ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

SEPTEMBER 2004 VOLUME 5 NUMBER 3

Official Publication of the African Society for Clinical Microbiology

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (ISSN 1595-689X)

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003031/2427

MATERNO-FETAL HAEMATOLOGICAL RELATIONSHIP IN MALARIA AT MONGOMO, GUINEA EQUATORIA

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This study is aimed to determine the effects of maternal and fetal parasitaemia on maternal and fetal haemoglobin. A nine-month (January - September 1997) prospective study was carried out at the labour unit of the Regional Hospital in Mongomo, Guinea Equatoria. One hundred and twenty-four patients with singleton deliveries were studied. The prevalence rates of maternal and fetal parasitaemia were 102 (82.25%) and 33 (26.61%) respectively. The mean maternal haemoglobin was 10.11 + 1.35 gm/dl, those with parasitaemia 9.26+0.85 gm/dl and those without parasitaemia 11.45+1.20 gm/dl (p<0.005). There is a close correlation between maternal parasitaemia, worsening maternal haemoglobin level and fetal parasitaemia (p<0.005 df=3 95% CI). Fetal parasitaemia is significantly commoner in fetuses with severe anaemia compared to those with negative fetal parasitaemia (p<0.005). The author emphasized curative treatment of all pregnant women at the first antenatal care visit to be supplemented by adequate prophylaxis throughout pregnancy. Choice of drugs for treatment and prophylaxis must be guided by the local sensitivity patterns and safety profiles of the drugs to the mothers and the developing fetuses.

INTRODUCTION

Malaria infection remains one of the most serious tropical diseases in many parts of the world. Currently, over 300 million cases of malaria are reported annually, about 85% of these from Africa (1-3). Two to three million deaths are estimated to occur annually with over 90% in Africa alone.

Malaria infection has profound effect on pregnancy outcome and neonatal life. *Plasmodium falciparum* infection prevalence increases in early pregnancy (9-16 weeks gestation) and parasite density is also increased in pregnant women (4-6). Consequently, materno-fetal complications such as anaemia, abortions, stillbirths, prematurity, intrauterine growth retardation, hypoglycaemia, cerebral malaria and even maternal mortality have been reported in pregnant women with malaria (1-5, 7-10).

The aims of this study are to determine the prevalence of maternal and fetal parasitization, and to determine the effects of maternal and fetal parasitaemia on maternal and fetal haemoglobin.

MATERIALS AND METHODS

This prospective cross-sectional study was carried out within a nine months period (January-September 1997) at the "Hospital provincial de Mongomo, Guinea Equatoria" on all women who delivered at the labour unit of the hospital. All the 124 women with singleton deliveries were unselected and included in the study as they presented at the labour unit. At delivery, a 4 millimeters venipuncture sample of maternal peripheral blood was collected and a standard glass microscopic slide preparation of the mother's peripheral blood was immediately prepared from this and labeled "A". Each newborn's peripheral blood sample was obtained through a heel prick, and a thick blood film sample was prepared on the glass microscope slide "B". Each slide was stained with Geimsa stain

after adequate dehaemoglobinization and examined under the light microscope using the X100 oil immersion objective. A positive slide is one that contains any of the parasites. No species identification was done. The cyanmethaemoglobin technique of Haemoglobin determination described by Decle and Lewis (11) was adopted.

RESULTS

The prevalence rates of maternal and fetal parasitaemia were 102 (82.25%) and 33 (26.61%) respectively. The mean age of the women in this study was 26.86 ± 8.09 years, those with parasitaemia 22.54 ± 4.30 years and those without parasitaemia 25.80 ± 3.36 years ($p<0.005$). (Table1). The mean maternal haemoglobin was 10.11 ± 1.35 gm/dl, those with parasitaemia 9.26 ± 0.85 gm/dl and those without parasitaemia 11.45 ± 1.20 gm/dl ($p < 0.005$). Severe anemia (haemoglobin < 7 gm/dl) was seen in two women both of whom had maternal and fetal parasitaemia. When compared with those with mild anaemia (72% and 54.5% maternal and fetal parasitaemia respectively) and without anaemia (63.3% and 22.2% maternal and fetal parasitaemia respectively), it is obvious that there is a close correlation between maternal parasitaemia, worsening maternal haemoglobin level and fetal anaemia. ($p < 0.005$, df = 3, 95%CI).

The mean fetal haemoglobin was 12.37 ± 1.64 gm/dl, those with parasitaemia 11.46 ± 1.23 gm/dl and those without parasitaemia 13.68 ± 0.82 ($p < 0.005$). Table 2 shows mean fetal haemoglobin concentrations in fetuses with or without parasitaemia. Parasitaemia is significantly commoner in fetuses with severe anaemia compared to those without parasitaemia ($p<0.005$).

Table 1: Prevalence rates of malaria parasitaemia in mothers and babies

Group	Positive Parasitaemia (%)	Negative Parasitaemia (%)	Total (%)
Maternal	102 (82.25)	22 (17.75)	124 (100)
Fetal	33 (26.61)	91 (73.39)	124 (100)

Table 2: Mean values of maternal and fetal indices in all women and in those with positive and negative parasitaemia

Variable	All Patients	Positive Parasitaemia	Negative Parasitaemia	P Value
Mean age (years)	26.86 + 8.9	22.54 + 4.30	25.80 + .26	P<0.005
Mean maternal haemoglobin (gm/dl)	10.11+ 1.35	9.26 + 0.85	11.445+ 1.20	P<0.005
Mean fetal haemoglobin (gm/dl)	12.37+1.64	11.46+1.23	13.68+0.82	P<0.005

DISCUSSION

The prevalence rates of maternal and fetal parasitaemia as recorded in this study were 82.25% and 26.62% respectively. These values are higher than the studies of Lamikanran (12), Uko *et al* (13), McGregor *et al* (6), Morgan (14) and Ezeoke and Braide (15) but lower than the values of Tanzanian (16) and Congolese (17) studies. A cursory look at these studies may suggest that the West African countries had relatively lower values than the East-Central African countries, this study inclusive.

This study has also shown that the maternal parasitaemia predisposes to maternal anaemia, placental parasitization and consequently fetal parasitaemia and anaemia (Tables 1 and 2). The presence of the parasite induces haemolysis of both parasitized and non-parasitized red blood

cells in both the maternal and fetal reticulo-endothelial systems (1, 4, 5, 7). Haemoglobin F (fetal haemoglobin) is said to confer some resistance to parasitization and subsequent haemolysis of fetal red blood cells (1, 4, 7, 18). This, coupled with the protective uteroplacental barrier, may be partly responsible for the lower prevalence rates for fetal parasitaemia and anaemia. Fetal anaemia has been suggested in this study to positively correlate with the level of fetal parasitaemia.

Increasing maternal parasitaemia, if not properly treated, increases the risk of poor fetal outcome. The authors support the line of management proposed by Ogunbode *et al* (19) which emphasizes curative treatment for all pregnant women at the first antenatal visit to be supplemented by adequate prophylaxis throughout pregnancy. It is hoped that this will reduce the prevalence rate of maternal malaria in pregnancy with its attendant maternal and fetal complications. Advent of drug resistance has made this proposal more difficult in clinical settings and consequently, the choice of drugs for treatment and prophylaxis must be guided by the local sensitivity patterns and safety profiles of the drugs to the mother and the developing fetus (20). Cost is also an important consideration since one of the goals of good antenatal care is to provide for as many women as possible. Supplementation of antimalarial prophylaxis with haematinics have been shown to be beneficial to all pregnant women in endemic areas, particularly primigravidae, some of whom were shown to significantly grow more and had less anemia than the control group who were not supplemented (21).

ACKNOWLEDGEMENT

The author acknowledge the cooperation of the entire management team of the 'Hospital regional de Mongomo', Mongomo, Guinea Equatoria, as well as the staff members of the maternity and the laboratory units. The name of Dr Jeronimo Ona Edu, the regional director of the hospital, deserves special mention for his untiring role in helping our medical cooperation team achieve our set out objectives in spite of the odds.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2004001/2428

PREVALENCE AND DISTRIBUTION OF RUMINANT TRYPANOSOMOSIS IN BOKKOS LOCAL GOVERNMENT AREA OF PLATEAU STATE, NIGERIA

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The seasonal prevalence of typanosomosis was investigated in Bokkos LGA of Plateau State, Nigeria. A total of 740 animals (684 cattle and 56 sheep) were bled during the dry and wet seasons. The standard methods used were simple random and jugular venipucture. Standard parasitological methods were used to determine the infection rate. In cattle, the findings showed an infection rate of 11.7% while in sheep it was 17.9%. Peak infection in animals was during the end of the rainy season and beginning of dry season (September-December). Complementary mice inoculation tests revealed 83 sub patent cases and are recommended as a confirmatory diagnostic technique

INTRODUCTION

Typanosomosis, caused by pathogenic trypanosomes *(Typanosoma spp)* transmitted through tsetsefly *(Glossina spp)* is a disease unique to Africa, affecting both humans and domestic animals. Symptoms of the disease are similar to those of other blood parasites, and include a general loss of condition, fever and anaemia. In acute cases, this might progress to internal haemorrhage and following invasion of the nervous system, coma and death (1). There have been reports of animal typanosomosis from different parts of the country including the high plateau of Jos: Bassa, Barkin-Ladi, Jos North and Jos South LGAs (3-9). This might have informed the inclusion of the Jos Plateau in a nationwide programme under the auspices of National Agricultural Research Project (NARP). Therefore, this study was part of the epidemiological survey of African animal typanosomosis carried out by Nigerian Institute for Trypanosomiasis Research.

MATERIALS AND METHODS

The survey was conducted in Bokkos Local Government Area of Plateau State during the months of June to January cutting across both dry and rainy seasons. The epidemiological survey took place in six villages. Samples were taken from cattle (Bovine) and sheep (Ovine). A total of 684 cattle and 56 sheep were sampled. The breeds of animals consisted of West African Dwarf (WAD), Yankassa, Uda and their crosses, for sheep and Zebu for cattle.

About 5 mls of blood was collected from the jugular vein of each animal into Bijou bottles containing EDTA. The bottles were labeled serially and the breed and sex of the animals bled indicated. Screening of the blood for typanosomes was carried out using the standard detection techniques (wet, thin and thick films) for quick assessment and by haematocrit centrifugation technique (HCT) for accurate diagnosis (2). Also, the packed cell volume (PCV) of all animals was recorded using the microhaematorit method. Mice ,inoculation test was carried out for all suspected blood even if the parasitological result was negative.

RESULTS

The results of the survey are shown in Tables 1, 2, 3 and 4. Out of a total of 684 cattle, 80(11.7%) were positive for various typanosoma species. In sheep, out of a total of 56, 10(17.9%) were positive. Tables 1 and 2 also show the distribution of infections encountered in the six villages outlined. Table 3 shows the sex differences and the PCV (packed cell volume). Table 4 shows that the peak infection in animals was during the end of the rainy season and beginning of dry season (September-December). Mice inoculation tests carried out revealed 83 subpatent and prepatent infections.

Table 1: Bovine trypanosomosis in six villages in Bokkos LGA of Plateau

Survey area	Number of animals	Number of positive animals			Total positive
		T. vivax	*T. congolense*	*T. brucei*	
Bokkos	115	8	-	7	15
Maltol	134	5	6	1	12
Ruwi	169	7	-	-	7
Richa	117	9	3	5	17
Kukuf (Marina)	81	19	4	-	23
Kamwai	68	-	6	-	6
Total (%)	**684**	**48 (60.0)***	**19 (23.8)***	**13 (16.3)***	**80 (11.9)****

* = % of species over total number of positive cases.

** = % of total positive cases over total sample size.

Table 2: Ovine trypanosomosis in six villages in Bokkos LGA of Plateau

Survey area	Number of animals	Number of positive animals			Total positive
		T. vivax	*T. congolense*	*T. brucei*	
Bokkos	13	-	-	3	3
Maltol	-	-	-	-	-
Ruwi	19	-	2	-	2
Richa	10	-	-		
Kukuf (Marina)	10	-	3	-	3
Kamwai	4	1	1	-	2
Total (%)	**56**	**1 (10.0)***	**6 (60.0)***	**3(30.0)***	**10 (17.9)****

* = % of species over total number of positive cases.

** = % of total positive cases over total sample size.

Table 3: Prevalence rates, packed cell volume and sex differences of trypanosome-infected animals

Animal species	Sex	No. of sample	No positive (%)	PCV (%) (Mean * SE)
Cattle	M	180	22 (12.2)	25.0 * 3.0
	F	504	58 (11.51)	27.1 * 4.0
	Total	684	80	
Sheep	M	20	3 (15. 0)	21. 3 * 1.2
	F	36	7 (19. 4)	24.2 * 2.0
	Total	56	10	
Grand Total		**740**	**90 (12.16)**	

Table 4: Prevalence of typanosomosis among cattle and during different months of the year

Month	Number examined	Number positive	% positive
June	120	6	5.0
July	85	7	8.2
August	110	9	8.1
September	111	11	10.0
October	103	20	19.4
November	62	17	27.4
December	98	15	15.3
January	51	5	9.8
	740	**90**	**12.16**

DISCUSSION

Sequel to the commencement of the NARP project, of which this study was part of, there has been report of menace of flies and typanosomosis from livestock farmers on the Jos Plateau. In this study, an overall infection rate of 12.16% for both cattle and sheep suggest that typanosomosis is no longer to be taken lightly on the Jos Plateau. It is not impossible to get a higher infection rate if more elaborate study is undertaken. Studies carried out earlier showed that in Barkin-Ladi and Bassa LGAs (4, 6) prevalence rates ranging between 6.4% and 9.1% were recorded. Acute typanosomosis was also reported in a Friesian herd residing on a farm in Jos South LGA (5). Earlier in 1982, Joshua and Ige (3) recorded a 5% incidence in sheep and goats slaughtered in the state government abattoir in Jos.

With the findings in this study, it has become imperative to carry-out more elaborate surveys on both the high and lower Plateau of Jos, to provide explanation on what is responsible for the change in the tsetse and typanosomosis status of the Plateau. The more significant and long term impact typanosomosis has imposed is the disruption caused to the development of sustainable mixed-farming systems and to the alleviation of poverty.

