bacteria isolated from infected wounds using standard microbiological techniques. Antibiotic susceptibility of the different isolates was determined.

Results: Majority (79.8%) of the wounds were infected with pathogenic bacteria. The germs globally showed multi resistant patterns to commonly used antibiotics in the study area, especially to co-trimoxazol, doxycycline, chloramphemicol, ampicilline and aztreonam. However appreciable sensitivity was noted to ofloxacillin, perflacin, and ceftriazone.

Conclusion: This study has revealed ofloxacin as the only antibiotic to which all the isolated bacteria from infected wounds were sensitive in the study area.

Keywords: Wound infection, Antibiotic susceptibility profile, Buea Health District, Cameroon

INTRODUCTION

Trauma, injections, invasive diagnostic procedures, intravenous therapy and urinary catheters can all break the first line of defense and make an individual more susceptible to infections (1). Although every effort is made to kill or check the growth of micro organisms in the hospital, the hospital environment is a major reservoir of pathogens (1). Gram-negative bacilli are the most common cause of opportunistic infections causing at least half of hospital-acquired infections in approximately 5% of all hospitalized patients (2). Early contaminants on wound surface are likely to be skin flora (e.g, *Staphylococcus epidermidis* and beta haemolytic streptococci) that adhere to the wound, proliferate and form healthy biofilm. Gram-negative bacilli then colonize the wound. These organisms utilize available oxygen and provide growth factors to enable anaerobes to establish within the biofilm (3). Bacteria pathogens reported to infect wounds include: *Staphylococcus aureus, Enterococcus sp, Corynebacterium diphtheriae, Clostridium sp, Neisseria meningitidis, Pseudomonas aeruginosa, Haemophilus ducreyi, Bacteriodes fragilis, Treponema palladium, E. coli, Klebsiella sp, proteus sp and Aeromonas sp* (3). Infections caused by these pathogens result in great morbidity and mortality and impose a major burden on the health care system worldwide (4). *S. aureus* bacteraemia extends length of hospital stay and increases antibiotic use, treatment cost and mortality (1).

Epidemiologically, acute wounds have a wide range of causes: often they are the unintentional result of motor vehicle accidents, falls, mishandling of sharp objects or sport related injuries. Intentional injuries are due to violence involving assault with weapons including knives and guns (1,5). Management of wounds involves dressing,

suturing, bandaging, use of antimicrobial agents for disinfection, physical therapy and educational strategies to prevent bacterial colonization from proceeding to the point of clinical infection. Sometimes skin graft is used to encourage healing. A number of methods for the diagnosis of wound infections caused by these organisms have been developed. These methods include: serum investigation which detects elevated white cell counts and quantitative analysis is done through wound biopsy (4).

Antibiotics used for treatment should cover the potential range of pathogens. Several studies have compared antibiotic regimens but no single regimen has emerged as clearly the best. The most commonly used antibiotics in the Unites Sates are cefazolin and cefozitin (6). The use of vancomycin is increasingly important for the treatment of hospital acquired Staphylococcus infections (7). Topical antibiotics such as silver sulphadiazine, compounds containing silver or iodine and honey have the potential to reduce the bacterial burden in wounds (8). Recent reports (9) have demonstrated the existence of pathogenic bacteria and the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) in wounds in several hospitals in Africa.

In Cameroon antibiotics (especially beta lactamines, aminolglycosides and sulfonamides) are used indiscriminately by some health personnel to manage wound infections. Besides, owing the fact that these antibiotics can easily be obtained from street vendors at low cost, there is a tendency for patients to purchase and use them repeatedly and indiscriminately without medical supervision. This practice steams from the lack of knowledge of antibiotic resistance pattern of the common pathogens but also from the scarcity of data on antimicrobial susceptibility profile of these organisms, hence this study was carried out.

MATERIALS AND METHODS

Study area and design

This study was carried out in Buea Health District, South West Region of Cameroon. The town of Buea is situated on the flank of Mount Cameroon (4100m) at 800m above sea level. Two thousand one hundred and twenty specimens comprising swabs from burns, ulcers, open or postoperative wounds were collected from hospitalized patients attending health institutions in the Buea Health District. These samples were collected from different anatomical sites of the patients. Patients were recruited into the study based on the following criteria: hospitalized with a wound in one institution in the area of study at the time of the study, acceptance to freely take part in the study by signing an informed consent. An ethical approval of the study was obtained from the Regional Delegation of Public Health, Buea. The study was a cross sectional prospective study. Patients were recruited from four health units. They were the Buea Regional Hospital Annex, the Mount Mary Hospital, the Buea Town Health Centre and the Muea Health Centre.

Sample collection

Samples were collected from hospitalized patients and transported according to methods previously described (1,7). Samples were aseptically collected using sterile swab sticks and inserted into sterile Amies Transport medium and carefully labeled. They were then transported to the microbiology laboratory of the University of Buea for processing and analysis, and in instances were delay was anticipated before analysis, the samples were preserved in a refrigerator at 4°C.

Sterilization and aseptic techniques

All glassware used were washed with detergent an rinsed with tap water, dried at 20°C, wrapped in aluminum paper and sterized in hot air oven (Gallenkam Britain) at 180°C for 60 minutes. Culture media, distilled water, specimen bottles, and normal saline were sterilized by autoclaving at 121°C for 15 minutes. The following culture media were used: Amies transport medium (ATM), MacConkey agar (MA), Blood agar (BA), Mannitol Salt agar (MSA) and Nutrient agar (NA). Media were prepared according to the manufacturer's instructions. Standard septic techniques were strictly respected.

Isolation and identification of bacteria

Specimens were inoculated aseptically into Blood agar, MacConkey agar, Nutrient agar and Mannitol Salt agar. Plates were incubated at 37°C for 18-24 hours, after which they were examined for growth. Colonies were then subjected to oxidase, catalase, coagulase, motility tests, Gram staining, as well as growth on Kliger iron agar. Colonies were presumptively identified based on their morphological and colonial characteristics (7,10). Their identity was confirmed using the Analytical Profile Index (API) 20E (Biomerieux SA, Marcy E'Etiole, France) (7).

Antibiotic susceptibility testing

The disc diffusion (Kirby Baur) technique was employed as previously described (3,7) using Mueller-Hinton (MH) agar (Schalau Chemie S.A, Spain), which is a standard medium for the disc diffusion assay. Antibiotics used in the study included: aminogycoside, [gentamicine (10µg)], macrolide, [erythromycin (10µg)], third generation cephalosporines, [ceftriazone (30µg), ceftazidime (30µg)], penicillins, [ampicillin (10µg), augmentin (30µg) and oxacillin (1µg)], folic acid synthesis

inhibitor, [co-trimoxazole (25µg)], fluoroquinolones, [ofloxacin (30µ), norfloxacin (10µg), pefloxacin (5µg)], tetracycline, [doxycycline (30µg)], protein synthesis inhibitor, [chloramphenicol (30µg)], monobactams, [aztreonam (30µg)].

A bacterial inoculum was prepared from subcultures and emulsified in 3ml sterile normal saline in bijou bottles to match with 0.5 Mc Farland turbidity standards (1.8 x 10^8 CFU/mL) by comparing visually. The optical density of the standard was monitored on a regular basis with a spectrophometer at λ = 625nm and 1cm length path (11). About 20µL of the inoculum was dispensed on the MH plate and ramified with a sterile spreader. The plates were allowed to dry for 3-5 minutes, then using a sterile forceps, antibiotic discs were applied on the surface of the inoculated plates and pressed gently to ensure complete contact with agar. The discs were placed at least 15mm apart from the edges of the plates to prevent overlapping of inhabitation zones. Within 15 minutes after discs were applied, the plates were inverted and incubated at 37°C for 24 hours after which the results were read. The diameter of the zones of inhibition was measured with a ruler. They were compared with recommended standards, which conform to those of the national committee of Clinical laboratory Standard (NCCLS) [now known as Clinical Laboratory Standards Institute (CLSI)]

Statistical Analysis

Statistical package for social science (SPSS) was used to analyze the data. The Chi-square (x^2) test was employed where appropriate for statistical analysis. Differences were considered significant at $p \leq 0.05$.

RESULTS

A total of 2120 patients presenting with various types of wounds were enrolled in the study. Out of the 2120 patients studied, 891 (42%) were females and 1229 (58%) were males. The ages of study participants ranged from 7 months to 80 years. Majority of these patients were presenting with open wound (1651/2120 = 77.8%) while those with burns (31/2120 = 1.4%) constituted the least. Some of the patients had diseases such as AIDS (140/2120 = 6.6%) and diabetes mellitus (60/2120 = 2.8%) which are predisposing factors to wound infection.