ACKNOWLEDGEMENT

We wish to thank the following members of staff for their invaluable help during the survey; Mrs P Okam, Messrs JA Ayandele, Moses Davou, Francis Doro and Mrs E Okorie for typing this script. Finally the Director, NITR, Dr I Halid for permission to publish.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003/044/2429

CHANGES IN PERIPHERAL LEUKOCYTE AND BODY FLUIDS OF ONCHOCERCIASIS PATIENTS TREATED WITH IVERMECTIN

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This study evaluated the peripheral leukocyte count and the presence of microfilariae in the body fluids of onchocerciasis patients treated with ivermectin. Fifty-three patients over the age 10 years were selected from Ipogun, an onchocerciasis endemic area in Ondo State, Nigeria. Before and after treatment, all patients received a parasitological and clinical examination that included physical examination, palpation of onchocercal nodules, assessment of microfilarial densities in iliac crest skin snips, diagnosis of concomitant parasitic infections in stool specimens and total leukocytes differential counts. Results indicated that ivermectin did not induce a decrease in the total number of peripheral leukocytes but there was a decrease in the number of eosinophils. Microfilariae were not found in increase frequency in the urine, blood and sputum, while the number of microfilariae per mg of skin snip decreased.

INTRODUCTION

Onchocerciasis is a major health problem in Nigeria. Epidemiological studies have shown that some communities are severely affected by the disease both in the Northern regions and in the Southern forested areas with foci of blinding disease found in the Northern parts of the country. Drug treatment and control of onchocerciasis have been unsatisfactory in the past. The available drug diethylcarbamazine (DEC) and suramin are too toxic for mass distribution.

The introduction of drug ivermectin has been responsible for the most recent dramatic advances both in disease control (1, 2) and in interruption of transmission (3, 4). Ivermectin has replaced DEC, and community based mass treatment campaigns against *Onchocerca volvulus* were initiated by the Onchocerciasis Control Program (OCP) in West Africa. Ivermectin is believed to paralyze susceptible nematodes by affecting neurotransmission mediated by gamma-amino-N-butyric acid (5), but total immobilization or killing of microfilariae *in vivo* has never been observed (6) and the exact mode of antifilarial action remain unclear.

Observations also indicate that ivermectin might not act on the filarial parasite directly, but rather, in synergism with the host immune response (7). Since ivermectin reduces microfilariae of *Onchocerca volvulus* and the drug acts in synergism with the host's immune response, it is expected that the drug would affect the clinical and systemic as well as immunological balance of the host.

The present study was designed to examine the eosinophilic leukocytic reactions to ivermectin provocation since filarial infections are usually followed by eosinophilia. It is also intended to correlate the signs and symptoms of the clinical response and the alterations in numbers of microfilariae in body fluids associated with a single oral dose of ivermectin in population of people with moderate to heavy infection with *Onchocerca volvulus*.

MATERIALS AND METHOD

Patient population and evaluation

The study was carried out in Ipogun, a town in Ifedore Local Government Area in Ondo State, Nigeria. Previous data from the study in the area showed that onchocerciasis is hyper endemic with a prevalence rate of 34% for leopard skin, and 16% nodular rate (8) which is representative of forest type disease. Ipogun is a rural community with a population of about 2000 residents.

Fifty three residents over the age of 10 years comprised the study patients. Children less than 10 years old, debilitated individuals, pregnant women and lactating mothers were excluded from the study. Patients were evaluated clinically and immunologically immediately before and after repeated 150 µg/kg doses of ivermectin given annually for 2 years. Samples (skin, blood, urine, and sputum) were collected before treatment, 2 days, 3 days, 3 months, 6 months, 12 months and 18 months after treatment. The patients co-operated all through in all aspects of the study except the blood sample at 48 hours and 72 hours. Clinical evaluation included physical examination, specific examination for the presence of onchocercal nodules, assessment of microfilarial densities in iliac crest skin snips and body fluids, total leukocyte and differential counts. All the subjects evaluated met the following criteria; positive history of exposure in endemic regions, clinical symptoms consistent with onchocerciasis and positive skin snips.

Skin snips were taken with corneo-scleral punch from both iliac crests and placed in wells of microtitre plates containing physiological saline. Emergent microfilariae was counted immediately and 24 hours after. The number of microfilariae were counted and scored quantitatively as reported by Crosskey and Crosskey (9).

Total and differential white blood cell count

Blood was anticoagulated with 1 mg/ml ethylene diamine tetra acetate (EDTA) and white blood cell count was done by conventional methods. Blood eosinophil count was done by the method of Discombe (10). Differential white blood cell count was done on smears stained with Leishmans stains.

Body fluid examination

The sediments of urine and blood specimens were treated according to previous methods (11, 12) and were checked for microfilaria of *Onchocerca volvulus*. Each sputum specimen was preserved with tincture of Merthiolate and examined similarly like the urine specimens.

Statistical analysis

Differences in means between time points were compared using student's test on logarithmically transformed data.

RESULTS

Pretreatment findings

Fifty three individuals aged 15-72 years (mean age 41.2 years) were evaluated before treatment. All were microfiladermic (geometric mean 22.72, 1-204/mg of skin) and 8 had detectable subcutaneous nodules. Thirty two of them were positive for leopard skin while ocular examination showed normal visual acuity except for 7 persons who complained of blurred vision (Table 1).

Complications during treatment

Fourteen patients (26.4%) had moderate side effects. Two patients experienced severe adverse reactions, of

which they received additional treatment during the follow up period of 3 days.

Skin microfilarial levels

Levels of microfilaraia, assessed by skin snips just before ivermectin administration, were measured at 6 months interval. There was a significant reduction ($p<0.05$) in the microfilarial level 6 months after the 1st dose of ivermectin (Table 2). Of the 53 patients, 22 (41.5%) had no skin microfilariae at the final sampling.

Total Leukocyte and Eosinophil counts

Ivermectin therapy had no effect on the total leukocyte counts. There was no significant difference at either the population or individual level between the pretreatment values and any of the subsequent time points (Table 2). In contrast, there was a rise in the absolute eosinophil counts two and three days after treatment. But by the third month after treatment, there was a considerable fall in the eosinophil count reaching statistical significance levels by 12 months. No correlation was seen between the reduction in eosinophil counts and the decrease in skin microfilarial levels, nor was there a correlation between eosinophil level and skin microfilarial density pretreatment.

Microfilariae levels in the body fluids

No onchocercal microfilaria was found in the urine, blood and sputum of patients before and after treatment. It is worthy to note that in 7 patients with concomitant *Schistosoma haematobium* infection, ivermectin was found not to have any effect *S. haematobium* ova was found in higher number in 2 cases of patients after treatment, while the number of *S. haematobium* ova remained the same in the other 5 patients before and after treatment.

Table 1: Baseline characteristics of patients treated with ivermectin

No of patients		53
Mean age (Range in kg)		41.2(15-72)
Mean weight (Range in Kg)		59(34-84)
No of patients with		
1.	Onchocerciasis	34
2.	Leopard skin	30
3.	Nodules	8
4.	Blurred vision	7

Table 2: Skin biopsy microfilaria counts and peripheral blood leukocyte levels in people with onchocerciasis over one year of therapy with ivermectin

Parameter	Before treatment	2days	3days	3mths	6mths	12mths	18mths
Mean no of MF/skin biopsy	22.72	21.5	4.5	17.7	5.5	9.6	6.8
Peripheral Blood Leukocytes	7662.4	8941.6	7936	7872.7	7522.8	7637.5	8428
Peripheral Blood Eosinophils	13.6	18.4	19.6	7.1	8.7	6.0	6.1

DISCUSSION

As expected from past studies (1, 13), levels of skin microfilariae decreased significantly during this period. Hematological assessment revealed no change in the leukocyte counts. Eosinophil numbers decreased significantly over the 18 months repeated observations. Such findings are particularly interesting since eosinophils have been known to be causally involved not only in the cytotoxicity to microfilariae after treatment (14) but also in the post treatment Mazzotti reactions (15) and the development of skin pathology (16).

Whether the decrease in blood eosinophil is the result of the hosts decreased antigen load (reduction in microfilariae) or whether if reflects some other possible treatment change in the host is unclear. By whatever mechanism, however, there was a reduction in eosinophil levels in these patients to less than half the pretreatment level. Within 2days after ivermectin was administrated, there was a rise in eosinophil and it has previously been demonstrated that there is a rise in eosinophil levels within the first month after treatment with either ivermectin or DEC (17). With ivermectin therapy the rise occurs more slowly than after DEC treatment, presumably because of a different mechanism of action (18) that results in decrease or clearance of the skin microfilariae.

The present finding indicate that after this initial early post treatment eosinophilia, there is a continual decline in eosinophil levels with repeated ivermectin treatment, suggesting that the patients' may be moving towards a normal (homoeostatic) state (19). The present study also demonstrated the effect of a single oral dose of ivermectin on migration of microfilariae in patients with a relatively heavy dose of infection on the skin. After administration of ivermectin the skin snips count tended to decrease with time while microfilariae were not found in the sputum, urine and blood as reported after DEC treatment by (20). This finding agrees with that of Richards *et al* (21) who also observed that the fall in microfilariae skip concentration after ivermectin treatment was not accompanied by any marked wave of microfilariae in the blood or urine.

The observations of Awadzi *et al* (17), Richards *et al* (21) and Basset *et al* (22) suggest that, after ivermectin administration although some microfilariae may enter the blood stream (presumably by way of the lymphatic system), their numbers are in no way comparable to those seen after DEC. Duke (23) also reported that ivermectin-affected microfilariae may be destroyed in

the lymph nodes and elsewhere more easily and with less reaction than those unmasked by DEC and fewer of them may survive the lymph node network to pass into the blood. They also observed that ivermectin caused microfilariae to move from the subepidermal layer into the deeper layers of the epidermis, subcutaneous fat, connective tissue and the regional lymph nodes. They concluded that the migration of microfilariae from superficial layers of the skin to the deeper connective tissues, fat lymph nodes, coupled with the mild cellular reactions that surround microfilariae dying from the effects on ivermectin are the main reasons why this effective microfilaricide for the control of onchocerciasis appears to be a promising chemotherapeutic strategy, particularly since it is not associated with severe side effects characteristic of DEC therapy.

Findings in this study demonstrated no increased symptoms that would preclude wide use of ivermectin to treat populations infected with generalized onchocerciasis.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003042/2430

EPIDEMIOLOGICAL MAPPING OF LYMPHATIC FILARIASIS IN SOUTHERN NIGERIA PRELIMINARY SURVEY OF AKINYELE LOCAL GOVERNMENT AREA

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Lymphatic filariasis caused by *Wuchereria bancrofti* is a major public health problem in tropical and subtropical countries including Nigeria. The real burden of the disease in most endemic regions remains unknown. The nocturnal periodicity of the parasites requires parasitological examination to be done at night and this is quite cumbersome. The World Health Organisation recently recommended two rapid methods for the assessment of lymphatic filariasis (RAGFIL). These RAGFILS methods i.e. using community health workers and key informants were used to rapidly map lymphatic filariasis in Akinyele Local Government area of Oyo State Nigeria. The prevalence of hydrocele and elephantiasis was highly sensitive in identifying this community as endemic for filariasis. The degree of association between finding by health workers and information obtained from the community key informants was high for the two major clinical manifestation of the disease used. Community key informants and health workers did provide useful information on the prevalence of clinical filariasis. These observations suggest that the mean number of cases obtained in the village through key informants and the examination of health workers for the clinical signs of the disease may be considered at an initial level to identify endemic areas. The need to extend this method to rapidly map lymphatic filariasis in Nigeria is discussed.

Key words: Lymphatic, Filariasis, Rapid, Mapping, Assessment and Health workers.

INTRODUCTION

Lymphatic filariasis is a major public health problem in tropical countries. Recent estimates suggest that 120 million persons are infected worldwide (1). The number of people with physical disabilities due either to lymphoedema and hydrocele or the new recognized sub clinical abnormalities of lymphatic and renal function are currently estimated at 4 million (1).

International task force on disease eradication identified lymphatic filariasis as one of the six potentially eradicable diseases since there are now good enough tools to combat the disease (1). Global efforts towards the control of lymphatic filariasis is now based on annual, single dose treatment of all eligible members of high risk communities with ivermectin and albendazole to prevent severe morbidity and ultimately interrupt transmission. In order to initiate any disease control programme based on mass distribution, one needs to understand the geographical distribution of the disease in the affected countries in order to know where to target mass treatment. Unfortunately, data on the distribution of the disease are not widely available primarily because the standard procedures for determining which countries are affected are cumbersome, time consuming, expensive and very intrusive. In areas (almost all) where the parasite exhibits a nocturnal periodicity, parasitological estimations need to be done at night. This becomes logically cumbersome to organize and communities often refuse to co-operate.

Throughout the tropical belt of Africa including Nigeria, very little is known about the prevalence or distribution of lymphatic filariasis such that the expert committee on filariasis in their 1992 (2) meeting suggested that efforts should be made to collect more information on the distribution and prevalence of disease and

the vectors especially from the African region. Thus one of the identified operation needs is the precise estimate of regional and national burden of illness caused by lymphatic filariasis in order to document the public health impact of the disease and formulate control strategies. There is also the need to have specific knowledge on distribution to ensure effective and lasting control particularly now that Merck and Co. Inc., the manufacturer of the drug ivermectin, are ready to donate the drugs free to needy countries.

METHODOLOGY

This study was carried out in Akinyele Local Government Area (LGA) of Oyo State Nigeria. Akinyele LGA is made up of 15 districts with 2 Primary Health Care (PHC) facilities. Two Rapid Geographical Assessment (RAGFIL) methods developed by WHO was used for the study; i. A method where a large sample of villages will be surveyed indirectly using questionnaires directed at key informants and health workers and ii. A method based on surveys using rapid assessment techniques viz, health workers examination for hydrocele and lymphoedema.

Questionnaires were administered to specific local key informants. The questionnaires were based on instruments developed for rapid assessment, which were evaluated at the Rap Workshop in July 1997 (3). Initial contact was made with district level authority i.e. LGA chairman, LGA medical officer. Key informants from the selected communities and means of contacting them were ascertained from the meeting with the LGA authority. Key informants include village head, teachers, health system personnel, market men and women leaders, religious leaders and other leaders identified in the community capable of responding to the questionnaire. Questionnaires were evaluated according to the analytical framework developed at the Rap Workshop in July 1998 (Absence or presence of lymphatic filariasis, number of cases or prominence as a health problem)

Health workers were trained to screen a random sample of 50-100 adult males for hydrocele and lymphoedema (this is the standard RAGFIL method developed by WHO). Only males who have been at least 10 years resident in the community were included in the examination and only obvious lymphoedema and hydrocele greater than a tennis ball were recorded as positive. The examination used the principles of "if in doubt, leave it out".

The years of residence in the community, age and clinical findings were entered for each subject. The presence of a single adult male with filarial disease was regarded as sufficient for classifying a community as endemic (4).

RESULT

Ninety-five key informants were interviewed in Akinyele local Government Area of Oyo State. Key informants were aged between 21-80 years with a mean age of 41. Fifty six were females while 39 were males. The occupational status of the key informants is given in Table 1. Of the 95 people interviewed, 25 (26.3%) have seen people with lymphoedema in the village. Of these, 1 (4%) person knew more than 6 people with hydrocele, while 24 (96%) knew between 1-5 people. Twelve (17.1%) of the key informants knew people with hydrocele. Of these, 11 (91.7%) knew between 1-5

people with it while only 1 (9.3%) person knew more than 5 people with hydrocele.

Tables 2 and 3 shows the records of the health workers after the screening of a random sampling of 50-100 adult males in the selected communities. They diagnosed 19 people as having lymphoedema and 7 people as having hydrocele. Agreement between findings of health workers and those of the key informants were significant ($r = 0.64$; $p < 0.05$).

The cases seen by the health workers complained of periodic fever, headache and chills particularly in the rainy season. Cases seen were between the age bracket of 28-80 years and all have resided in the communities for more than 21 years of their life. Of the 44 health workers interviewed, 10% have encountered fever that persists in patients treated with antimalarial. Twenty percent of the health workers observed that very few people in the village sleep under mosquito bed nets, while 76% observed that no one has bed nets. Majority of the health workers interviewed did not know the right cause of the disease and attributed it to witchcraft and sorcery, while 4.2% of those interviewed said mosquitoes were the source of infection.

Table 1: Occupational status of key informants

Occupation	Frequency	Percentage
Farmer	13	14
Health Worker	44	47.3
Teaching	2	2.2
Artisan	2	2.2
Trading	18	19.4
Civil Service	9	9.7
Others	5	5.4

Others- Non-working village leaders

Table 2: Clinical identification of hydrocele by trained health workers in Akinyele LGA

Age	Year of residency in the community	Clinical findings
52	42	Hydrocele
45	36	"
55	55	"
40	40	"
63	28	"
70	45	"
68	31	"

Table 3: Clinical identification of lymphoedema by trained health workers in Akinyele LGA

Age	Year of residency in community	Clinical Finding
80	44	Lymphoedema
45	45	"
55	32	"
35	35	"
80	35	"
42	21	"
22	22	"
45	26	"
28	28	"
45	33	"
54	31	"
27	27	"
46	42	"
58	25	"
37	33	"
48	48	"
79	63	"
58	36	"
48	38	"

DISCUSSION AND CONCLUSION

The use of peripheral health staff and community key informants in community level data about lymphatic filariasis has been explored in many parts of the world and has been found to be a good predictor of communities at risk of filariasis (5, 6, 7). The observation in this study, like in those mentioned above, suggest that the mean number of cases obtained through key informant technique may be considered at a

primary level to identify endemic areas, followed by clinical examination by health workers for clinical filariasis.

This study will go further to rapidly map filariasis in the whole of Southern Nigeria. The cost effectiveness of using the different RAGEIL methods will be estimated and recommendations made.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003039/2431

KNOWLEDGE, ATTITUDE AND PERCEPTIONS OF ONCHOCERCIASIS IN A HYPER-ENDEMIC COMMUNITY OF EDO STATE, NIGERIA

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An evaluation of the knowledge, attitude and perception of Ekpan, a rural community in Edo State of Nigeria towards onchocerciasis after 3 years of ivermectin distribution was undertaken. The structured questionnaire administered to the respondents focused on specific aspects of knowledge, attitude and perception related to the disease, its mode of transmission and control activities in the village. Results from the survey showed that 133 (68.6%) had fair knowledge of the disease. All subjects knew the bite of the blackflies was followed by itching but none knew that bites were accompanied by *Onchocerca volvulus* transmission. Level of education influenced knowledge of the disease and the relationship was statistically significant ($P<0.05$). Knowledge of the clinical manifestation was however poor. Majority of the respondents (83.0%) perceived the disease to be due to other causes other than *Onchocerca volvulus*. Knowledge of the side effects of ivermectin treatment was good while knowledge of those excluded from treatment was generally fair. Their attitude to ivermectin distribution was strongly influenced by adverse reactions to the drug, leading either to outright rejection or discontinuation of the treatment after initial acceptance. The most prevalent reactions were swelling of leg/feet (22.2%), followed by itching (17.5%) and weakness (9.8%). The respondents perceived the different clinical manifestation of onchocerciasis to be specific disease entities. Onchocercal nodules were believed to be blood clots; leopard skin was thought to be healed scars of wounds and cuts accidentally acquired in the course of farming while hanging groin was regarded as hernia. In conclusion, ignorance of the cause of the disease negatively influenced their attitude and perception. Therefore, for maximum impact on morbidity and transmission to be achieved with ivermectin treatment, knowledge of the disease and control activities should be imparted to the residents. Such health education should of necessity take into consideration community attitude and culture, which promote health-seeking behaviour.

Key words: Knowledge, attitudes, perception, onchocerciasis, ivermectin

INTRODUCTION

Onchocerciasis is still a public health problem where it occurs in high intensity and endemicity (1, 2). Eighteen million people are infected world-wide and half the infected live in the rainforest zones where the non-blinding form, onchocercal skin disease (OSD) is prevalent (3, 4, 5). Seven million of the world's infected are Nigerians, of which about 114,000 are already blind and a high proportion are suffering untold hardship as a result of the OSD (3, 4, 6, 7). The disease, which is acquired through the bite of infected *Simulium spp*, has posed a serious threat to the health of the inhabitants and impediment to socio-economic development of those living in endemic communities (1, 7).

Ignorance of the cause and mode of transmission of onchocerciasis has hampered the control of the disease in endemic communities. Various authors have reported the lack of knowledge about the vector, parasite and afflictions (5, 8, 9), which could affect attitude towards the disease and its control efforts. Since lack of knowledge perpetuate disease, appropriate health education strategy will be necessary to improve the situation. This could be achieved through the Primary Health Care (PHC) system to encourage participation and acceptance of control programmes aimed at improving the health status of the affected communities.

METHODS

Study area

The study was undertaken in Ekpan Village, a farming community in Uhunmwode Local Government Area of Edo State, Nigeria from 1998-2000. The village is located between longitude 5°56.660'E and latitude 6°43.210'N and at an altitude of 279m ASL. The community lacks social amenities such as electricity, potable water supply and health facility. The only source of water supply for the community is the Ekpan River, which is fast flowing and provides ideal breeding environment for *Simulium* flies. The vegetation of the area is typical tropical rainforest, characterized by tall trees with thick undergrowth.

Advocacy and mobilisation

Advocacy visits were paid to the community head, seeking consent for the survey. Consent to undertake the survey was granted after the objectives of the survey were highlighted. Mobilization of the community for the survey was undertaken by the Community Based Distributor of Ivermectin (CBDI) on the directives of the village head and the elders' council.

Sampling method

There were 529 individuals enumerated in the 84 houses in Ekpan village, which constitute the entire population. A sample size of 264 was derived from a microfilaria prevalence of 77.5% (10). The mean number of residents per house was 6. Using a systematic sampling method and a sampling ratio of 1:2, 40 houses were selected for the survey. All residents 15 years and above who have been domicile in the community for upwards of 5 years were eligible to participate in the study. Although a total of 202 eligible individuals were resident in the selected houses, only 194 of them who were available participated in the survey. The structured questionnaire administered to the respondents by trained interviewers focused on specific aspects of knowledge, attitude and perception, related to onchocerciasis mode of transmission and control activities in the community. The duration of interview varied, depending on the educational level of the respondents, but the mean interview time was 25 min.

Knowledge of the respondents was assessed on; (i.) Vector of onchocerciasis, (ii.) Recognition of the clinical manifestation of the disease, (iii.) Drug used for onchocerciasis control and its source, (iv.) Mode of distribution of the drug, (v.) Population excluded from treatment, (vi.) Reaction to ivermectin, (vii.) Attitude to ivermectin treatment, (viii.)Perception of onchocerciasis. Knowledge was classified as either poor, fair or good. There were 5 sets of questions on knowledge, 2 sets of 5 each and 3 sets of 7. All questions asked were correct and scored as follows: < 3 = poor, 3 = fair and > 3 = good for the 2sets of 5 while the 3sets of 7 were scored as follows; < 4 = poor, 4-5 = fair and > 5 = good.

Data collected were analyzed using EPI INFO 6 statistical package. Test of significance was determined by the Chi-square test at 95% confidence limit.

RESULTS

Knowledge

Of the 194 persons interviewed, 106 (54.6%) were males while 88 (45.4%) were females. Knowledge of the disease was generally fair as shown in Table 1. All the subjects knew that the bites of the blackflies were followed by itching but none knew that bites were accompanied by *Onchocerca*

volvulus transmission. They knew that ivermectin was given free of charge but none knew the source of the drug. The level of education was however found to influence knowledge of the disease and this relationship was found to be statistically significant (X^2 = P < 0.05). The knowledge of the symptoms of onchocerciasis was poor. Majority (83.0%) perceived the disease to be due to other causes other than *Onchocerca volvulus* (Table 1). In fact, no specific name was ascribed to the disease in the community. Table 2 showed that 102 (52.5%) of the subjects had good knowledge of the side effects of *ivermectin*. Knowledge of the population excluded from taking ivermectin was generally fair as 54 (27.8%) of respondents had good knowledge while 44 (22.6%) had fair knowledge.

Attitude

Of the 194 respondents eligible to take ivermectin in the survey, a total of 176 (90.7%) actually took the drug at various times of the distribution. According to them, they accepted ivermectin because it improved their health. However, a few (28.3%) rejected the drug for fear of adverse reactions. Others (1.5%), after initial acceptance of the drug, refused further treatment, when it failed in their case to expel intestinal worms as reported by other treated residents. Another group (18.0%) claimed they failed to take drug because they were absent from home at the time of distribution. About 17% of the respondents could not advance any specific reason for not taking the drug. Only 3.1% claimed they did not take the drug because they were pregnant at the time of administration.

Among those who took the drug, 104 (53.6%) claimed to have had reactions to the drug after the first dose while 90 (46.4%) did not experience any side effects. Table 3 shows that among those who had reactions, 86 (44.3%) experienced swelling of different parts of the body. The predominant reaction was swollen leg/feet (22.2%) followed by itching (17.5%) and weakness (9.8%). On the other hand, some respondents expressed satisfaction with ivermectin treatment. Among this group, 163 (84.0%) were pleased with the treatment because it improved their health and vigour. Interestingly, 10 (5.2%) of the respondents claimed they experienced improved visual acuity following treatment. Others (5.2%) cited pain relief as their ground for satisfaction with the treatment while 11(5.7%) experienced intestinal worm expulsion.

Perception

In Ekpan community, onchocercal nodules were perceived to be blood clots resulting from falls. Among them, leopard skin was believed to be part of the aging process peculiar to certain families. Others thought them to be healed scars of cuts and wounds accidentally acquired in the course of their occupation as farmers. Hanging groin was perceived as hernia in the community.

Table1. Knowledge of onchocerciasis and symptoms of the disease by educational level

Level of education	% Frequency of knowledge of the disease			% Frequency of knowledge of the symptoms			
	Good	Fair	Poor	Good	Fair	Poor	Total
No formal Education	3(1.5)	26(13.4)	16(8.2)	2(1.0)	3(1.5)	40(20.6)	45(23.1)
Primary	10(5.2)	83(42.8)	19(9.8)	6(3.1)	12(6.2)	94(48.5)	112(57.8)
Secondary	6(3.1)	24(12.4)	7(3.6)	3(1.5)	7(3.6)	27(13.9)	37(19.0)
Total	**19(9.8)**	**133(68.6)**	**42(21.6)**	**11(5.6)**	**22(11.3)**	**161(83.0)**	**194(100)**

Table2: Knowledge of those ineligible to take ivermectin and reaction to the drug among the respondents by educational level

Level of education	% Frequency of knowledge of the disease			% Frequency of knowledge of reactions to ivermectin			
	Good	Fair	Poor	Good	Fair	Poor	Total
No formal Education	9(4.6)	8(1.4)	28(14.4)	21(10.8)	4(2.1)	20(10.3)	45(23.2)
Primary	30(15.5)	29(14.9)	3(27.3)	59(30.4)	8(4.1)	45(23.2)	112(57.7)
Secondary	15(7.7)	7(3.6)	15(7.7)	22(11.3)	5(2.6)	10(5.2)	37(19.1)
Total	54(27.8)	44(22.6)	96(49.4)	102(52.5)	17(18.8)	75(38.7)	194(100)

Table 3: Different types of reactions experienced by respondents

Types of reactions	% Frequency of reactions
Swollen leg/feet	43 (22.2)
Swollen hand	13 (6.7)
Swollen body	23 (11.8)
Swollen face	7 (3.6)
Itching	34 (17.5)
Weakness	19 (9.8)
Dizziness	7 (3.6)
Sleepiness	2 (1.0)
Heavy/legs	2 (1.0)
Fever	1 (0.5)
Eye redness	1 (0.5)
Excessive eating	1 (0.5)
Intestinal worm expulsion	2 (1.0)

DISCUSSION

This survey revealed that majority of the respondents lacked adequate knowledge about onchocerciasis. This finding is similar to reports from other studies (6, 9, 11, 12, 13). Although they knew that blackflies constituted a great biting nuisance, the bite was not associated with disease transmission. This inability to associate onchocerciasis transmission with *Simulium* bites is not peculiar to Ekpan village because other investigators have similarly observed this problem in other locations (5, 8, 14, 15). As have been previously reported by other investigators (8, 9), ignorance of the disease and its mode of transmission could be the reason why no specific name was ascribed to onchocerciasis in the community. In contrast, the vector, *Simulium damnosum* is well-known and is called 'Isikpor' in the village and the surrounding communities.