Table 1 shows the prevalence of bacteria in different sample sources. Of the 2120 specimens cultured, pathogenic bacteria were isolated from 1690 giving an overall prevalence of 79.7%. Four hundred and thirty (20.3%) specimens yielded no bacterial growth. Among the different types of samples analyzed, burns had the highest isolation rate of 100% (30/30) while open wounds had the least (78.8%). However, the difference in isolation between specimens was not significant (X^2=1.302; df = 3; p = 0.729).

TABLE 1: PREVALENCE OF BACTERIA IN DIFFERENT SAMPLE SOURCES

Specimens	Number(%) with positive culture	Number (%) with negative culture	Total (%)
Burns	**31 (100)**	**0 (0)**	**31(1.4)**
Post operative wound	**230 (79.2)**	**59 (20.8)**	**289 (13.7)**
Ulcer	**130 (86.6)**	**19 (13.4)**	**149 (7.1)**
Open wound	**1300 (78.7)**	**351 (21.3)**	**1651 (77.8)**
Total (%)	**1691 (79.8)**	**429 (20.2)**	**2120 (100)**

(X^2 = 1.302; df = 3; p = 0.729)

Table 2 shows the frequency of isolation of bacterial pathogens. Twelve species of bacteria were isolated from the specimens. *Staphylococcus aureus* (24.8%) was the commonest organism isolated followed by *Pseudomonas aeruginosa* (23.1%), while the least isolated was *Serratia sakazkii* (0.6%).

The distribution of bacteria isolates based on specimen source is shown in Table 3. All infections of burns were by *Pseudomonas aeruginosa* (5.1%) and *Staphylococcus aureus* (2.1%) though there was no statistical difference in the distribution of bacteria isolates based on specimen source (P = 0.972).

The antimicrobial susceptibility of isolates to some commonly prescribed antibiotics is shown in Table 4. Ofloxacin (100%) and pefloxacin (100%), were the most active drugs. Isolates also demonstrated high sensitivity to ceftriazone (94.2%), gentamicin (92.0%) and ceftazidime (89.6%). However, all isolates showed complete resistance (100%) to oxacillin. Other inactive drugs included co-trimoxazole (18.7%), aztreonam (40%) and ampicillin (43.8%). *Staphylococcus aureus,* the

most common isolate, showed resistance to co-trimoazole (100%), aztreonam (100%), ampicillin (95%), erythromycin (80), doxycyclin (80%), chloramphenicol (60%), and ceftazidime (54.4%).

TABLE 2: FREQUENCY OF ISOLATION OF BACTERIAL PATHOGENS

Isolates	Frequency of isolation	Percentage of Isolation
Staphylococcus aureus	481	28.4
Pseudomonas aerinosa	390	23.1
Klebsiella pneumonia	219	13.0
Enterobacter cloacae	210	12.4
Escherichia coli	99	5.9
Serratia marcescens	99	5.9
Proteus mirabilis	81	4.7
Serratia rubideae	31	1.8
Enterbacter aerogenes	30	1.8
Streptococcus sp	19	1.2
Hafnia alvei	21	1.2
Serratia sakazakii	11	0.6
Total	1691	100

TABLE 3: DISTRIBUTION OF BACTERIA ISOLATE BASED ON SPECIMEN SOURCE

Isolates	Sample source				Total (%)
	Positive patients (%)				
	Burns	Ulcers	Postoperative wounds	Open wounds	
E. coli	0 (0)	20 (20)	19 (20)	61 (60)	100 (5.9)
E. cloacae	0 (0)	11 (4.7)	11 (4.7)	188 (90.5)	210 (12.4)
E. aerogenes	0 (0)	0 (0)	0 (0)	30 (100)	30 (1.8)
P. mirabilis	0 (0)	11 (12.5)	29 (37.5)	40 (50)	80 (4.7)
K. pneumonia	0 (0)	20 (9.1)	41 (18.2)	160 (72.7)	221 (13.0)
H. alvei	0 (0)	0 (0)	0 (0)	21 (100)	21 (1.2)
P. aerginosa	20 (5.1)	39 (10.3)	31 (7.7)	297 (76.9)	387 (23.1)
S. marcescens	0 (0)	0 (0)	19 (20)	81 (80)	100 (5.9)
S. rubideae	0 (0)	0 (0)	10 (33.3)	21 (66.7)	31 (1.8)
S. sakazakii	0 (0)	0 (0)	0 (0)	11 (100)	11 (0.6)
S. aureus	11 (2.1)	29 (6.3)	70 (14.6)	368 (77.0)	478 (28.4)
Strep. Sp.	0 (0)	0 (0)	0 (0)	22 (100)	22 (1.2)
Total %	31 (1.8)	130 (7.7)	230 (13.6)	1300 (76.9)	1691 (100)

($X^2 = 19.299$; df = 33; p = 0.972)

Ninety two (5.44%) of the 1691 isolates were found to be resistant to at least two antibiotics (Table 30 patterns of multi-drug resistance emerged l resistance to two or more antibiotics, excluding oxacillin. Seventy-one (4.2%) isolates were resistant to three or more antibiotics and, of these, 66 (3.9%) were resistant to at least five. The predominant resistant patterns SXT^R DXT^R C^R AMP^R ATM^R was observed in *K. pneumoniae* and *P. aeriginosa* and constituted 40.2% (370/920) of the isolates. SXT^R NOR^R, SXT^R AMP^R ATM^R and GEN^R SXT^R NOR^R AMP^R exhibited by *S. sakazakii*, *E. aerogenes* and *Streptococcus sp.* respectively were the least. Multi-drug resistance was commonly encountered in *S. aureus* with 31.5% of this organism being resistant to seven drugs.

DISCUSSION AND CONCLUSION

This study was carried out to determine the antibiotic susceptible profile of the common germs that currently cause surgical wound infection in the Buea Health District, South West of Cameroon.

Pathogenic bacteria were isolated from 1691 of the 2120 (79.8%) specimens cultured. Bacteria may enter wound by direct contamination from patient's skin or internal organs, through the hands and instruments. These infections are the biological summation of several factors: the implantation of bacteria introduced into the wound during the procedure, the unique virulence of contaminants, the microenvironment of each wound, and the integrity of the patient's defense mechanisms (11). Four hundred and twenty nine (20.2%) of the 2120 specimens had no bacteria growth. This could be due to normal healing process where the bacteria have been over-powered by the body's defense mechanism. It is also possible that some organisms could have been anaerobic and, as such were missed as cultures were incubated aerobically. This condition could therefore not support growth of such organisms.

TABLE 4: ANTIBIOTIC SUSCEPTIBILITY TESTING OF ISOLATES

Bacteria Isolates	Percentage (%) susceptible to:													
	OFX	CAZ	GEN	SXT	PEF	CRO	DXT	NOR	C	E	AUG	AMP	OX	ATM
E. coli	100	100	100	60	100	100	100	100	100	100	100	80	0	100
E. cloacae	100	100	100	6.2	100	100	90	100	100	100	100	10	0	50
E. aerogenes	100	100	100	4	100	100	98	100	100	100	100	7	0	7.8
P. mirabilis	100	100	100	10	100	50.7	92	100	100	100	100	70	0	0
K. pneumonia	100	90.6	100	0	100	100	11	92.1	39	54	77	6.2	0	0
H. alvei	100	91.2	100	70	100	100	100	100	100	100	100	83	0	7
P. aerginosa	100	85.7	70.1	0	100	100	15	100	36	89	75.5	7	0	0
S. marcescens	100	100	100	60	100	100	12.5	25	24	64.3	95.5	58	0	60.4
S. rubideae	100	100	100	8.3	100	100	55.6	100	80	100	76.5	64	0	9
S. sakazakii	100	100	100	0	100	100	94.1	5.9	79	100	55.6	78	0	79
S. aureus	100	45.6	87.5	0	100	80	20	51	40	20	60	5	0	0
Strep. Sp.	100	61.8	46.0	6	100	99.1	96	39.6	75	83.3	66.7	45.8	0	58
Total %	100	89.6	92	18.7	100	94.2	65.4	76.1	72.8	84.2	83.9	43.8	0	40

Abbreviation: OFX, ofloxacin; CAZ, ceftazidime; GEN, gentamicin; SXT, co-trimoxazole; PEF, pefloxacin; CRO, ceftriazone; XT, doxycyclin; NOR, norfloxacin; C, chloramphenicol; E, erythromycin; AUG, augmentin; AMP, ampicillin; OX, oxacillin; ATM, aztreonam.