The fact that none of the respondents knew the source of the drug reveals that community participation in the procurement and distribution of ivermectin as recommended under the African Programme for Onchocerciasis Control (APOC) is not yet in place or at best in the rudimentary stage in the community. According to Katabarwa and Mutabazi (16), it is cheaper to train the community to achieve a sustainable control programme. At the moment, only the CBDI is trained and empowered for onchocerciasis control in Ekpan village.

According to WHO (2), those excluded from ivermectin intake include children under 5 years, the sick, pregnant and nursing mothers. The subjects were knowledgeable in this aspect as they may have been repeatedly informed by the CBDI. Although ivermectin coverage in Ekpan village was high (85.7%), a number of the inhabitants did not participate in or discontinued treatment with ivermectin for various reasons, prominent among these being the development of drug-related adverse reactions, after the first dose of the drug. Investigators including Abanobi (15), Richard-Lenoble *et al*, (17) and Oparaocha *et al*, (18) found similar reactions to ivermectin in the first dose recipients in their investigations. These adverse effects following the first dose of ivermectin in Ekpan village, as observed also by the other authors (19, 20) decreased and gradually stabilized with successive treatments. In Ekpan village, one other factor that contributed to a section of the community not participating in the treatment programme was the disagreement on who holds the position of CBDI. The aggrieved section of the community felt the position of the CBDI should be held by the first son of the reigning village head even though he had

no training for the job. When the community-directed mode of ivermectin distribution as adopted by APOC takes root in the community, such wrangling will be overcome as the entire community and a not few individuals will decide how ivermectin is procured and distributed in the village.

Ekpan village residents perceived the different clinical manifestations of onchocerciasis as distinct disease entities, which is indicative of ignorance of the disease. This observation is in agreement with the reports of Johnston *et al*, (8) in Malawi and Abanobi (15) in Imo State, Nigeria. Above all, onchocerciasis was not perceived as a health problem, since the affected persons could engage in their normal socio-economic pursuits with minimal incapacitation.

In conclusion, it is obvious from the results of this survey that ignorance of onchocerciasis has influenced the community perception and attitude towards the disease. Therefore, for the ivermectin treatment programme to have the desired impact on morbidity and transmission, appropriate education on the subject should be undertaken. Such health education should take into consideration community attitudes and culture which promote health seeking behaviour.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003043/2432

THE USE OF IMMUNOCHROMATOGRAPHIC TECHNIQUE (ICT) IN THE DIAGNOSIS OF MALARIA IN ILORIN, NIGERIA

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Malaria is a major global health problem with about 2.4 billion people at risk. It is the commonest cause of outpatient consultations and one of the leading causes of paediatrics medical admission. Prompt and accurate diagnosis is the key to effective disease management and one of the main interventions of the global malaria strategy. We assess the sensitivity, specificity, predictive values and diagnostic accuracy of immunochromatographic technique (ICT) with the aim of assessing its relevance to the diagnosis of malaria in the North Central part of Nigeria. The study population, which comprised of 39 subjects aged 1 to 49 years, was sent to the hospital laboratory after clinical assessment. Thirty-five (89.7%) of the 39 subjects with fever had parasite count by thick blood film (TBF) ranging from 60-7,200 parasites/μL of blood. Twenty-five of these were positive by the dipstick technique giving a sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the ICT of 71.4%, 100%, 100%, 28.5% and 74.3% respectively when compared with the TBF. All four (4) subjects that were negative microscopically also tested negative with the ICT kit. We therefore conclude that ICT kits is a good alternative in diagnosis of malaria especially in adult in an endemic environment, because it is fast, requires simple manpower and no need of heavy equipment, However, before antigen tests can replace the thick and thin film, it should cover and differentiate between all Plasmodia species and detect lower level of parasitaemia.

INTRODUCTION

Malaria is a major global health problem with about 2.4 billion people at risk. It is estimated world wide that 200-300 million cases occur annually with about 1 million deaths, 90% of which occur in Sub-Saharan Africa (1). Malaria accounts for 10% of Africa's disease burden (2). It is the commonest cause of outpatient consultations and one of the leading causes of paediatrics medical admission (3, 4, 5). Falciparium malaria is the commonest cause of malaria in the Sub-Saharan region, while species other than *Plasmodium falciparium* account for less than 5% of infection (6).

It is estimated that up to 44% of fevers are attributable to malaria (7, 8). Therefore excluding malaria infection as a cause of ill health is central to improved health in this sub-region since other infectious diseases that cause high morbidity and mortality can be treated early following exclusion of a more common disease like malaria (9).

Prompt and accurate diagnosis is the key to effective disease management and one of the main interventions of the global malaria control strategy (10). Clinical diagnosis of malaria is inexpensive to perform and requires no special equipment or supplies. The symptoms of malaria are however non-specific and overlap with those of other febrile illnesses. A diagnosis of malaria based on clinical ground alone is therefore unreliable and when possible should be confirmed by laboratory tests. However, in endemic areas with considerable levels of acquired immunity, asymptomatic infection is common especially in the adult population (11). As high parasite count are more likely to coincide with fever, an alternate approach is to diagnose clinical malaria for fever episode when the parasite count is above a defined value which may

vary according to the level of acquired immunity (12, 13).

Accurate diagnosis of malaria is central to effective disease management and the goal standard is microscopic examination of stained blood film of malaria parasite. However, microscopy is labour intensive and can be inappropriate for some settings due to logistical burden such as lack of trained staff, time and technical equipment. Immunochromatographic test (one step malaria card test) is a rapid test that qualitatively detects the presence of *Plasmodium falciparum* Histidine-rich Protein 2 (HRP-2) antigen in whole blood using two antibodies, one attached to a visible colloidal gold in the test strip and the other immobilized to a membrane strip. It is an easy to use and rapid diagnostic test that requires simple manpower and no equipment support.

Our objective is to determine the sensitivity, specificity, predictive values and diagnostic accuracy of immunochromatographic technique (ICT) with the aim of assessing its relevance to the diagnosis of malaria in the North Central part of Nigeria.

PATIENTS AND METHOD

The study population comprised 39 subjects aged 1 to 49 years who had clinical malaria presenting at the outpatient department of a leading private hospital (Malaria Research Centre, Olanrewaju Hospital) in Ilorin located in the North Central region of Nigeria. The study was conducted between August and October 2002.

Clinical malaria was diagnosed as symptoms of fever, headache, body pains, loss of appetite, nausea and vomiting, abdominal discomfort and dizziness. Those who have commenced therapy elsewhere for malaria were excluded. The controls were patients who had other complaints not related to malaria on presentation.

The subjects were sent to the hospital laboratory after clinical assessment for full blood count, thin and thick blood films. Two milliliters of venous blood were collected from each subject for FBC and blood films. Full blood count was done using Leishmans stain on smeared slide. The thin blood film was used for speciation of the malaria parasite and was read on the same slide used for the FBC. The thick blood films were stained with Geimsa stain. All readings on the light microscope were done using high power 100 X oil immersion objective.

Malaria parasites were counted until 200 white blood cells (WBC) were encountered or up to 500 WBC when the number of parasite per 200 WBC was less than 10. Subjects were considered negative for malaria parasite if 500 WBC were counted without a parasite. The number of malaria parasite per ml was calculated using the formula; Number of parasite encountered (P) X (WBC count of patient)/Number of WBC counted (14). The WBC count used in this study was the average WBC for all the patients under investigation. The 'plus system' was also employed on the thick blood film examined with oil immersion objective and the results recorded using one to four pluses; + = 1-10 parasites per 100 thick film fields, ++ = 11-100 parasites per 100 thick film fields, +++ = 1-10 parasites per thick film fields, ++++ = More than 10 parasites per thick film fields.

Subjects positive for malaria parasite by the blood film were subsequently

tested with the rapid ICT diagnostic kit (Acon laboratories, USA). Other patients negative on blood films but with clinical features of malaria were also tested with the ICT kit. The ICT was performed according to the manufacturer's instruction. Briefly, a disposable specimen dropper was used to pick one drop (about 10 µL) of whole blood which was freshly obtained by finger prick from each subject and transferred to the specimen well of the kit. Three drops of buffer (about 120 µL) were added and the timer started. Results were read after 15 minutes. A pink line at the test and control region of the strip indicate positive result, a pink line at the control lane alone indicate negative result while absence of any pink line or pink line at the test alone indicate an invalid result.

Data were entered into EPI-INFO version 6.0 computers for analysis. Test of significance between variables was ascertained using Chi square test. The main limitation of the study was the cost of the kit.

RESULTS

Thirty five (89.7%) of the 39 subjects with fever, had parasite count by thick blood film (TBF) ranging from 60-7,200 parasites/µL of blood. Twenty-five of these were positive by the ICT dipstick technique giving sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for the ICT of 71.4%, 100%, 100%, 28.5% and 74.3% respectively when compared with the TBF as "gold standard". All four (4) subjects who were negative microscopically also tested negative with the ICT kit. (Table1).

Table 2 shows a comparison of 2 malaria parasite counting method; parasite count per liter and the "plus" method commonly used in this environment. Seven of the 10 patients with negative ICT had parasite count below 100/µL while 2 had parasitaemia level between 100 and 300/µL. When the 7 patients were removed from the analysis, the sensitivity and negative predictive values increased to 92.6% and 100% respectively. The test was negative at parasite count below 300/µL showing 0% sensitivity. There were no false positives and no invalid results. The WBC used in calculating the parasite count was 6,000/µL (6 X 10^9/L), which is the average WBC count recorded among the patients.

Table 1: Laboratory result of thick blood film (TBF) and immunochromatographic technique (ICT)

Diagnostic method		TBF		Total
		Positive	Negative	
ICT	Positive	25	0	25
	Negative	10	4	14
Total		35	4	39

Table 2: Comparison of two malaria parasites counting methods; Parasite count per microliter and the "Plus" method

Parasite count / µL	"Plus" system	No positive (TBF)	No positive (ICT)
> 4,500	++++	2	2
1,800-4,500	+++	8	8
900-1800	++	4	4
300- 900	+	12	11
100-300	scanty	2	-
< 100		7	-
Total		35	25

DISCUSSION

The average WBC among the patients recruited into this study is 6.0 x 10^9/L. This is in contrast with findings in caucasians in which an average of 8.0 x 10^9/L has been generally accepted (14). This calls for reassessment of average values in health and disease among people in this environment. The finding of 100% *Plasmodium falciparium* among the patients in this study agrees with other reports in which over 95% of plasmodia causing malaria is *Plasmodium falciparium* (5, 15, 16). However, this contrasts the findings of Wolday *et al* (17) in Ethiopia in which 32.8% were *Plasmodium falciparium* and 66.4% were *Plasmodium vivax*.

In this study, the dipstick method was persistently positive at a parasite count above 300/μL. This is less sensitive than the work done earlier by Olanrewaju *et al* (5) in which the kit was persistently positive at a count above 100/μL. Though, only 71.4% sensitive when compared with the thick film, the sensitivity of this kit approach 100% at a parasite count above 300/μL. The sensitivity (71.4%), PPV (100%) and NPV (28.5%) are comparable with the findings of Olanrewaju *et al* (sensitivity 66.7% and PPV 100%) and Wolday *et al* (sensitivity 97.2%, PPV 77.8%. Similar to findings of Olanrewaju *et al*, there was no invalid result and no doubtful case unlike that of Proux *et al* (18). The larger number of subjects rather than kits used by Proux *et al* can be said to account for this difference.

There remained an unresolved issue that for a patient in malaria endemic area where asymptomatic infection is commonly seen (11), what level of parasitaemia will be clinically relevant to the diagnosis of malaria (15). Further and more elaborate studies will help in providing information on the level of parasitaemia at which malaria infection can be conveniently diagnosed. Possible effort at having multiple lines on the kit that can determine an increasing plasmodium parasite/μL of blood (graduated kit) on the part of the manufacturers of this product will be relevant to enhance the usefulness of the kit. Moreover, ICT kit will be more relevant to malaria diagnosis if the parasite count at which the kit becomes positive can be noted and reflected in each batch by the manufacturer.

We therefore conclude that ICT kit is a good alternative in the diagnosis of malaria especially in adult in an endemic environment, because it is fast, requires simple manpower and no need of heavy equipment. There is however the need for us to determine the level of parasitaemia corresponding to malaria disease in this area to reduce the problem of over-diagnosis of malaria. Finally, before antigen tests can replace the thick and thin film, it should differentiate between other plasmodia species and detect lower level of parasitaemia.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003033/2433

INVESTIGATION OF AN EPIDEMIC OF MENINGITIS IN BARUTEN LOCAL GOVERNMENT AREA OF KWARA STATE, NIGERIA

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This is a report of an investigation into an outbreak of meningitis in three communities of Baruten Local Government Area (LGA) of Kwara State, Nigeria. A total of 41 cases of cerebrospinal meningitis (CSM) were reported. There was a preponderance of males (78%). Thirty-eight (92.7%) did not receive CSF vaccine while the remaining 3 (7.3%) that receive the vaccine were vaccinated less than a week before the onset of illness. Case Fatality Rate (CFR) was 7.3%. About half (46.3%) of the cases were aged between 6-15 years. The outbreak could have been prevented if adequate supplies of CSM vaccine were provided and vaccination conducted early enough. Government policy of mass immunization against meningitis in November of every year should be implemented and properly monitored to prevent future outbreaks of meningitis and its resultant morbidity and mortality.
Key words: Cerebrospinal meningitis, Outbreak, Kwara State, Nigeria.

INTRODUCTTION

Meningococcal infections are endemic in the northern savannah region of tropical Africa and approximately every 10 years, severe epidemics occur. This area is traditionally called 'the meningitis belt of Africa' and extends from the Gambia, in the west, to Ethiopia, in the east. This region is characterized by an annual rainfall of 300-1100 mm (1). In the sub-Saharan region, meningitis occurs on an annual basis. Epidemics occur at the start of the long, dusty dry season, which is associated with very low humidity, and ends abruptly at the onset of the rains.

Epidemic meningitis in sub-Saharan Africa is primarily due to group A *Neisseria meningitidis*, which causes the cycles of meningitis in the region. During an epidemic, one person in every hundred may become ill (2). Meningitis presents clinically usually as an acute illness, with fever, intense headache, nausea and vomiting, stiff neck and often a skin rash. Delirium and coma commonly occur and case fatality rate in untreated cases is about 50-80% (3). Epidemics of meningitis have been reported in the recent past in some areas of Kwara State. These include Kaiama LGA, which shares a boundary with Baruten LGA in 1997 and in Edu LGA in 2000 (4).

Prevention of meningococcal disease has been attempted by the administration of bivalent meningococcal A + C polysaccharide vaccine and there is evidence supporting the efficacy of group A vaccine from a number of African countries (5). This is a report of the investigation of the outbreak of meningitis that occurred in some communities in Baruten LGA between January and March 2001 with highlights of constraints found during the investigation and control of the epidemic.

MATERIALS AND METHODS

Study area

Baruten Local Government Area is located in the northwestern part of Kwara State of Nigeria. It is one of the largest LGAs in Kwara State both in terms of land mass and population. The projected population for the year 2001 is about 265,000. The LGA is divided into 4 districts namely Okuta, Yashikira, and Gwanara and Ilesha-Baruba districts. The LGA headquarters is located at Kosubosu in Yashikira district. The LGA is bounded in the north by Niger State, in the south by Oyo State, in the east by Kaiama LGA

of Kwara State and in the west by Republic of Benin.

Three communities in the LGA were visited; Ilesha-Baruba, located in the southern part, Shiya, located north of Ilesha-Baruba and Okuta, located between the two. There were two types of houses built in the LGA; modern brick houses roofed with zinc/asbestos sheets and mud houses roofed with thatched leaves. While the former is commonly seen in the more urban settings, the latter abound more in the rural communities and farm settlements. The brick houses had windows and doors and therefore are more ventilated than the mud houses that are poorly ventilated. There were only two medical doctors practicing in the whole LGA, one at the government-owned comprehensive health centre in Ilesha-Baruba and the other in the missionary-owned Baptist hospital in Okuta. In addition, there is a cottage hospital, 4 basic health centres and several private clinics, dispensaries and village health posts.

Outbreak investigating team

An emergency health team consisting of epidemiologists and staffs of the Disease Surveillance and Notification (DSN) unit of the Epidemiology Division of Kwara State Ministry of Health were mandated to investigate and control the outbreak of meningitis in some of the communities in Baruten LGA. The team also included a resident doctor from the Department of Epidemiology and Community Health, University of Ilorin Teaching Hospital, who provided technical support.

Data collection

Data were obtained from the records of the various health facilities in the communities affected. The Federal Ministry of Health case definition for cerebrospinal meningitis, which describes a case as acute illness with fever, intense headache, nausea and vomiting, stiff neck and a skin rash with delirium and coma often occurring and death common in untreated cases, was employed (2). Information obtained from patients who fit the case definition included name, age, sex, address, history of previous CSM immunization and time, date of presentation to the health centre, state of consciousness at and during admission and outcome/complications. Additional information was also obtained from the Baruten Local Government Council and the Kwara State Ministry of Health.

Sample collection/processing

Cerebrospinal fluid (CSF) samples were collected from 2 cases that were admitted less than 12 hours before the investigating team arrived. The specimens were transported and processed at the medical microbiology laboratory of the University of Ilorin Teaching Hospital using standard bacteriologic techniques.

Intervention measures

Intervention measures include mass CSM vaccination and administration of long-acting oily chloramphenicol injection (Tifomycin) to cases seen during the investigation.

RESULTS

A total of 41 cases presented at the health facilities located in the 3 communities visited with case definition of CSM (Table 1). Thirty-two (78%) of these were males while 9 (22%) were females. Over 95% of cases were less than 30 years and less than 5% were above 30 years. The median age was 14.8 years. (Table 2 and 3)

In the communities, 12 of the 18 patients on admission at the time of visit

were cases of meningitis, constituting 66.7% of total hospital admission. Of these, only 3 patients claimed to have received the CSM vaccine and all 3 took the vaccine less than 1 week before presenting with the illness. They all recovered consequently and were discharged without complications. The remaining 38 cases gave no history of previous CSM vaccination.

Of the 41 cases, 3 died giving a case fatality rate of 7.3%. Eight of the 41 cases were fully conscious before, during or after treatment. Thirty-three were however unconscious at one time or the other during the illness. Only one out of the 38 surviving cases developed complication. The case was a 22 year-old male who developed left knee arthritis.

The two CSF samples collected were turbid and both showed Gram-negative cocci on Gram-staining. The samples were obtained from 2 males aged 12 and 15 years presenting 6 and 18 hours respectively before specimen were taken. CSF white blood cell count was 292 cells/ml (60% polymorphs, 40% lymphocytes) for the first patient and 208 cells/ml (76% polymorphs, 24% lymphocytes) for the second patient. None of the sample grew any pathogen on culture.

Record of CSM vaccines supplied to Baruten LGA from the beginning of year 2000 showed that about 27,200 doses were supplied from Kwara State Ministry of Health. This will only immunize about 10% of the estimated population. Mass vaccination was however not commenced in the area until the first few cases were recorded from the southernmost district of Gwanara in late January 2001. Mass vaccination actually began in the LGA in early February 2001.

Table 1: Cases presenting at the health facilities that met the criteria for case definition of Meningitis in Ilesha-Baruba, Okuta and Shiya communities

Date	Ilesha-Baruba	Okuta	Shiya
February 1-7 2001	0	0	0
February 8-14 2001	3	1	0
February 15-21 2001	7	6	0
February 22-28 2001	7	14	3
Total	**17**	**21**	**3**
Last patient admitted	25-02-2001	28-02-2001	28-02-2001

Table 2: Sex distribution of people affected

Sex	Frequency	Percentage (%)
Male	32	78
Female	9	22
Total	**41**	**100**

Table 3: Age distribution of people affected

Age group (Years)	Frequency	Percentage (%)
1-5	5	12.2
6-10	5	12.2
11-15	14	34.1
16-20	6	14.6
21-25	5	12.2
26-30	4	9.8
Above 30 years	2	4.9
Total	41	100

DISCUSSION

The pattern of meningococcal infections in African epidemics is similar to that in developed countries where meningitis is by far the commonest presentation in over 80% of cases (6). There has been no reported outbreak of meningitis in the entire Local Government investigated in several years. The first community to be affected among the three was Ilesha- Baruba in the second week of February 2001. Okuta community was later affected the same week. Shiya community did not record any case until the fourth week of February. While the epidemic peaked in Ilesha-Baruba community in the third week of February and appeared to be subsiding by the fourth week, it was still raging in Okuta community by the third and fourth week of February. Shiya community, located north of both Okuta and Ilesha-Baruba communities recorded its first few cases in the 4th week of February. This spread to contiguous regions was observed in a similar epidemic in Katsina State in 1996 where the occurrence of cases in a community was one of the major criteria used in assessing priority on the need to vaccinate neighboring communities (3). The spread through droplet infection could have been aided by the weekly major market days in Ilesha-Baruba and Okuta where people from far and near come to carry out commercial activities.

The higher number of males (78%) affected in this epidemic may be due to an increased opportunity to acquire the infection by males compared to females rather a genetic sex predisposition to the infection (7). However, while some studies showed almost equal sex distribution of the cases, others give results showing higher affectation of male. For example, the 1996 epidemic in Katsina state (3) showed that half of the 20,927 cases registered were males. In the same epidemic around the same period in neigbouring Kano State (1), the male: female ratio of cases was 1.94: 1 compared to 3.56:1 recorded in our study.

The age group most affected is the 11-15 years age group constituting over one-third of all the cases. The median age affected was 14.8 years. This is similar to the report of the 1996 Kano epidemic study where 50% of all cases seen at the Infectious Diseases Hospital, Kano were between 6-15 years. About 46.3% of cases seen in the various health facilities in the 3 communities assessed in the study were also within the 6-15 years age group. Whereas 8% of cases were older than 30 years in the Kastina study, 4.9% of cases seen were above 30 years in the present study. It has been documented that relatively more cases occurs in the 5-19 years age group during epidemic than non-epidemic period (8). The fact that only one patient was aged less than 2 years corroborated the observation that children less than 2 years old may be spared during epidemics (5). Also, the fact that about two-third of the patients seen in the various hospitals were cases of meningitis is an indication of the amount of pressure the outbreak had placed on the available health facilities.

Of the 41 cases, 38 (92.7%) did not receive the CSM vaccine in the past 5 years. All the remaining 3 cases only took the vaccine less than a week to the date of presentation. It is in line with the observation that the vaccine may not offer protection until after 7 days of its administration (9). The observed Case Fatality Rate of 7.3% among the cases presenting at the various health centres is

similar to the 7:8% found in the Kano study. Most of the fatal cases died within few hours of admission. All the cases were among those who were unconscious before or at admission. Although no record of coma scale rating was available, level of consciousness could still serve as a prognostic index (1). Only one patient developed complication of left knee arthritis after apparent recovery from the infection. This was thought to be an immune-complex mediated reaction, which usually resolves without any sequelae (10).

The laboratory results of the CSF samples from the 2 patients, though confirmed the diagnosis of pyogenic meningitis from microscopy, did not yield any growth on culture. The result of the Gram-stain showing Gram-negative cocci suggests the likely organism in epidemic meningitis, *Neisseria meningitidis*. The organism is known to be very fastidious and dies rapidly on exposure to heat or cold (7) hence this may explain our failure to isolate it on culture. Lack of appropriate media for transportation of specimen and the long distance between the communities and University of Ilorin Teaching Hospital, where the specimens were cultured could be additional factors responsible for our inability to isolate the organism. This raises the need to equip zonal public health laboratories in the states.

The housing condition of the communities if improved with better ventilation will go a long way in long-term control of meningitis in the areas. The prolonged delay in carrying out mass vaccination in Baruten LGA contributed to the occurrence of the epidemic. Also, the delay in reporting to the hospital by most patients resulted in a higher number of patients presenting with unconsciousness before or during admission and with consequent adverse effect on the prognosis. Future outbreaks can be better prevented by ensuring that mass vaccination is carried out by the month of November every year as recommended by the Federal Ministry of Health. Improving housing condition in the rural communities and involving the communities in early recognition, detection and immediate reporting to the hospital will assist in these areas where health facilities are very limited.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003037/2434

A STUDY OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY IN ILE - IFE, SOUTHWESTERN NIGERIA

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Asymptomatic bacteriuria presents a considerable risk to the mother and may lead to onset of acute pyelonephritis in about 5% of pregnant women and also increase the risk of fetal mortality. Apart from one previous study, no other study has been carried out in this environment hence our study. The objectives are to determine the prevalence of asymptomatic bacteriuria amongst pregnant women in the three trimesters of pregnancy, to isolate and characterise the bacteria agents involved in this condition and recommend methods of reducing incidence and possible attendant sequelae. A descriptive study with purposive sampling carried out at the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife Southwestern Nigeria between May 2000 and April 2001 examined two hundred and one consecutive pregnant women attending the antenatal clinic. This included women in the three trimesters of pregnancy. Those with urinary tract infections were excluded. Each subject was given a sterile universal bottle and requested to collect midstream urine. Each sample was plated onto Cystein-Lactose-Electrolyte-Deficient (CLED) medium and chocolate agar (CA). The major bacterial colonies were isolated and characterised employing standard bacteriologic methods. The prevalence rate was 26%. *Staphylococcus aureus* was predominant (43.8%), of which 58.8% were beta-lactamase producers. Forty six point six percent of total isolates were Gram-negative rods; *Klebsiella pneumoniae* (6.8%), *Escherichia coli* (4.5%), *Citrobacter freundii* (4.5%) and others. The study recorded a relatively high prevalence of asymptomatic bacteriuria. While the bacterial isolates were multi-resistant to drugs traditionally employed to treat uropathogens, they were relatively sensitive to nitrofurantoin *in vitro*. Because of the high prevalence of asymptomatic bacteriuria, we recommend routine screening for this condition in all antenatal clinics in this environment to reduce the incidence and probable attendant sequelae.

INTRODUCTION

Pregnancy is a predisposing factor to urinary tract infections and pregnant women suffering from this condition are at risk of various complications of pregnancy including low birth weight and preterm birth. Asymptomatic bacteriuria refers to significant bacteriuria in a patient without symptoms (1) while significant bacteriuria is the number of bacteria in voided urine that exceeds the number usually associated with contamination from the anterior urethra i.e. $>10^5$ bacteria/ml of urine (2). In a review by Miller and Cox (3), asymptomatic bacteriuria, cystitis and pyelonephritis are frequently encountered complications of pregnancy. In their studies, Grio *et al* (4, 5) noted that non-treated asymptomatic bacteriuria present a considerable risk to the mother and may lead to the onset of acute pyelonephritis in approximately 5% of pregnant women which may increase, to some extent, the risk of fetal mortality.

The incidence of asymptomatic bacteriuria varies with study population as well as the method of collection of samples. Asymptomatic bacteriuria was reported in 5.6% of 181 black pre-natal patients in Durban, South Africa (6) while a rate of 7% was reported among 326 pregnant women in Ethiopia (7). Other investigators (8) in Ibadan, South western Nigeria have reported 12% prevalence rate. Apart from a study done by Okonofua *et al* (9) in Ile-Ife, there is no other study hence our study. Besides, this condition may manifest in a subtle-form for the entire duration of pregnancy and complications do arise. This study addresses the incidence of asymptomatic bacteriuria in the three trimesters of pregnancy in Ile-Ife

and its environs and characterized the bacteriological agents involved. This will assist clinicians in effective management of this condition to prevent the attendant possible sequalae.

MATERIALS AND METHOD

Location of study

The study was carried out at the Obafemi Awolowo University Teaching Hospital Complex, (OAUTHC) Ile-Ife, Southwestern Nigeria between May 2000 and April 2001. The hospital is a referral centre for over half a million people within 40 km radius of the city.

Subject selection

A purposive selection consisting of pregnant women attending the ante-natal clinic was taken. This included women in the three trimesters of pregnancy. Those with overt urinary tract infection were excluded. A total of 201 pregnant women were studied. Informed consent of the women was obtained at the ante-natal clinic. A questionnaire was admitted on each patient before collection of urine specimen. Information required on the questionnaire includes the age, level of education, parity, gestational age and symptoms relating to urinary tract infection.