TABLE 5: ANTIBIOTICS RESISTANCE PATTERNS OF ISOLATES

Isolates	Drugs resistant patterns	Number of positive isolates showing patterns (%)	The number of isolates per pattern
E. cloacae	SXT^R AMP^R	140 (15.2)	140
P. mirabilis	SXT^R ATM^R	39 (4.3)	60
S. rubideae	SXT^R NOR^R	21 (2.2)	
S. sakazakii	SXT^R AMT^R AMP^R ATM^R	10 (1.1)	10
E. aerogenes	SXT^R AMP^R ATM^R	10 (1.1)	10
S. marcescens	DXT^R NOR^R AMP^R	31 (3.3)	31
Strephtococcus. Sp.	GEN^R SXT^R NOR^R AMP^R	9 (1.1)	9
K. pneumonia	SXT^R DXT^R C^R AMP^R ATM^R	159 (14.4)	370
P. aerginosa	SXT^R DXT^R C^R AMP^R ATM^R	211 (22.8)	
S. aureus	CAZ^R SXT^R DXT^R C^RE^R AMP^R ATM^R	290 (31.5)	290
Total	80	920 100	

Abreviation: SXT, co-trimoxazole; AMP, ampicillin; ATM, aztreonam; NOR, norfloxacin; DXT, doxycyline; C, chloramphenicol; GEN, gentamicin; CAZ, ceftazidime; R, resistance.

The highest and least occurrences were in burns (100%) and open wounds (78.8%) respectively, although differences between specimens were not significant. This corroborates the finding of Bjornhagen and Bragderyd(14) who reported burn wound infection as he most common infection in hospitals in Sweden. Approximately 73% of all deaths within the first 5 days post-burn have been shown to be directly or indirectly caused by septic processes. A high rate of isolation from burns can be attributed to a large surface barrier loss hence this increases risk of contamination by bacteria(15).

Twelve species of bacteria were recovered from the isolates. They were *S. aureus* (28.4%), *P. aerginosa* (23.1%), *Klebsiella pneumonia* (13%), *Enterbacter cloacae* (12.4%), *E. coli* (5.9%), *S. marcescens* (5.9%), *Proteus mirabilis* (4.7%), *Enterbacter aerogenes* (1.8%), *S. rubideae* (1.8%), *Hafnia alvei* (1.2%), *Streptococcus sp*. (1.2%) and *S. sakazakii* (0.6%). This result corroborates those of Anguru and Olila (16) who isolated similar organisms from septic postoperative wounds in a regional referral hospital in Uganda. Recent studies (13, 17) have also isolated similar organisms from ulcers and surgical wounds. As all patients recruited in the study were hospitalized patients, isolation of these organisms suggests they could be of nosocomial origin. The dysfunction of the immune system, a large cutaneous bacterial load, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization and invasive diagnostic and therapeutic procedure all contribute to infections (9, 14).

Antimicrobial usage in an indiscriminate manner and at non pharmacological doses is considered the most important factor promoting the emergence, selection and dissemination of antimicrobial-resistant microorganisms (3, 8). The study also determined the susceptibility patterns of isolates to antibiotics and revealed marked susceptibility (100%) to ofloxacin (fluoroquinolones) used in this study. This could be attributed to the fact that the high cost of these drugs in the study area limits their abuse. Nonetheless, resistance to these drugs has been reported in other regions of the world(15), and was thought to be due to improper dosage prescription. Similarly, high sensitivity was observed for ceftriazone (94.2%), gentamycin (aminogycoside) (92%) and ceftazidime (89.6%). This finding contradicts the results of Angura and Olilia (16) who reported high bacterial resistance to these drugs. Isolates however exhibited complete resistance to oxacillin, which correlates previous findings (15). The ability of both nosocomial and community-acquired pathogens to develop resistance to powerful broad-spectrum agents presents a great challenge for prescribing patterns and in the development of new drugs to be relatively resistant to inactivation. The high resistance observed in co-trimoxazole (81.3%) could be partly due to the excessive use of this drug in the locality. However, susceptibility of *H. alvei*, *E. coli* and *S. marcescens* to this drug has been reported by Mascaretti (10). Some authors reported low sensitivity of amoxicillin, oxacillin, and ampicillin to *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (7).

Eight distinct resistance patterns were observed. Pattern SXT^R DXT^R C^R AMP^R ATM^R was the most prevalent (40.2% of the isolate), while SXT^R NOR^R, SXT^R AMP^R ATM^R and GEN^R SXT^R NOR^R AMP^R were the least prevalent (1.1%). Approximately 77.2% of the isolates were resistant to three or more

antibiotics. Of these, 71.7% were resistant to five or more drugs. Multi-drug resistance might be linked to high abuse resulting from constant and indiscriminate usage. Advances in control of infections have not completely eradicated the problem because of the development of resistance. Antimicrobial resistance can increase complication and costs associated with procedures and treatment. Multidrug-resistant bacteria have frequently been reported as the cause of nosocomail outbreaks of wound infection (9). *Staphylococcus aureus* the most common isolate such as honey, sugar etc, which have been shown to be broad-spectrum topical antimicrobial agents, eradicating antibiotic resistant strains of bacteria from wounds (1, 8), could play a significant role in treatment of wound infection in study area. Also other topical new antimicrobial agents such as citric acid (8) have been reported to be cost effective in treatment of wound infections caused by resistant bacteria. There is no simple cure for resistance but opportunities for control lie to a lesser and better use of antibiotics, backed by swifter and more accurate diagnosis and susceptibility testing, developing new antibiotics and in protecting old ones from developing resistance. This is because antimicrobial susceptibility testing is intended to predict whether an antimicrobial therapy will be clinically effective and results may directly affect the therapy chosen for treatment of an infection. The use of antibiotics in hospital and the community at large serves as a major selection process for antibiotic resistant bacteria, especially when the use is massive, indiscriminatory, not carefully tailored and monitored, and when standard doses are not respected.

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(31.5%), exhibited resistance to more than six drugs. Other isolates that also largely exhibited multidrug resistance were *P. aeruginosa* (22.8%) and *K. pneumoniae* (14.4%). Drug resistance of these organisms in other parts of the world has reached a worrying level(17). The progressive reduction of therapeutic efficacies of the available antibiotics underlines the need for the development of new therapeutic strategies for the treatment of infected wounds and other infections. Thus, natural products

The most commonly encountered germ in the study area, *S. aureus*, was resistant to most commonly prescribed antibiotics. This study has revealed predominant multidrug resistant pathogenic bacteria isolates from infected wounds in the study area, with dominant resistant patterns being SXT^R, DXT^R, C^R, AMP^R, ATM^R. Appreciable sensitivity of the isolated pathogenic bacteria was shown to ofloxacillin, pefloxacin, followed be ceftriazone, in decreasing order of potency.

Limitation

A total of 391 (15.57%) patients dropped out of the study for various reasons. This may have ultimately affected the results.

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MICROBIAL STATUS OF SMOKED FISH, *SCOMBIA SCOMBIA* SOLD IN OWERRI, IMO STATE, NIGERIA

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ABSTRACT

As one of the common sources of protein available to man, fish is highly consumed due to its lower cholesterol content and price. So it forms a rich protein source for both poor and rich. As a part of checkmating the public health risks associated with this general dependence of the population on fish, the microbiological assessment of smoked fish, *Scombia, scombia* sold in Owerri was embarked on with the aim of ascertaining the microbial quality, the presence and prevalence of microorganisms of public health importance. A total of one hundred and eight (108) samples were collected from the smoking Factory, Open Market and Hawkers. These were analyzed microbiologically for viable heterotrophic bacteria and fungi count on Nutrient and Potato dextrose agar respectively, using pour plate method and coliform count in MacConkey broth by multiple tube method (MPN). The mean value results from the analysis revealed high microbial contamination in all the samples. The resultant data were analyzed statistically using randomized block design of Analysis of Variance (ANOVA) at 95% level of confidence and the difference were separated using the least significance difference (LSD). The mean results of viable heterotrophic bacteria and fungi count showed no significance difference for the collection sites; but the coliform mean results for the three sites showed marked variation at 95% level (P>0.05). Identified bacteria, include: *Staphylococcus aureus, E. coli, Bacillus sp., Klebsiella sp.*, whereas fungi are *Penicillium sp., Aspergilusl sp., Fusarium violaceum, Biospora sp., Candida sp, Botryodioplodia sp., Alternania sp.* This high level of microbial contamination can be traceable to handlers, and environment to which this fish is exposed during smoking and selling exercises, and considering the danger it portends to human health, public health and food safety authorities should intensify their monitoring efforts towards controlling such contamination.

Key words: **Bacteria, Yeast, Mould, Smoked fish, Contamination.**

INTRODUCTION

Fish is a vertebrate animal, living in fresh and seawater. It is one of the main sources of animal protein foods available for human consumption (**1**). Most of the catch comes from oceans, seas, rivers and lately from man-made ponds (**2**). It is a highly nutritious food of about 60-80% water, 15-25% protein, 11-22% fat, 20% mineral and 1% carbohydrate (**3**). It is often cheaper than meat and so it is a rich protein source for both the poor and the wealthy.