Collection of specimens

Each of the women was given a sterile universal bottle and was requested to collect mid-stream urine and to submit the specimen with the questionnaire.

Processing of specimen and isolation

The culture media used for isolation were Cystein-Lactose Electrolyte-Deficient (Difco Co, USA) and chocolate agar plates. Each urine sample was inoculated and streaked with the aid of heat-flamed standard wireloop (delivering 0.001ml urine) on to the agar plates. The plates were incubated aerobically at 37°C overnight and then examined. Only plates with significant growth (i.e. at least 100 colonies) were considered significant and further analyzed. The cultural and morphological characteristics of distinct and isolated colonies were studied. This included size, elevation, opacity and colour. Distinct and isolated colonies from each significant growth were Gram stained. Those resembling staphylococci were inoculated onto Mannitol Salt agar (MSA) and colonies that fermented mannitol were presumptively identified as *Staphylococcus aureus* and confirmed by the coagulase slide and tube agglutination tests with pooled human plasma. Coagluase negative staphylococci (CONS) were noted. Gram-negative rods were identified as lactose or non-lactose fermenters using Eosin Methylene Blue (EMB) and MacConkey agar. Further speciation of the isolates was based on their activities on conventional media such as Triple Sugar Iron agar (TSI), Koser's citrate medium, Sulphide Indole Motility agar (SIM) and Urea agar and according to methods described by Cowan and Steel (10).

Detection of beta-lactamase

Each isolate was tested for β-lactamase activity by the starch paper method. Starch paper was soaked for 10 minutes in a solution of benzyl penicillin containing 10^5 units/ml and then spread smoothly in a Petri dish. Using a fine bacteriological loop (2 mm diameter), each colony of bacteria was collected from the surface of the culture plate and transferred onto surface of test paper and spread over an area of 2-3 mm. The inoculum was placed at least 1.5cm apart. Plates were

incubated for 30 minutes after which the papers were flooded with iodine solution (Gram's iodine diluted 1 in 2). Beta-lactamase producing strains were detected by the discoloration of the blue-black colour of iodine surrounding each organism with the widening of the white-halo in the course of the ensuing 5 minutes while the surface of the inoculum remained whitish. Penicillinase-negative isolate did not produce any discoloration of the surrounding area.

Antibiotic sensitivity test

The disc diffusion method of Bauer *et al* (11) was employed in this study. Five colonies of each strain of the isolate were suspended in a sterile bijou bottle containing 5mls of peptone water (Lab M) and incubated overnight at 37°C. The overnight broth cultures were diluted to 10^6 colony-forming units per ml. A sterile cotton-tipped applicator was introduced into standardized inoculum and used to inoculate dried plate of sensitivity test agar (STA) for each isolate. The antibiotic discs used were ampicillin (AMP) 10 µg, ciprofloxacin (CIP) 10µg, gentamicin (GEN) 10µg, erythromycin (ERY) 10µg, nalidixic acid (NAL) 30µg, nitrofuration (NIT) 200µg, ceftriaxone (CRO) 30µg, colistin (COL) 25µg, tetracycline (TCN) 10µg, penicillin G (PEN) 1 iu, cloxacillin (CLX) 5µg, chloramphenicol (CMN) 10µg, cefuroxime (CXM) 30µg, ofloxacin (OFX) 5µg, ceftazidime (CAZ) 30µg, and cotrimozaxole (COT) 25µg. Control organisms, *Staphylococcus aureus* ATCC 25923 and *Enterobacter aerogenes* ATCC 10342, were used.

Statistical analysis

The prevalence of bacteriuria in relation to trimester among subjects was determined using SSPS 8.0 statistical package.

RESULT

Of 27 women whose gestational age was 1-13 weeks, 14 were bacteriuric while of 87 subjects within 14-26 weeks of pregnancy, 26 were bacteriuric. Of 82 subjects in the third trimester of pregnancy, 33 had significant bacteriuria. The differences in bacteriuria in relation to gestational age were not statistically significant ($p = 0.158$). Although the prevalence of bacteriuria varies with parity with the highest rates occurring among nullipara (47.5%), followed by multipara (42.5%) and lowest among primipara (21.1%), the differences were not statistically significant ($P=0.064$). The prevalence of bacteriuria in relation to trimester among subjects also shows no statistically significant differences ($p = 0.158$ $t = 2.206$, $df = 2$).

Bacterial isolates

The bacteria isolates recovered from urine of the subjects are shown in Table 1. Altogether, a total of 73 isolates were recovered. Gram-positive organism constitute 53.4% made up of *Staphylococcus aureus* (43.8%) followed by coagulase negative staphylococci (31.5%). Gram-negative rods accounted for 46.6% of the total isolates made up of *Klebsiella pneumoniae* (6.8%), *Escherichia coli* (5.5%), *Citrobacter freundii* (4.1%), coliforms and *Proteus mirabilis* (2.7% each). *Pseudomonas aeruginosa* accounted for only 1.4% of the total isolates. Table 2 shows the profile of the β-lactamase producing isolates.

Table 1: Distribution of bacterial isolates

Microbe	No of isolate (%)
Staphylococcus aureus	32 (43.8)
Coagulate negative staphylococci	23 (31.5)
Klebsiella pneumoniae	5 (6.8)
Escherichia coli	4 (5.5)
Citrobacter freundii	3 (4.1)
Coliforms	2 (2.7)
Proteus mirabilis	2 (2.7)
Streptococcus faecalis	1 (1.4)
Pseudomonas aeruginosa	1 (1.4)
Total	**73 (99.9)**

Table2: Profile of beta-lactamase production amongst isolates

Microbe	No tested	β-lactamase positive (%)
Staphylococcus aureus	32	22(68.8)
Coagulate negative staphylococci	23	10(43.5)
Klebsiella pneumoniae	5	2(40.0)
Escherichia coli	4	2(50.0)
Citrobacter freundii	3	3(100)
Proteus mirabilis	2	0(0)
Coliforms	2	0(0)
Streptococcus faecalis	1	0(0)
Pseudomonas aeruginosa	1	0(0)

DISCUSSION

This study shows that of the 196 women examined, only 73 (37.2%) showed significant bacteriuria at the first collection. According to Kincaid-Smith and Bullen (12), only 70% of women who have positive culture at the first examination displayed this symptom in the second collection. This suggests that in reality the prevalence rate in this study can be adjudged as 26%. The prevalence of asymptomatic bacteriuria varies from one study to another. Little in 1996 (13) found an incidence of 5.3% in 5,000 women which was similar to that of Sleigh *et al* (14) who reported an incidence of 6.6% in a survey of 4,349 patients. In a study carried out by Olusanya *et al* (15) among 510 pregnant women and 304 non-pregnant women at Ogun State University Teaching Hospital, Sagamu South-West Nigeria, 23.9% of the population examined showed significant bacteriuria. The value obtained in our study is a little higher than the 23.9% in their study, which is within the same geographic zone. Investigators in Trinidad (16) recorded a prevalence rate of 16.7% among Trinidadian women, which is similar to that reported by Reddy and Campbell (17) in a racially mixed community in Gisborne, New Zealand. However, Al-Sibai (18) reported 14.2% among Saudi-Arabian women, which was about the rate reported by Okubadejo *et al* (8) in Ibadan, South-Western Nigeria, underscoring the variation of prevalence of bacteriuria from one locality to another.

Our result also showed that the incidence of symptomatic bacteriuria in the three trimesters of pregnancy was not significantly different. In a study carried out

by Nnatu *et al* (19), the incidence of bacteriuria was highest in first trimester of pregnancy in which 3.3% of women screened displayed this symptom compared with 4.1% and 2.8% in the second and third trimester respectively.

The aetiologic agents of asymptomatic bacteriuria also vary (20, 8, 15). In our study both Gram-positive and Gram-negative organisms were cultured from the urine. Out of the total 73 isolates encountered, 56 (76.7%) were Gram-positive cocci of which *Staphylococcus aureus* accounted for 32 (57.1%), coagulase negative staphylococci 23 (41.1%) and *Streptococcus faecalis* 1(1.8%). The predominance of *Staphylococcus aureus* in the urine sample examined in this study corroborates a study done in Sagamu Southwestern Nigeria by Olusanya *et al* (20), who reported *Staphylococcus aureus* as the predominant organism in their study. Most studies done in Nigeria have reported Gram-negative rods as the major organisms in bacteriuria in pregnant women (8, 21). Isolation of enteric organisms in the urine may be due to the proximity of the perineum to the vulva and urethra (the organism being normal flora in the bowels readily colonize the perineum and then the vulva). Nnatu *et al* (19) in Lagos recorded *Escherichia coli* in 45% of bacteriuric patients while Okubadejo *et al* (8) reported *Escherichia coli* in 41% followed by *Klebsiella pneumoniae* 19.4% and *Proteus mirabilis* 16.1%. The present study shows *Klebsiella pneumoniae* (23.5%), *Citrobacter freundii* (17.6%), *Proteus mirabilis* and coliforms (11.7% each) and *Pseudomonas* aeruginosa (5.7%).

The bacteria isolated from pregnant women in this study are remarkably similar to those reported by Olusanya *et al* (20). In their study, coagulase positive staphylococci were recorded in 27.9% of pregnant women compared with our study of 57.1% which doubled their value, followed by coagulase negative staphylococci 19.1% compared 41.1% in our study. The variation in their value and that of our study may be due to their relatively large sample size of 510, which is more than double the sample size of 196 in our study. The significance of *Staphylococcus aureus* as predominant organism in bacteriuric pregnant women in this study is not apparently clear. However, in a study of urinary tract infection in Ile-Ife carried out in the same hospital in 1993, *Staphylococcus aureus* was responsible for 15.4% of the total bacteria isolated from females. In addition, about 20-40% of individuals in the environment are known to be carriers of *Staphylococcus aureus* (22, 23) and might therefore acquired the organism by auto-infection. Similarly, like in Olusanya *et al* study (20), coagulase negative staphylococci were rated second among Gram-positive cocci encountered. The isolation of coagulase negative staphylococci from urine may be significant only when *Staphylococcus saprophyticus* is involved. Other species may be seen as contaminants.

Klebsiella pneumoniae and *Escherichia coli* were also encountered in this study. The isolation of Gram-negative organisms as in pyelonephritis may suggest ascending infection (8). Our study showed that more than half (39) of the bacteria isolated produced beta-lactamase. This enzyme hydrolyzes the beta-lactam drugs such as the penicillins and cephaloporins, though the cephaloporins are reported to be more stable. This finding may in part

explain the rate of resistance to beta-lactam drugs in this study. Sixty percent of the *Staphylococcus aureus* isolates were resistant to ampicillin, 87.5% to penicillin G and 89.3% resistant to cloxacillin respectively (Table not shown). Sensitivity to gentamicin was only moderate being 44.5% among *Staphylococcus aureus* isolates, 31.8% for coagulase negative staphylococci, 40% for *Klebsiella pneumoniae*, and 50% each for *Citrobacter freundii, Proteus mirabilis* and *Escherichia coli*. Susceptibility to augmentin was similar, 38.1% of *Staphylococcus aureus*, 58.3% of coagulase negative staphylococci, 25% of *Klebsiella pneumoniae* and 75% of *Escherichia coli* strains were sensitive to the drug. These data suggest that resistance to beta-lactam drugs such as penicillin may also be co-transferred with resistance to other antibiotics such as gentamicin, tetracycline and even chloramphenicol. Such findings have been reported among bacterial isolates from cases of acute otitis media in Ile-Ife, Southwestern Nigeria (26).

It is also noteworthy the high frequency of resistance to cotrimozaxole by all the isolates. All the *Staphylococcus aureus*, coagulase negative staphylococci, *Klebsiella pneumoniae* and *Escherichia coli* isolates tested against cotrimozaxole were resistant. Other workers (25, 26, 27) have reported similar findings. Ako-Nai *et al* (26) reported only 45%, 43%, 40% and 40% susceptibility for *Escherichia coli, Klebsiella spp*, coliforms and *Proteus spp* respectively. This finding suggests possible abuse of cotrimozaxole in this environment based on its over-the counter availability.

It is interesting to note that virtually all the organisms tested against nitrofuration were susceptible. The susceptibility value ranged from 50% amongst coagulase negative staphylococci, to 80% amongst *Klebsiella pneumoniae* and 100% among *Staphylococcus aureus, Citrobacter freundii* and *Pseudomonas aeruginosa* isolates. Dempsey *et al* (28) reporting the characteristics of bacteriuria in a homogenous maternity hospital population noted that the most effective antibiotic in their study was nitrofuration, with over 90% of isolates sensitive to it. The implications of this finding, is that nitrofuratoin, a traditional urinary antiseptic, is still effective in the treatment of urinary tract infection in this environment.

Finally, there are conflicting views as to the rationale of routine screening pregnant women for significant bacteriuria. Al-Sibai *et al* (18) advocated screening on a selective basis (i.e. for young teenage parous women, those coming from disadvantaged socio-economic conditions and those with a past history of urinary tract infection). Olusanya *et al* (20) on the other hand advocated routine screening for all pregnant women at least during the first visit to the ante-natal clinic. Our results which shows that about one in every four (26%) pregnant women in this environment have asymptomatic bacteriuria, is rather high. It may be a worthwhile exercise therefore, if routine screening for bacteriuria of pregnant women is part of antenatal clinic facilities in this environment. This is even more important since early detection of asymptomatic significant bacteriuria has been reported to prevent eclampsia in pregnancy and reduce the incidence of prematurity and pyelonephritis later in life among women with such problem (29).

Similarly, untreated pregnant women with significant bacteriuria have been reported to have higher complication in pregnancy than the ones treated (21).

ACKNOWLEDGEMENT

The authors acknowledge the effort of Mrs. M. O. Disu for the preparation of manuscript.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003038/2435

VIBRIO CHOLERAE 01 INFECTIONS IN JOS, NIGERIA

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A study to determine the prevalence of *Vibrio cholerae* 01 in stool sample submitted for routine examination of enteric pathogens, as well as identify the serotypes and antibiogram of the isolates to commonly used antibiotics was undertaken. The survey involved the examination of 774 (763 stool and 11 rectal swabs) specimens obtained from different patients seen at the Jos University Teaching Hospital (JUTH). Of the total number examined, 34 (4.39%) yielded *Vibrio cholerae* 01. All of them were Inaba serotype of El-Tor biotype. The age group 20-29 years had the highest rate, 21 (6.95%). Rectal swabs yielded a higher number of isolates, 9 (81.82%) from 11 specimens compared to 25 (3.28%) from 763 stool specimens. The organism is most prevalent during the mid-rainy season (June/July) since most of the isolates 29 (85.29%) of the 34 isolates were isolated during this period ($P < 0.05$). Isolates were very sensitive to ofloxacin, erythromycin and tetracycline but resistant to chloramphenicol, ampicillin, cloxacillin and penicillin G. This study demonstrates that *Vibrio cholerae* 01 is endemic in our environment.

INTRODUCTION

Cholera (Greek, chole, bile) is caused by the Gram-negative *Vibrio cholerae* bacterium of the family Vibrionaceae. Although, there are many serogroups, only 01 and 0139 have exhibited the ability to cause epidemics (1). *Vibrio cholerae* 01 is divided into 2 serotypes, Inaba and Ogawa, and 2 biotypes, Classic and El-Tor. Throughout recorded history, Cholera has caused seven pandemics in various areas of the world especially in Asia, the Middle East, and Africa. The first reported case in Nigeria occurred in 1970 (2). Since then, the disease has remained endemic with occasional outbreaks in some states of the country primarily due to lack of good water supply and poor personal and environmental hygiene.

An average of 3,000 stool specimens is processed annually at the medical microbiology department of Jos University Teaching Hospital (JUTH). From the results obtained from these specimens over the past one decade, it was observed that the rate of isolation of enteric pathogens has been very low. Due to the limited range of facilities in JUTH, only a selected few of the enteric bacterial pathogens are sought for routinely; *Salmonella, Shigellae,* and occasionally enteropathogenic *Escherichia coli.* Others such as *Vibrio cholerae,* Helicobacter and Campylobacter are never sought for routinely. This has led to the always-recurring result "no pathogen isolated".

This study was therefore undertaken to examine the prevalence of *Vibrio cholerae* 01 in stool samples routinely processed in JUTH and identify the prevailing serotype and antibiogram of recovered isolates in the light of reported cases of clinical cholera.

MATERIALS AND METHODS

Samples

The samples analysed in this study included a total of 774 (763 stools and 11 rectal swabs) of patients attending the Jos University Teaching Hospital. These were

brought in clean, transparent, wide mouthed bottles. In suspected cholera cases, rectal swabs were collected by nurses in the wards and inoculated into bottles of sterile alkaline peptone water. Both out patients and nurses were instructed on the mode of collection.

Processing of specimens

The specimens were processed according to the guidelines on laboratory methods for the diagnosis of *Vibrio cholerae* by the Centres for Disease Control, National Centre for Infectious Diseases and Prevention (CDC/NIVD) Atlanta Georgia, United States of America and as described by Collee and Miles (3). These include macroscopy, microscopy, motility testing, Gram stain, culture and biochemical testing. Others are serology and antimicrobial susceptibility testing. Specimens were inoculated into thiosulphate citrate sucrose bile salt (TCBS) agar (Antec Diagnostics, UK) and alkaline peptone water and incubated at 37°C. After 6 hours of incubation, subcultures were made from the surface growth on alkaline peptone water onto TCBS and incubated overnight. Colonies from TCBS agar were inoculated onto Brain Heart Infusion (BHI) agar for biochemical and serological identification.

Biochemical testing

Suspected organisms were identified as *Vibrio cholerae* 01 from growth on BHI agar using the following standard tests; oxidase test, stringing test, citrate utilization test, lysine decarboxylase test, direct haemagglutination test, nitrosol-indole test and immobilization by distilled water. All tests were as described by Collee and Miles (3) and Porter and Duguid (4).

Antimicrobial susceptibility testing

Sensitivity of isolates to antimicrobial agents was determined on BHI agar plates using the disc diffusion method of Scott (5). From a pure culture of the isolate to be tested, a uniform streak was made on the agar plate. The antibiotic discs (Antec Diagnostics, UK) were then placed on the plates and incubated at 37°C overnight. Interpretation of results was done using the zone sizes. Zone diameters of inhibition of ≥ 18mm were considered sensitive, while 13-17 mm intermediate and < 13 mm were considered resistant. All isolates were tested for sensitivity to the following antibiotics; ampicillin (10mcg), chloramphenicol (10mcg), streptomycin (10mcg) tetracycline (10mcg), cotrimoxazole (25mcg), erythromycin (5mcg), ofloxacin (95mg) and penicillin G (10 units).

Statistical analysis

The data obtained were subjected to the Chi-squared test using a probability of $P < 0.05$ as the level of significance.

RESULTS

A total of 774 samples made up of 763 stool and 11 rectal swabs were examined over a period of 8 months (January-August 1996). The age range of the patients was 0- 69 years. Of the total number of specimens examined, 34 (4.39%) were positive for *Vibrio cholerae* 01. The highest numbers of isolates were recovered from the age group 20-29 years, followed by age group 30-39 years with 5 isolates. The age brackets 0-9 years, 10-19 years, and 40-49 years had 2 isolates each. Only one isolate each was recovered from the age groups 50-59 and 60-69 years (Table 1). The difference is not statistically significant ($p > 0.05$).

Macroscopic examination of the specimens showed that 189 were watery, 321 soft formed, 15 blood-stained, 206 hard formed and 32, mucoid. The watery samples yielded the highest number of isolates (21), soft-formed specimens 3 and hard formed 1. The blood stained and mucoid specimens yielded no isolates. The remaining 9 positive were from rectal swabs (Table 2).

Table 3 shows the prevalence of *Vibrio cholerae* 01 in in-patients and out-patients. Of the 580 samples taken from out-patients, 6(1.03%) were positive for *Vibrio cholerae* 01, while 28 (14.43%) of the 194 samples from in-patients yielded *Vibrio cholerae* 01. This difference is statistically significant ($p < 0.05$). The monthly isolation of *Vibrio cholerae* 01 is shown in Table 4. Only one isolate was recovered in January, none in February, March, and April. Two isolates were obtained in May, 20 in June, 9 in July, and 2 in August. Serological screening of all the 34 isolates showed that all were of the Inaba serotype and El-Tor biotype.

Table 5 shows the *in-vitro* susceptibility pattern of the isolates. All the isolates were sensitive to tetracycline, erythromycin and ofloxacin. The only strain showing the widest range of susceptibility was isolated in January. It was sensitive to cotrimoxazole and gentamicin in addition to the other three antibiotics. This difference is statistically significant ($p < 0.05$).

Table 1: Prevalence of *Vibrio cholerae* 01 isolated in relation to age

Age groups (Years)	No examined	No positive (%)
0-9	142	2(1.41)
10-19	109	2(1.83)
20-29	302	21(6.95)
30-39	108	5(4.63)
40-49	55	2(3.64)
50-59	37	1(2.70)
60-69	21	1(4.76)
Total	**774**	**34(4.39)**

$X^2 = 8.97$ $df = 6$ $p > 0.05$

Table 2: Type of samples treated and the number of *Vibrio cholerae* 01 isolated

Types of stool sample	No examined	No positive (%)
Watery	189	21(11.11)
Soft formed	321	3(0.93)
Blood stained	15	0(0.00)
Hard formed	206	1(0.49)
Mucoid	32	0(0.00)
Total	**763**	**25(3.28)**
Rectal swab	11	9(81.82)

Table 3: Prevalence of *Vibrio cholerae* 01 isolated from the various specimen sources

Source of specimen	No examined	No positive (%)
Out patients	580	6(1.03)
In-Patients	194	28(14.43)
TOTAL	**774**	**34(4.39)**

$X^2 = 50.81$ $df = 1$ $p < 0.05$

Table 4: Monthly incidence of *Vibrio cholerae* 01 in JUTH

Period	No examined	No positive (%)
January	25	1(4.00)
February	29	0(0.00)
March	125	0(0.00)
April	111	0(0.00)
May	103	2(1.85)
June	163	20(12.2)
July	100	9(9.00)
August	68	2(2.95)
Total	**774**	**34(4.39)**

Table 5: *In-vitro* antibiotic susceptibility pattern of *Vibrio cholerae* 01

Antibiotic (mcg)	No examined	No positive (%)
Ofloxacin (5)	34	34(100)
Erythromycin (5)	34	34(100)
Tetracycline (10)	34	34(100)
Cotrimoxazole (25)	34	1(2.94)
Gentamicin (10)	34	1(2.94)
Chloramphenicol(10)	34	0(0.00)
Ampicillin (10)	34	0(0.00)
Cloxacillin (5)	34	0(0.00)
Penicillin (10 unit)	34	0(0.00)

$X^2 = 130.35$ $df = 8$ $P < 0.05$

DISCUSSION

A total of 774 samples were analysed in this study, in which 34 (4.39%) were positive for *Vibrio cholerae* 01.The percentage is low compared to the 18% documented by Shapiro *et al* (6) in rural western Kenya in diarrhoea specimens. Our finding is however significant since the specimens included non-diarrhoea stools. All the 34 isolates were Inaba serotype of the El-Tor biotype. This is contrary to the 1991 outbreak in Jos, in which Ogawa was the predominant serotype. Most strains causing epidemics in Nigeria have been Ogawa serotype (2, 7). Reports of one serotype displacing another have been on the increase. Gomez *et al* (8) reported a case in which Inaba strains, which were dominant in Mexico in 1991, were later sub-planted by Ogawa serotype in 1992. In Calcutta, India, *Vibrio cholerae* 0139 displaced El-Tor *Vibrio cholerae* 01 (causative agent of the seventh pandemic), an event that has never happened in recorded history of cholera (1). The toxigenic Inaba serotype of *Vibrio cholerae* 01 biotype El-Tor however reappeared in India in 1998 and 1999, almost a decade after its last dominance in the Calcutta episode (9). Antigenic variations have also been observed *in-vitro* and that more than one antigenic variant may be isolated from the stool of cholera patients (10).

The highest number of isolates were from the age group 20-29 years with 21 (6.95%), while the least 1 (2.7%) and 1(4.76%) were from the age brackets 50-59 years and 60-69 years respectively. The age group 0-9 years yielded 2 (1.4%) isolates. This finding differs from the report of Samir (11) who stated that in endemic areas, clinical infections are most common among the "unsalted" pre-school children. It however agrees with that of Bhattacharya *et al* (12) who found majority of cases in adults.

Macroscopic examinations of the stool samples showed that watery stools yielded the highest number of isolates 21(11.11%). This agrees with reports in the literature, which showed that cholera is characterized by watery stools often called "rice water stool". Mucoid and blood stained samples yielded no *Vibrio cholerae* 01. This is not unexpected since the organism is non-invasive (1, 13). The percentage isolation from rectal swabs was 81.82%. This agrees with Porter and Duguid (4), who advocate the collection of rectal swabs, especially when screening for carriership.

The percentage isolation from outpatients was low (1.03%) compared to 14.43% in in-patients. This is not surprising since clinical cholera cannot be treated on outpatient basis. The few isolates from the outpatients could be from contacts or carriers. The periodic pattern of *Vibrio cholerae* 01 showed that the highest numbers of isolates were recovered in June. This is probably due to the fact that June

corresponds to the middle of the raining season in Jos during which rivers and streams begin to flood and as such carry along waste from the country side. Also, dams are flooded supplying more water than the treatment facilities can cope with. In addition, because of torrents associated with rains, water pipes become exposed, damaged and contaminated by faecal material.

The isolates were very sensitive to ofloxacin, erythromycin and tetracycline. The only isolate recovered in January showed a wider range of susceptibility to the other antibiotics. It is possible that this strain mutated and later developed multiple drug resistance thereby leading to the epidemics of June and July. Coppo *et al* (14) had reported the introduction of cholera into Somalia by an initial drug susceptible strain. An alternative explanation could be that this strain was supplanted by a new strain brought to the state from neighbouring Kano where an epidemic had been reported since January.

This study has shown that cholera is endemic in our environment. It is therefore recommended that stool specimens should be routinely examined for *Vibrio cholerae*.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003040/2436

EFFECT OF ESSENTIAL LEAF OIL OF *OCIMUM GRATISSIMUM* ON CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA*

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***Ocimum gratissimum* leaf oil, which has been reported to possess *in vitro* and *in vivo* efficacy against enteric bacteria was tested against forty six clinical isolates of Pseudomonas aeruginosa in Lagos, Nigeria. The effect of the essential oil (EO) on pyocyanin production among these strains was also investigated. Agar well diffusion assay revealed susceptibility in 40 (87%) of the 46 tested strains with inhibition zone diameter (20-36 mm) comparable with the effect of tobramycin. Of the 40 susceptible isolates, 34 strains were quantitatively demonstrated to show susceptibility when further tested with the essential oil in broth and on agar yielding MIC and MBC values of 36 – 54 mg/ml and 42 – 66 mg/ml respectively. The values were higher than the 12 mg/ml (MIC) and 24 mg/ml (MBC) observed in *Escherichia coli* ATCC 25922. Compared with the control, the essential oil was found to reduce pyocyanins production significantly ($p < 0.01$) at 15 mg/ml (30.1 – 30.5 vs 259.2 – 276. 7 µg/ml) and 75 mg/ml (2.5 – 3.5 vs 259.2 – 276.7 µg/ml) in both sensitive and resistant strains, suggesting that *Ocimum gratissimum* leaf oil may inhibit expression of virulence factors and progression of *Pseudomonas* infections caused by the tested strains.**

Key words: ***Ocimum gratissimum* leaf oil, *Pseudomonas aeruginosa*, Nigeria**

INTRODUCTION

On a global scale, *Pseudomonas aeruginosa* is responsible for 16% of all nosocomial infections and 5% of all community-acquired infections (1). In Nigeria, hospital acquired pseudomonal infections are common among patients on in-dwelling devices and those with chronic discharging ears and bronchopulmonary disorders (2). These diseases often lead to complications such as septicaemia with eventual death (3). The large numbers of diseases caused by *Pseudomonas aeruginosa* as an opportunistic pathogen is premised on many factors, which include multi-drug resistance and production of virulence factors such as pyocyanin.