Microbial flora of fish depends on the microbial content of the water in which they live as the slime that covers the surface of fish has been found to contain great variety of bacteria genera (**4**). Many dangers therefore exist if fish harvested from polluted water is eaten raw, and because of the high microbial load of freshly harvested fish it is susceptible to rapid spoilage. Hence preservation of fresh fish becomes very important. This can be achieved by freezing, drying through smoking and sun-drying, canning, etc.

Smoking simply means a heating process that dries the fish to preserve it from spoilage (**5**). Most dry fish consumed in Nigeria are smoked (**6**). Smoking of fish from smoldering wood for its preservation dates back to civilization (**7**). The steps in the smoking process are necessary not only for safe preservation, but also to produce good flavor and aroma (**8**). Hence smoked fishes are less prone to microbial spoilage than fresh fish. However spoilage still occurs as a result of growth of microbes due to partial dehydration during smoking (**9**).

Contamination of fish and other fishery products by microbes has been a serious threat to human health. There are four main factors responsible for fish spoilage once it is out of its natural habitat (water) and these include: Autolysis which usually precedes bacterial spoilage and involves the breakdown of

protein and lipids to amino acids and fats by muscle enzymes. The activity of microorganism is another factor which uses the amino acid produced by autolysis for proliferation (**10)**. Others are chemical deterioration and insect attack which cause considerable deterioration.

However, spoilage of fresh and highly preserved fish products is mostly caused by microbial action. Foods of high sugar/salt contents are therefore most likely to be spoiled by any kind of microbe (**5)**. It has been reported that serious disease outbreak had occurred in both man and animals after consuming some dried fish feed and food (**11)**. This could be as a result of disease causing microorganism like *Escherichia coli, Salmonella typhi, Vibrio cholera* which results from poor handling/cross-contamination and improper processing practice of ready to eat "smoked fish" products. Other microorganisms of primary concern are *Listeria monocytogen* and *Clostridium botulinium*. Extensive handling provide opportunities for other food borne pathogens to contaminate products if sufficient attention is not given during smoking process (**12)**.

This work therefore tends to investigate the level of sanitation maintained by handlers during processing and storage of smoked fish sold in Owerri.

MATERIALS AND METHODS

Sample collection

A total of 108 smoked fish samples "*Scombia scombia*" were collected from the smoked fish factories, Open markets and Hawkers all within Owerri. Three weekly samples were collected for 3 Months between the months of February and April 2006. These were analyzed for microbial load at the Microbiology Laboratory of the Department of Medical Laboratory Science, Imo State University, Owerri.

Sample preparation and Laboratory analysis

Ten (10) grams of the smoked fish was weighed into a stomacher bag and 90ml of sterile physiological saline was added. This was thoroughly homogenized in the stomacher for 90 second. Then ten-fold serial dilution was prepared in 9ml of solvent using 1ml sterile pipette. The viable heterotrophic bacterial counts, yeast and mould counts were done using pour plate method on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media respectively, while the Most Probable Number (MPN) of coliform was determined in MacConkey broth using Multiple Tube method.

Pour Plate Method

An aliquot of 0.1ml from each dilution was aseptically transferred to the centre of sterile Petri-dishes in duplicates. Then sterile molten nutrient Agar/Potato Dextrose Agar at about 45^0C was poured on them accordingly. These were mixed by a combination of rotational movement: To and fro, clockwise and anticlockwise direction for 5-10 seconds. The plates were allowed on the bench to solidify, inverted and properly labeled. These were incubated at 37^0C for 24hrs and room temp for 3-7days for bacterial and fungal growths respectively.

Most Probable Number (MPN) Method

Eleven test tubes with Durham's tubes inverted inside them were used. The tubes were labeled and grouped into three batches of one, five and five tubes for each batch. Batch one contained 50mls, batch two contained 10mls and batch three contained 5mls each. Each tube in the three batches was inoculated with 50mls, 10mls and 1ml of each sample respectively.
The inoculated tubes were corked and incubated at 44^0C for 24hrs. Positive test tubes were indicated by colour change from red to yellow showing acid production and gas production was shown by the displacement of broth in the Durham's tube inside the tubes. Most Probable Number (MPN) of coliform was determined as stipulated by Chesbrough (2000) (**13)**.

The bacteria and fungi isolates were sub-cultured and preserved in Nutrient and potato Agar slants accordingly for further characterization. These were tested for Grams reaction, Motility and biochemical characteristics such as Catalase, Oxidase, Indole, Urease and Carbohydrate (sugar) utilization as stipulated by Baron *et al,* (1994) (**14)**.

STATISTICAL ANALYSIS

The data obtained from this investigation were analyzed statistically using randomized block design ANOVA and the means separated using Least Significance Difference (LSD).

RESULT

The result of microbial status of smoked fish, *Scombia scombia* fish sold in Owerri, Imo State are as follows: Microbial analysis was done to determine the general viable count, the coliform count as well as yeast and moulds count.

Table 1 shows the mean of viable heterotrophic bacteria count of smoked fish samples from the three sources and showed no significant difference at 95% level ($P<0.05$). Factory smoked fish recorded $8.83x10^5$ (cfu)/g, while market smoked fish recorded $1.35 x10^6$ (cfu)/g and Hawkers $2.50x10^6$ (cfu)/g.

Table 2 presents the Mean results of the coliform count which showed significant difference at 95% level ($P>0.05$). There were high coliform counts in all the samples. Factory smoked fish had mean count of $2.8 x 10^4$ (cfu)/g, Market smoked fish had $3.8x10^4$ (cfu)/g and Hawkers, $5.57x10^5$ (cfu)/g.

TABLE 1: THE MEAN RESULT OF VIABLE HETEROTROPHIC BACTERIA COUNT OF SMOKED FISH SAMPLE (X10³ CFU/G)

Weekly Replication	smoked fish sources		
Factory	Market	Hawkers	
1	50	70	175
2	120	225	350
3	95	110	225
Total	265	405	750
Mean	(88.3)	(135)	(250)

F 0.05 (2, 6) = 5.14, P<5.14. *Not significant at 95%

TABLE 2: THE MEAN RESULT OF COLIFORM COUNT OF SMOKED FISH (X10³ CFU/G), USING MOST PROBABLE NUMBER (MPN) METHOD.

Weekly Replication	smoked fish sources		
Factory	Market	Hawkers	
1	30	41	57
2	28	35	55
3	26	38	55
Total	84	114	167
Mean	(28)	(38)	(55.7)

F 0.05 (2,6) = 5.14, P>5.14. *Significant at 95%. LSD = 4.69

Table 3 displays mean values of fungal loads for the sites. The highest,0.2 x 10^4 (cfu)/g was observed in samples from Hawkers, whereas samples from Factory and open market had the lowest,1 x10^4 (cfu) /g each. However there was no significant difference among the samples at 95% level (P>0.05).

TABLE 3: FUNGAL COUNT (X10³CFU/G) FOR THE THREE SITES

Weekly Replication	Smoked fish sources		
	Factory	Market	Hawkers
1	1	1	3
2	1	1	2
3	1	1	1
Total	3	3	6
Mean	(1)	(1)	(2)

F 0.05 (2, 6) = 3.0, P>5.14. Not Significant at 95%

Table 4 shows sample location specificity for Bacteria isolates. This indicates that *Bacillus spp.* and *Staphylococcus aureus* were isolated from all the sources, while *E. coli* and *Klebsiella spp.* were isolated from Market and Hawkers respectively.

TABLE 4: SPECIFIC SITES OF BACTERIAL CONTAMINANTS' ISOLATION.

Sources	No. of samples collected	No. contaminated	No of organisms	Bacterial Isolates isolated
Factory	**36**	**36**	**2**	***Bacillus spp., Staph. aureus***
Market	**36**	**36**	**3**	***Bacillus spp., Staph. aureus,E. coli***
Hawkers	**36**	**36**	**3**	***Bacillus spp., Staph. aureus,Klebsiella spp.***

Table 5 shows fungal contaminants of smoked fish from the different sources. Fishes from Hawkers were more contaminated with *Candida spp., Fusarium spp.* and *Aspergellus spp.,* followed by those from market and factory which have *Alternaria spp.* and *Penicillum spp;* and (*Botryodioplodia spp* and *Biospora spp.,* respectively

TABLE 5: SPECIFIC SAMPLES OF FUNGI CONTAMINANTS ISOLATION.

Sources	No. of samples collected	No. contaminated	No of organisms	Fungal Isolates isolated
Factory	**36**	**6**	**2**	***Biospora spp, Botryodioplodia spp.***
Market	**36**	**10**	**2**	***Alternaria spp, Penicillum spp.***
Hawkers	**36**	**20**	**3**	***Candida spp, Fusarium spp, Aspergellum spp***

DISCUSSION AND CONCLUSION

This work primarily aimed at investigating the maintenance of proper sanitary levels of processing and storage conditions by handlers of smoked fishes sold in Owerri. There were marked variations between the means of viable bacteria counts. Result showed high coliform contamination, compared with the standard (10^3). The high count, especially on the factory source could be attributed to improper pre/post handling /smoking procedures. This is in agreement with Maga, (1988) (15) who considered smoking process, a mild preservative treatment, which kills bacteria and prevents microbial proliferation due to combined effects off heating, drying, pH and Anti microbial smoke components. Hence, as a mild treatment, smoking does not achieve complete elimination of microbial load of a fresh fish which has been proved to be naturally high due to the high microbial load of their habitat (water) (4).

The highest counts observed among the samples from Hawkers can be attributed to the fact that hawking exposes the fish to more possibilities of contamination than any of the other sources. This supports the observation of Eklund *et al,* 1993 (12), which stated that any handling of fish and the associated sanitary practice from the point of harvesting can potentially contribute to the micro flora on the final product. Moreover, hawkers move from one place to and other hence, the possibility of exposing the fish to different microbial inhabitant of the different areas. This is unlike those of the factory and market that have limited exposition to microbial environments. Again hawkers are mostly children under the age of twelve. Who are not yet used to hygienic practices compared to their adult counterparts in the factory and some of the markets.

The isolation of *E. coli* and *Klebsiella spp.* are indications of feacal contamination and this is in agreement with the report of Frazier and Westhoff (1995) (4) which states that microbial flora of fish depends on the microbial contents of the waters in which they lived in. Dike*et al,2007*(16) has proven that water sources in Owerri, especially the streams and rivers from where these fishes were obtained are contaminated with coliform organisms. Hence the isolation of these feacal contaminant from fishes sold in Owerri is likely to be from those water sources.

Again the isolation of *Staphylococcus aureus and Bacillus spp.* is an indication of poor handling or cross contamination of smoked fish products, since the two organisms have been indicted in food poisoning (17). *Biospora spp., Botryodioplodia spp., Alternaria spp., Penicillum spp., Candida spp., Fusarium spp.,* and *Aspergellum spp.* as identified in this work have all been incriminated in food spoilage and are traceable to water and soil with which the fish is in contact (18). Also the isolation of these microorganisms from the smoked fish indicate partial dehydration during

smoking which is in agreement with Schewan (1977) (9) who attributed species of fungi observed in smoked fish samples to microbial spoilage as a result of partial dehydration during smoking.

The result of this work has proven that smoked fish sold in Owerri are contaminated right from the factory point. This implies that smoking is not an effective means of preservation and prevention of microbial proliferation in fish. This work also has shown that bacteria and fungi are responsible for the microbial contamination of smoked fish.

Based on these findings, we are recommending the use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture. Also we are recommending that regulatory authority such as NAFDAC should look into the environmental condition of our food handlers as it concerns the smoking factories, the markets where our foods are sold and even the hawkers that carry the food from one place to another. Their hygienic condition must be ascertained before authorizing them to handle public food.

Finally, we are recommending that people should properly cook their fish before eating even when it is smoked to avert food poisoning.

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PAEDIATRIC MALARIA: A TEN-YEAR RETROSPECTIVE STUDY AT THE NATIONAL HOSPITAL, ABUJA, NIGERIA

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ABSTRACT

A ten year study of malaria amongst paediatric patients was carried out in the Federal Capital Territory, Nigeria, West Africa from 2000 to 2010. Giemsa staining methodology was used. Of the 24 289 blood samples analyzed (comprising of 13 435 male children and 10 854 female children), 8668 (35·7%) were positive for malaria parasites. 267 (3·1%) had parasite density of > 5000 parasites/µl of blood; 382 (4·4%) had between 500 - 5000 parasites/µl of blood; 1262 (14·6%) had between 50 - 500 parasites/µl of blood; while 6757 (77·9%) had between 5 - 50 parasites/µl of blood. The 11-15 years age group had the highest prevalence of 40·6%, while neonates (<1 - 28 days), 1 month – 5 years, and 6 – 10 years age groups recorded 27·2%, 34.5% and 36·5% respectively. Of the 13 435 male children, 4845 (36·1%) had positive malaria result as against 35·2% (3823) of positive cases recorded among the 10854 female children. There is need to enhance parasitological diagnosis by way of providing diagnostic tolls at all levels of health care – primary (rural settings), secondary and tertiary. There are negative implications associated with the continued use of malaria rapid diagnostic tests (M-RDTs) methodologies which includes underdiagnosis, misdiagnosis of malaria and mismanagement of non-malarial fever, which wastes limited resources, erodes confidence in the health care system, and contributes to drug resistance. Finally, appropriate antimalarial drugs for treatment should be given free to all malaria positive children.

Keywords: Malaria, paediatric patients, parasite density, prevalence, laboratory diagnosis, treatment.

INTRODUCTION

The bare facts are that malaria is a public health problem in more than 90 countries inhabited by 40% of the world's population and that there are 300 – 500 million cases annually with a mortality of 1·5 – 2·7 million, mainly children in Africa. Malaria infections produce the most severe disease in those with the least immunity. 90 % of the world's malaria is transmitted in the sub-Saharan Africa, and immunity is acquired over time, requiring repeated exposure to the bites of infective Anopheles mosquitos. As a result, young African children are the group at highest risk of developing severe disease. Patwari (1985) (1) reported malaria accounting for between 5 – 15% of deaths of children in endemic areas. However, ten years later, Defo (1995) (2) reported 20 – 30% of deaths in childhood being attributed to malaria.

Based on documented cases, the WHO estimates that there were 219 million cases of malaria in 2010 resulting in 660 000 deaths (3). This is equivalent to roughly 2000 deaths every day (4). A 2012 study estimated the number of documented and undocumented deaths in 2010 was 1·24 million. The majority of cases (65%) occur in children under 15 years old (5, 6). Pregnant women are also especially vulnerable: about 125 million pregnant women are at risk of infection each year. In Sub-Saharan Africa, maternal malaria is associated with up to 200 000 estimated infant deaths yearly (7).

In Nigeria, the disease is responsible for 60% of outpatient visits to health facilities, 30% childhood deaths, 25% of death in children under one year and 11% maternal death (8). WHO estimates there were 655 000 malaria deaths in 2010, 91% in the African Region, and 86% were children under 5 years of age.

More cases of malaria in the neonatal period are being reported which may be due to congenital malaria through transplacental infection, malaria acquired postnatally from mosquito bites or exchange transfusion (9, 10).

The problem of malaria had not abated though; Artemisinim-combination therapy (ACT) drugs are in circulation in the drug market aimed at taking care of resistant strains of ***Plasmodium*** species.

The objective of this study therefore is to determine the burden of malaria amongst paediatric patients in the Federal Capital Territory, Nigeria.

METHODS

Study Area

The Federal Capital Territory (FCT), Abuja is the study area. Abuja is located between latitude 8·25 and 9·20 north of the Equator and longitude 6·45 and 7·39 east of the Greenwich Meridian. The Federal Capital Territory has a total landmass of about 8000 sq kms; located geographically at the centre of the country. The current population size of the FCT according to the 2006 census figures stands at 1 405 201 (11).

Two seasons are experienced in FCT. These are the wet (rainy) season and the dry season. The rainy season lasts between April to October (with a mean total rainfall of 62·8 – 262·7 mm; August being the highest). The dry season is between November and March (with a mean total rainfall of 1·2 to 11·3 mm).

Abuja has a daily temperature range between 20·4 – 34·7^{o}c with an average of 27·2^{o}c/81^{o}f in January; and between 21·9 – 29·1^{o}C with an average of 25·6^{o}c/78^{o}f in July (12).

Study Population

The population studied consisted of 24 289 children (comprising of 13 435 males and 10 854 females) whose age ranged from day of birth to 15 years whose blood samples were processed at the Parasitology Laboratory of National Hospital, Abuja for malaria parasite diagnosis. This study was carried out from May 2000 to December, 2010.

Parasitological Techniques

Thick and thin blood films were made on clean grease-free slides and stained appropriately using Giemsa's staining method. The stained films were then examined microscopically using 100x objective to count the parasites.

Parasites were counted by estimating the parasite numbers/µl of blood from the thick film. This was carried out by multiplying the average number of parasites per thick film fields (100x objective) by 500. Between 10 -100 fields (depending on parasiteamia) were examined to determine the average number of trophozoites per thick film fields. Ten fields are sufficient when the parasite density is high.

The factor of 500 was proposed by Greenwood and Armstrong (1991) [13]. They calculated that 5 - 8 µl is the volume of blood required to make a satisfactory thick film and that the volume of blood in one thick film fields (100 x objectives) of a well-prepared thick film is about 0.002µl. Therefore the number of parasites per thick film field multiplied by 500 gives the estimated number of parasites/µl of blood.

This method, Greenwood and Armstrong (1991) (13) found to be more accurate and quicker than counting the parasites against 100 white blood cells in a thick film using WHO method as used by Molineaux and Gramiccia (1980) [14]. In 2002, Ikeh *et al*, [15] used this technique in their study at Jos.

For designation of the relative parasite count on a thick film, a simple code of from one to four crosses or the plus sign scheme is used to report parasite numbers:

- + (1+) = 1 – 10 parasites per 100 thick film fields
- + + (2+) = 11 – 100 parasites per 100 thick film fields
- + + + (3+) = 1 – 10 parasites per one thick film field
- + + + + (4+) = > 10 parasites per one thick film field

RESULTS

Of the 24 289 samples analyzed, 8668 (35·7%) were positive for malaria parasites. 267 (3·1%) had parasite density of > 5000 parasites/µl of blood (4+); 382 (4·4%) had between 500-5000 parasites/µl of blood (3+); 1262 (14·6%) had between 50-500 parasites/µl of blood (2+); while 6757 (77·9%) had between 5-50 parasites/µl of blood (1+) as shown

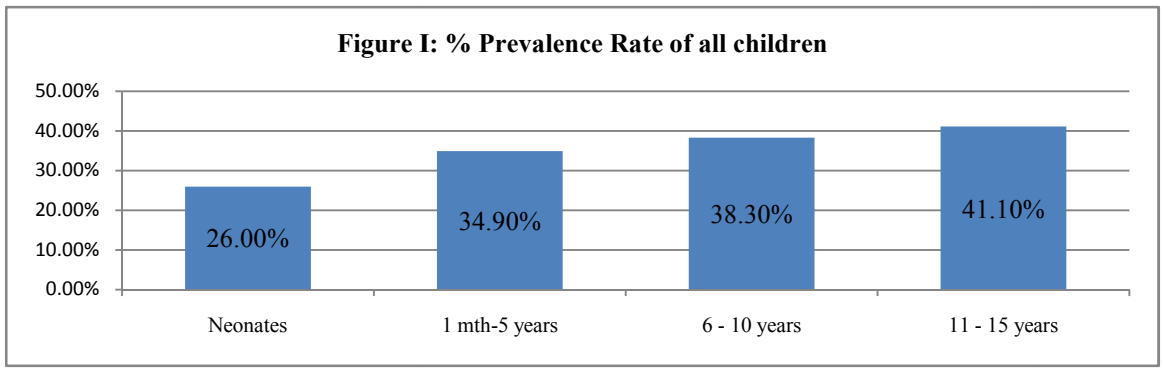
The 11-15 years age group had the highest prevalence of 40·6%, while neonates (<1 - 28 days), 1 month – 5 years, and 6 – 10 years recorded 27·2%, 34·5% and 36·5% respectively. The parasite density of each age group is shown in table 1.

Of the 13 435 male children, 4845 (36·1%) had positive malaria result as against 35·2% (3823) positive cases recorded among the 10 854 female children

TABLE I: PREVALENCE AND PARASITE INTENSITY OF ALL CHILDREN

Age Group	4+	3+	2+	1+	Total Positives	Negatives	Total No. Examined
Neonates	0(0%)	2(0·6%)	51(14·2%)	306(85·2%)	359(26%)	1022	1381
1mth – 5years	108(1·9%)	166(3·0%)	759(13·7%)	4510(81·4%)	5543(34·9%)	10362	15905
6 – 10 years	77(5%)	91(5·9%)	233(15·1%)	1144(74%)	1545(38·3%)	2484	4029
11 – 15 years	82(6·7%)	123(10·1%)	219(18%)	797(65·3%)	1221(41·1%)	1753	2974
Total	267(3·1%)	382(4·4%)	1262(14·6%)	6757(77·9%)	8668(35·7%)	15621	24289

Key: 4+ => 5000 parasites/µl of blood; 3+ = 500 – 5000 parasites/µl of blood; 2+ = 50 – 500 parasites/µl of blood 1+ = 5 – 50 parasites/µl of blood



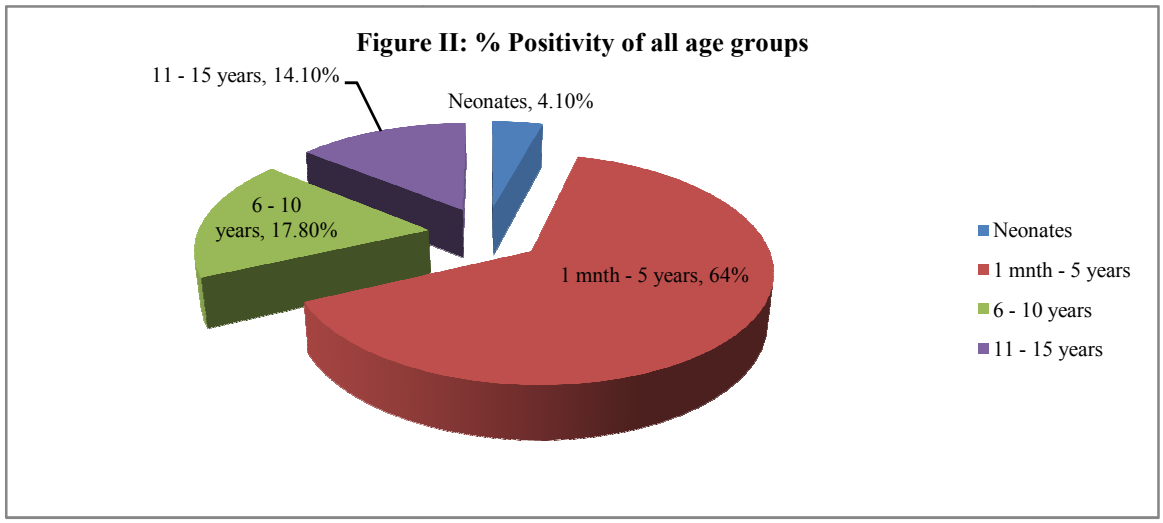


TABLE II: PREVALENCE AND PARASITE INTENSITY OF MALE CHILDREN

Age Group	4+	3+	2+	1+	Total Positives	Negatives	Total No. Examined
Neonates	0(0%)	1(0·5%)	29(15·5%)	158(84%)	188(25%)	565	753
1mth – 5years	65(2·1%)	91(2·9%)	443(14·2%)	2528(80·8%)	3127(35·1%)	5783	8910
6 – 10 years	49(5·6%)	56(6·4%)	133(15·2%)	636(72·8%)	874(40%)	1315	2189
11 – 15 years	52(7·9%)	75(11·4%)	112(17·1%)	417(63·6%)	656(41·4%)	927	1583
Total	166(3·4%)	223(4·6%)	717(14·8%)	3739(77·2%)	4845(36·1%)	8590	13435

Key: 4+ - > 5000 parasites/µl of blood; 3+ - 500 – 5000 parasites/µl of blood; 2+ - 50 – 500 parasites/µl of blood
1+ - 5 – 50 parasites/µl of blood

FIGURE III: % PARASITE DENSITY OF MALE CHILDREN

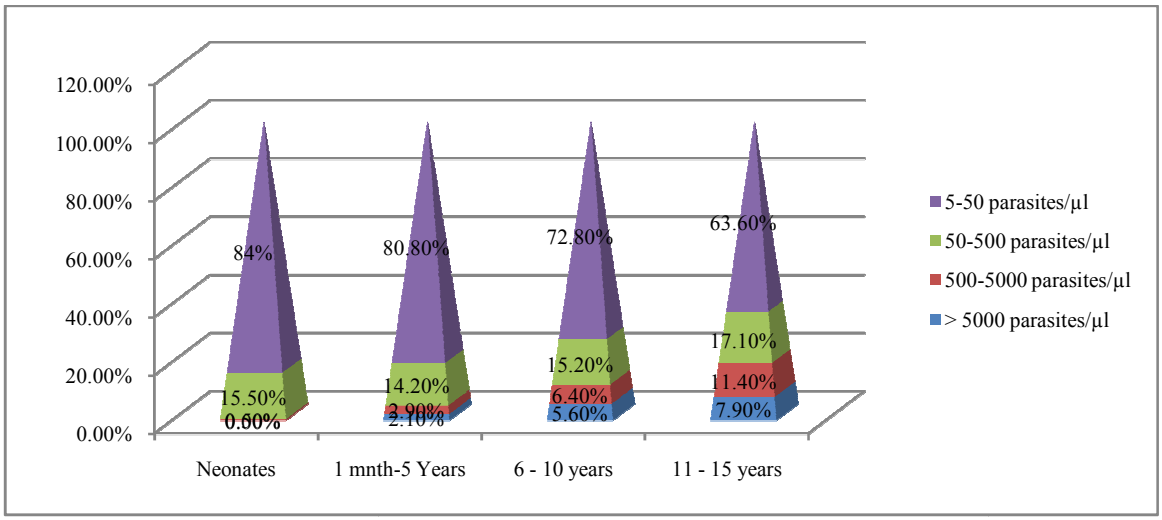


FIGURE IV: % POSITIVITY OF AGE GROUPS AMONG MALE CHILDREN

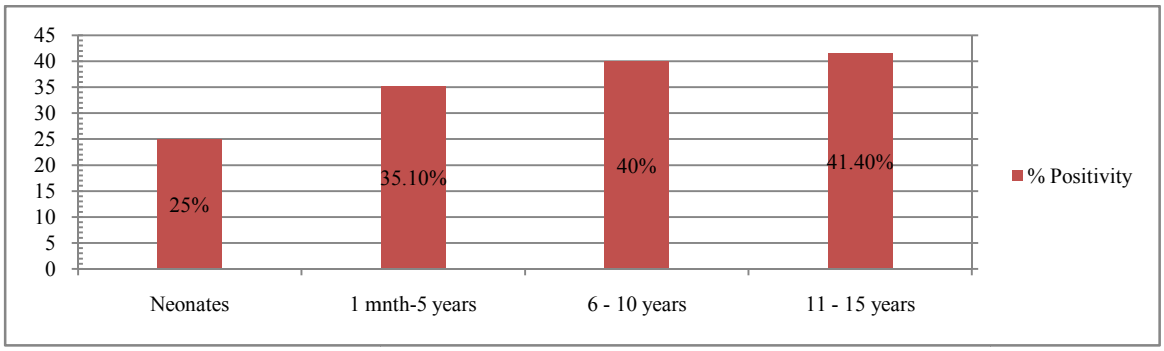


TABLE III: PREVALENCE AND PARASITE INTENSITY OF FEMALE CHILDREN

Age Group	4+	3+	2+	1+	Total Positives	Negatives	Total No. Examined
Neonates	0(0%)	1(0·9%)	22(12·9%)	148(86·5%)	171(27·2%)	457	628
1mth – 5years	43(1·8%)	75(3·1%)	316(13·1%)	1982(82%)	2416(34·5%)	4579	6995
6 – 10 years	28(4·2%)	35(5·2%)	100(15%)	508(75·7%)	671(36·5%)	1169	1840
11 – 15 years	30(5·3%)	48(8·5%)	107(19%)	380(67·3%)	565(40·6%)	826	1391
Total	101(2·6%)	159(4·2%)	545(14·3%)	3018(78·9%)	3823(35·2%)	7031	10854

Key: 4+ = > 5000 parasites/µl of blood; 3+ = 500 – 5000 parasites/µl of blood; 2+ = 50 – 500 parasites/µl of blood; 1+ = 5 – 50 parasites/µl of blood

FIGURE V: % PARASITE DENSITY OF FEMALE CHILDREN

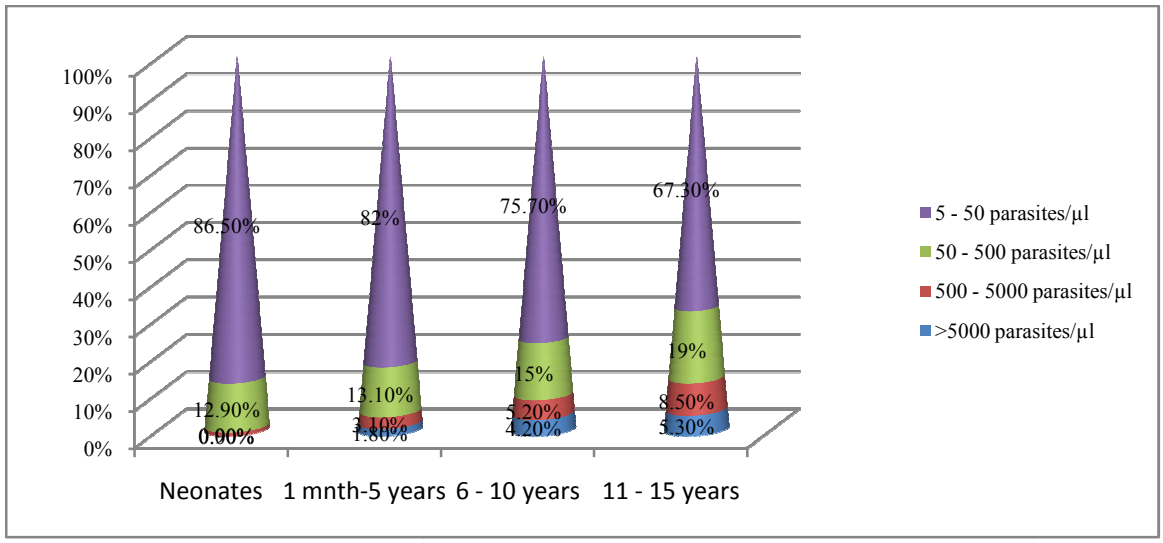
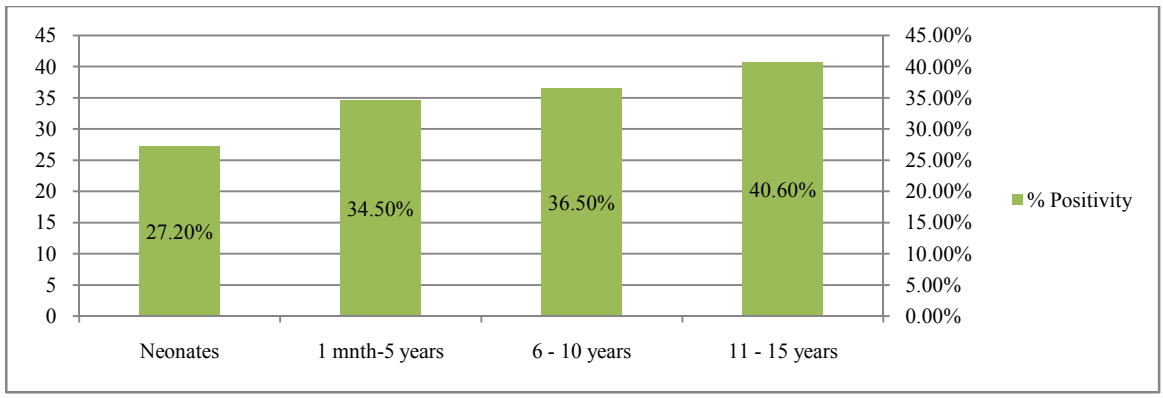


FIGURE VI: % POSITIVITY OF AGE GROUPS AMONG FEMALE CHILDREN



DISCUSSION

This study highlights the importance of prompt and accurate parasitological diagnosis of malaria amongst the paediatrics. Malaria parasites were found in 35·7% of all age groups.

In 1971, Hendrickse *et al* (16) reported a 33·3% prevalence rate in their study in Ibadan, Western Nigeria (now, South West Nigeria). In 1993, Pam *et al*, (17) reported a 41% prevalence rate for malaria among children that attended maternal and child welfare clinics in northern Nigeria. Ikeh *et al,* (2002) (15) had reported 29·3% positivity among paediatric patients in Jos. In 2006, Runsewe-Abiodun *et al*, (18) reported 24·8% malaria positivity among neonates and 17·4% for congenital malaria at Sagamu, Ogun State, South-west Nigeria. Oyedeji *et al*, (2010) (19) in their report stated that 20·3% of paediatrics had positive malaria test at Osogbo, South-West Nigeria. All these reports indicate that Nigeria is a mesoendemic country having a stable transmission being based on the WHO classification of endemicity revised by Metselaar and Van Thiel (1959) (20).

However, Okogun & Amadi (2005) (21) reported that 95·2% of paediatric patients were confirmed positive for malaria parasites microscopically at a Nigerian tertiary health institution. We have not seen any other report that supports such very high percentage positivity among paediatrics in Nigeria.

In this study, although, the 1 month – 5 years age group (under fives) had most of the samples analyzed, 15,905 (65·5%) of the 24,289; it had a % positivity of 34·9%. The 11 – 15 years age group had the highest % positivity of 41·1%, though with the second to last total number of samples examined, 2,974 (12·2%). From our data, it showed that a greater number of the blood samples analyzed (71·2%) were those of the under fives including the neonates. This indicates that this group is more vulnerable or more hospital bound due to their low immune status than the older children between 6 – 15 years. 26% of neonates had malaria positivity rate while of the 6 – 10 years age group a % positivity of 38·3% was recorded.

The highest % positivity of 41·1% among the 11 – 15 years age group may be attributed to the theory of innate immunity (9). In 2002, Ikeh *et al*, (15) reported 38·1% positivity amongst this age group.

The parasitaemia among neonates in this study - 26%, most likely represents first infection and a good indication of recent transmission of malaria through transplacental infection or blood transfusion. We did not differentiate between congenital malaria from other neonatal malaria because we could not lay our hands on the folders of these patients as to identify those that had exchange blood transfusion.

In this study, no significant differences were found in prevalence and % positivity in relation to gender. While the male children had a % positivity of 36·1%, the female children had 35·2%.

Meanwhile, the continued use of malaria rapid diagnostic tests (M-RDTs) methodologies in Nigeria for individual diagnosis of malaria should be highly discouraged because of its negative implication. The implication of the continued use of these malaria rapid diagnostic tests (M-RDTs) methodologies includes underdiagnosis, misdiagnosis of malaria, mismanagement of non-malarial fever, which wastes limited resources, erodes confidence in the health care system, contributes to drug resistance, and eventual administration of unnecessary antimalarial drugs aimed at mopping up all negative test results where patients may still present with symptoms.

Several studies have shown that malaria rapid diagnostic tests (M-RDTs) methodologies have malaria parasites detection limit of 397 – 500 parasites/µl of blood and in some cases even more than 500 parasites/ µl of blood (22, 23, 24, 25, 26, 27, 28) as against the 5 parasites/µl of blood detectable with the Giemsa stained slide microscopy examined by microscopists who are experienced and competent in the preparation and staining of blood films, as well as in the recognition and identification of the characteristic stages of malaria parasites usually found in human blood.

Conclusion

The missing component of the Roll Back Malaria (RBM) strategy - Laboratory Diagnosis should be incorporated. The vision of the current five-year strategic plan (2009 – 2013) is to ensure that Malaria no longer becomes a major public health problem in Nigeria as illness and death from malaria gets significantly reduced. This is to be achieved by ensuring that families will have universal access to malaria prevention and treatment. This latter aspect should rather read "access to malaria prevention, laboratory diagnosis and treatment.

There is need to enhance parasitological diagnosis by way of providing diagnostic tolls (both man-power and materials) at all levels of health care – primary (rural settings), secondary and tertiary. Above all, the turn-around time for malaria parasitological diagnosis should be drastically reduced so that such patients get their results same day and those with positive results see their doctors again for appropriate

treatment before going back home. Appropriate antimalarial drugs for treatment should be given free to all malaria positive children.

Finally, if we must win this war against malaria, all unnecessary politicking associated with the prevention, accurate diagnosis at all levels of healthcare and effective treatment must be put aside.

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GUT FERMENTATION SYNDROME

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ABSTRACT
Fungi have not been recognized to cause such notable syndromes until very recently. It has been documented among few individuals who became intoxicated after consuming carbohydrates, which became fermented in the gastrointestinal tract. These claims of intoxication without drinking alcohol, and the findings on endogenous alcohol fermentation are now called Gut Fermentation Syndrome. This review will concentrate on understanding the aetiology, clinical manifestations, laboratory diagnostic options and treatment of the syndrome.

KEYWORDS: Gut Fermentation Syndrome, Fungi, Yeast, *Saccharomyces cerevisiae.*

INTRODUCTION

Infectious diseases physicians are familiar with several syndromes associated with bacterial and parasitic diseases. Examples of such are rheumatic fever, rheumatic heart disease, and acute glomerulonephritis (following Lancefield group A streptococcal infections); toxic shock syndrome, Ritter's disease and scalatiniform rash (following infections by *Staphylococcus aureus*); Reiter's disease (following infections by *Chlamydia trachomatis*) (1). Fungi have not been recognized to cause such notable syndromes until very recently.

RECENT FINDINGS

Kaji and others described the case of a 24 years old female who became intoxicated after consuming carbohydrates, which fermented in the gastrointestinal tract (2). In this situation the causative organisms were determined by cultures to be *Candida albicans* and *Candida krusei*.

Claims of endogenous fermentation of this type have been used as a defense against drunken driving charges (3).

Most bacteria that ferment sugars do so through glycolytic, pentose phosphate and Entner- Doudoroff pathways, with lactic and pyruvic acids as their major end products (4). Common yeasts (*C. albicans, C. tropicalis and Torulopsis glabrata)* and *Saccharomyces cerevisiae* are able to ferment sugars through the homolactic, heterolactic or mixed acid fermentation pathways, with ethanol as the major end product (4). Candida is common yeast found in the environment, on our bodies and in our bodies. It competes for space with the normal flora on the skin and in warm and moist environments especially digestive tract. Candida can overgrow almost anywhere in the body and cause an infection. *Candida species* are the most common cause of invasive fungal infections in humans, producing infections that range from non-life threatening mucocutaneous disorders to invasive disease that can involve any organ.

Because of the claims of intoxication without drinking and the findings on endogenous alcohol fermentation, a new syndrome called Gut Fermentation Syndrome was described (5). This syndrome also goes by several other names, such as Auto-Brewery Syndrome, Syndrome A and Endogenous Ethanol Fermentation (5). The underlying mechanism is thought to be an overgrowth of yeast in the gut whereby the yeast ferments carbohydrates into ethanol.

Gut Fermentation Syndrome is a relatively unknown phenomenon in modern medicine. The earliest cases of this phenomenon were described prior to 1976 and only a few cases have been reported in the last three decades (2, 6,).

Dahshan and Donovan (7) described the case of a 13 years old girl with short gut syndrome who became intoxicated after ingesting carbohydrates. She had been placed in a rehabilitation facility with no access to alcohol. Aspirates from her small intestines grew *Candida glabrata* and *Saccharomyces cerevisiae*.

Barbara Cordell and Justin McCarthy (5) presented a case study of a 61 years old male with a well-documented case of Gut Fermentation Syndrome verified with glucose and carbohydrate challenges. The stool cultures demonstrated the causative organism as *Saccharomyces cerevisiae*.

DIAGNOSTIC PROCEDURES

A clinical test was proposed in 1990 for dietary fermentation (8). Subjects suspected of gut fermentation syndrome were given a fasting glucose challenge of 5 gm glucose orally. One gram of glucose was given in a hardened gelatin capsule to ensure passage into the duodenum. Blood glucose and ethanol levels were measured at 1 hour. Fasting blood alcohol levels were zero in nearly all normal subjects; but 61% of the 510 subjects showed an increase in blood alcohol levels on the average of 2.5 mg/dl (range 1.0 - 7.0 mg/dl). This compared to near zero blood alcohol levels in the control group given the same challenge (8).

Another experiment was also conducted by combining five infant food formulas and/or supplements with four common yeasts (*C. albicans*, *C. tropicalis*, *Torulopsis glabrata*, and *S. cerevisiae*) to measure ethanol production *in vitro*. All of the mixtures of yeast and carbohydrate produced ethanol, with the *Saccharomyces. cerevisiae* preparations being the highest (9). *Saccharomyces cerevisiae*, also known as brewer's yeast, has a very well-known history and life cycle because of the brewing industry. More recently the entire genome of *S. cerevisiae* has been identified due to its important use in fermenting corn into ethanol for fuel consumption.

S. cerevisiae has mostly been identified as a pathogen in critically ill patients who are immuno-compromised (10, 11). Not one single article could be found on an *S. cerevisiae* infection in an otherwise healthy, immuno-competent adult (5).

MANAGEMENT OF PATIENTS

Individuals with this syndrome should be given an oral course of fluconazole 100 mg daily for three weeks followed by a course of Nystatin 500,000 IU 4 times daily for three weeks. Acidophilus tablets could also be given to re-colonize the gut (5). These antifungal drugs tend to put an end to the condition.

CONCLUSION

Gut Fermentation Syndrome, also known as Auto-Brewery Syndrome, Syndrome A and Endogenous Ethanol Fermentation, is a recently described clinical condition amongst patients who feel drunk after a heavy carbohydrate meal. The underlying mechanism is an overgrowth of yeast in the gut whereby the yeast ferments carbohydrates into ethanol. Oral antifungal chemotherapy, given for at least three weeks is the treatment of choice. Further studies are needed to improve our understanding of the pathogenesis of this syndrome and definitive diagnostic procedure(s).

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