Infections caused by multi-drug resistant strains of *Pseudomonas aeruginosa* have severally been reported in Nigeria (4, 5, 6). The gross resistance of *Pseudomonas aeruginosa* to antibiotics has paved way for an extensive exploration of plants of folkloric medicine for phytochemicals against this organism throughout the globe (7, 8, 9).

Plants' parts such as the seeds of *Moringa oleifera* and *Nigella sativa* (10, 11), roots of *Diospyros mespiliformis* (12), fruits of *Juniperus oxycedrus* (13) and leaves of *Synclisia scariba* and *Bryophyllum pinnatum* (14, 15) have been found to possess antibacterial activity against *Pseudomonas aeruginosa*. However, the use of these plants for complementary therapies is limited by their narrow geographical spread and inadequate folklore medicinal belief mostly in unfamiliar rural communities.

Ocimum gratissimum is one of the medicinal plants that are widely used in Nigeria and acclaimed to have a large clinical coverage of diseases in folk medicine (16). The extracts of the plants have severally been demonstrated to possess bacteriologic and clinical efficacy against infections due to *Enterobacteriaceae* (17, 18). *In vitro*, we have found the essential oil of *Ocimum gratissimum* inhibitory to extracellular protease elicited as a virulent factor by *Shigellae* (article in-press) and a study by Fakae *et al* (19) demonstrated the

ability of the oil to inhibit glutathione – S - transferase activity in helminthic infections. Elsewhere the anti-pseudomonal effect of basil oil chemotypes on *Pseudomonas spp* has been reported (20) amidst contradictions from a related study (21).

In Nigeria, there are no documented studies demonstrating the interactions between *Ocimum gratissimum* leaf oil and *Pseudomonas aeruginosa*. We hypothesize that knowledge of the efficacy of *Ocimum gratissimum* leaf oil on *Pseudomonas aeruginosa* may improve and cheapen intervention measures against Pseudomonal infections. Hence, the present study was conducted to investigate the effect of *Ocimum gratissimum* leaf on growth and pyocyanin production among clinical strains of *Pseudomonas aeruginosa* isolated in Lagos, Nigeria.

MATERIALS AND METHODS

Ocimum gratissimum

Fresh leaves of *Ocimum gratissimum* (*Labiatae*) attached to their stems with leaves were authenticated at the Forestry Research Institute (FRIN), Ibadan, Nigeria. After authentication, the specimen was given a voucher number FHI 106506 and deposited in the forestry herbarium.

Essential leaf oil extraction

Three hundred grammes of *Ocimum gratissimum* leaves cut into pieces were steam distillated with the aid of a distillation apparatus (Borhringer, Germany). The resulting distillate was fractionated with petroleum ether (40–60°C) and extracted oil was dried on anhydrous sodium sulphate (Sigma, USA). Petroleum ether was removed by evaporation in vacuo at 50°C using a rotary evaporator. The amount of oil obtained as a percentage the quantity of leaf distillated was determined and recorded. The oil extract was reconstituted by dissolution in 10ml of 2% Tween-80 and filtered sterilized by passage through a 0.45µm filtration apparatus (21).

Bacterial isolates

Pure stock cultures of 46 clinical isolates of *Pseudomonas aeruginosa* maintained on nutrient agar slants within 4 weeks of isolation at 4°C and obtained from the Genetics units of the Nigerian Institute for Medical Research (NIMR), Lagos were used in this study. These isolates were identified and purified by culturing samples (blood, urine, stool, wound exudates and ear discharge) first on MacConkey and then on *Pseudomonas* agar medium supplemented with cetrimide (200 µg/ml) and sodium nalidixate (15 µg/ml). *Escherichia coli* ATCC 25922 was employed to validate outcomes of antibacterial susceptibility testing. All the bacterial strains were obtained from the Microbiology and Genetic division of the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria.

Antibacterial susceptibility testing

The sensitivity of the selected *Pseudomonas aeruginosa* strains to *Ocimum gratissimum* essential oil (EO) was determined by agar-well diffusion method. Cultures were grown in nutrient broth to late exponential phase (12 – 18 hours). The resulting turbidity was adjusted to 0.5 McFarland standard (1 x 10^8CFU/ml) using phosphate buffered saline (pH 7.4). 10 µL of standardized inoculum (10^5CFU/plate) was then used to streak Mueller Hinton agar (20 ml). Four 6 mm holes placed 4 cm apart were immediately bored on each inoculated plate using heat sterilized capillary tubes. After air-drying, the holes were seeded with

100 µL each of EO at 5000 µg/hole and 2% Tween-80 diluent for test and control. The plates were further mounted with a standard antibiotic tobramycin disk (20 µg) from Oxoid, UK, to serve as a negative control. Control plates inoculated with *Escherichia coli* ATCC 25922 were also prepared in parallel with the test experiment to validate sensitivity. All the plates were incubated aerobically at 37°C for 24 hours. Zones of inhibition were measured in millimeters (mm) and recorded.

Minimum inhibitory concentration (MIC)

The inhibitory effect of EO on each of the 46 *Pseudomonas aeruginosa* isolates was determined based on dilution in broth according to Ilori *et al* (17) with a little modification. A concentration range of 0 – 72 mg/ml of EO was prepared using Mueller Hinton broth as the diluent. The susceptibility test broth tubes were then made up to 4 ml by adding 100 µL of culture (1×10^6 CFU/ml) of the tested strains. EO negative tubes and EO tubes inoculated with *Escherichia coli* ATCC 25922 served as controls. The tubes were plugged with sterile cotton wool and incubated aerobically at 37°C for 24 hours. The MICs were regarded as the lowest concentration of EO at which no visible growth or turbidity occurred. Turbidity of cultures was monitored spectrophotometrically at 620 nm against sterile nutrient broth.

Minimum bactericidal concentration (MBC)

Here, 10µL sample taken from MIC tubes without bacterial growth or turbidity was inoculated onto Mueller Hinton agar and plates were subsequently incubated accordingly for 24 hour. MBCs were defined as the lowest concentration of EO that yielded no colonies of strains tested after incubation.

O.gratissimum effect on pyocyanin production

Each *Pseudomonas aeruginosa* strain was grown under iron limiting condition in tris-minimal succinate medium containing 25 µM ethylenediaminedi-o-hydroxyphenylacetic acid (EDDA), with and without 20 or 72 mg/ml EO. Culture negative broths were used as control. After 24 hours incubation at 37°C, pyocyanin production was estimated as described by Rogers (22). In brief, pyocyanin producing strains were grown aerobically overnight at 37°C with shaking (120 rpm) in tris-minimal succinate solution without iron and glucose but containing $MgCl_2$ (500µM), $CaCl_2$ (100µM) and methionine (700 µM). Cultures were then centrifuged at 4000 rpm for 10 minutes and supernatant acidified with ethyl acetate in volume 5:2 ratio. The acidified pyocin fraction was then concentrated under reduced pressure using a rotary evaporator at 50°C. The crude pyocin preparation obtained was then dissolved in 400µL sterile water, sterilized by passage through a 0.45 µM filtration unit and the yield measured in µg/ml. A non-pyocin strain of *Pseudomonas putida* PUC 34 was used as control.

Statistical analysis

Pyocyanin production among the *Pseudomonas aeruginosa* strains tested measured in µg/ml was expressed as means ± standard deviation. Differences between mean values were further subjected to student's t-test with level of significance investigated at 99% confidence limits. $P < 0.01$ was considered significant.

RESULTS

The data obtained following the extraction of essential oil (EO) from *Ocimum gratissimum* leaves and effects on growth of 46 clinical isolates of *Pseudomonas aeruginosa* are summarized. Table 1 revealed the production of 720 mg of essential oil equivalent to 0.24% oil yield from 300g of *Ocimum gratissimum* leaves following steam distillation with petroleum ether. Forty of the 46 tested *Pseudomonas aeruginosa* strains showed susceptibility to the essential oil and produced an inhibition zone (18 – 34 mm) that was comparable to the bactericidal effects of tobramycin (20 – 32 mm). All the tested strains formed colonies resulting in the absence of inhibition zones when grown on 2% Tween-80 agar (plates not shown). Results in table 2 indicated that *Ocimum gratissimum* EO at 36 – 54 mg/ml and 42 – 66 mg/ml concentrations inhibited the growth of 36 of the 40 sensitive strains in broth and on agar *in vitro*. Minimum inhibitory concentrations (MICs) of the remaining 6 sensitive strains were observed at ≥ 72 mg/ml EO. MIC of 12 mg/ml and MBC of 24 mg/ml EO were obtained following interactions with the control strain, *Escherichia.coli* ATCC 25922.

A decrease in pyocyanin production was also observed in the order of 259 ± 86.7, 30.1 ± 10.2 and 2.5 ± 1.5 µg/ml as a result of the growth of the *Pseudomonas aeruginosa* sensitive strains in media containing tris succinate and EDDA, 15mg/ml EO and EDDA and 75mg/ml EO and EDDA. The disparity in pyocin yield as a result of growth in the presence of EO was found to be significant ($p < 0.01$) (Table 3).

The data presented in Table 4 also showed a significant decrease ($p < 0.01$) in pyocyanin production (276.7 ± 23.1 vs 3.5 – 30.5 ± (1.0 – 2.3) µg/ml) among the EO resistant strains when cultured in the three categories of media used.

Table 1: Essential oil yield and antibacterial susceptibility testing of *Pseudomonas aeruginosa* strains with *Ocimum gratissimum* essential oil (EO) by agar well diffusion method

	O. gratissimum EO	Tobramycin	Tween-80
Organism tested			
P. aeruginosa (N = 46).	18 – 34[a]	20 – 32[b]	0*
E. coli (ATCC2592) (N = 4).	22 – 30[b]	20 – 35[b]	0*

- Essential oil yield = 720mg per 300g leaf.
- Percentage oil yield = 0.24%

N = number of strains tested or determinations; a = 40 *P. aeruginosa* strains tested were susceptible to EO; b = all the strains tested were susceptible to EO or tobramycin. Figures were a range of inhibition zone diameters measured in millimeters (mm). * = All the tested strains formed colonies on 2% Tween-80 control agar plates.

Table 2: Determination of MICs and MBCs of *Ocimum gratissimum* EO on the susceptible *Pseudomonas aeruginosa* strains

	Pseudomonas aeruginosa		*Escherichia coli* ATCC25922
	N = 34	N = 6	
Parameters			
MIC (mg/ml)	36 – 54	≥ 72	12
MBC (mg/ml)	42 – 66	ND	24

ND = growth inhibition not detected within the EO concentration tested.
E. coli ATCC 25922 inoculations was done in quadruplicate.

Table 3: Effects of *Ocimum gratissimum* essential oil on pyocyanin production in susceptible *Pseudomonas aeruginosa* strains

Tests	Pyocyanin production (µg/ml ± SD)
	N = 40
15mg/ml EO + EDDA	30.1 ± 10.2@
72mg/ml + EDDA	2.5 ± 1.5@
Tris-succinate + EDDA	259.2 ± 86.7

N = number of strains tested; @ = Significantly reduced pyocyanin yield at 99% confidence limits

Table 4: Effects of *Ocimum gratissimum* essential oil on pyocyanin production in resistant *Pseudomonas aeruginosa strains*

Test	Pyocyanin production (µg/ml ± SD)
	N = 6
15mg/ml EO + EDDA	50.5 ± 2.3@
72mg/ml + EDDA	3.5 ± 1.0@
Tris-succinate + EDDA	276.7 ± 23.1

N = number of strains tested; @ = Significantly reduced pyocyanin yield at 99% confidence limits

DISCUSSION

Ocimum gratissimum has been credited to have tremendous clinical benefits in folk medicine prior to the exploration of its chemotypic potentials and the propagation of modern medicine (16). The present study has an intention to expand the scientific basis of *Ocimum gratissimum* essential oil as an antibacterial agent. The 0.24% oil obtained in this study is comparable with yields reported in previous works (21, 23). The essential oil was further found to inhibit the growth of 40 of 46 tested strains on agar medium with 18 – 34 mm zone of inhibition. The MIC and MBCs values of 36 – 54 mg/ml and 42 – 66 mg/ml observed among the susceptible strains are contrary to the report of Nakamura *et al* (21). These workers found the eugenol containing EO un-inhibitory to *Pseudomonas aeruginosa* at 24 mg/ml. Other causes of disparity may include the chemotype variation common to basil plants including *Ocimum gratissimum* (24), experimental design and the strains of *Pseudomonas aeruginosa* tested. The latter could result from clonal variation in the acquisition of R-plasmids and other virulence determinants as demonstrated in the work of Mucha and Farrand (25). However, our result is comparable to the efficacy reported for the essential oil of *Cinnomonium osmophloeum* (26). It also aligns with the microbiological safety

appraisals given to the plant essential oils of Australian origin (27, 28).

Furthermore, in support of our result is the finding of Orafidiya *et al* (29) in which liquid and semi solid formulations of *Ocimum gratissimum* EO were found efficacious as a topical agent in the treatment of boil, pimples and wounds. Strains of *Pseudomonas aeruginosa* have been found responsible for foot puncture wound infections in children (30) and many studies have implicated *Pseudomonas aeruginosa* as among the aetiologic agents of skin infections (31). Keita *et al* (32) also reported concentrations of *Ocimum gratissimum* EO greater than 60 mg/ml in the control of *Callosobruchus maculatus* while in another study, concentrations as high as 62 mg/ml were found inhibitory against enteric bacteria (17).

The reduction in pyocyanin production among the strains tested irrespective of the susceptibility outcomes further demonstrates the spectrum of activity inherent in *Ocimum gratissimum* EO. Pyocyanins confer virulence to pseudomonas in a manner that employs cell-to-cell signaling system through which cell multiplication and further production of virulence determinants are ensured (33). Hence, inhibition of pyocyanin production may prevent the development of these processes. This observation further mimics the finding of Tateda *et al* (34) in which sub inhibitory concentrations of macrolides inhibit protein synthesis and suppress virulence factors in *Pseudomonas aeruginosa.*

The realization of *Ocimum gratissimum* essential oil as an antipseudomonal agent will be highly rewarding especially in rural areas that are far from adequate health facilities. The oil can easily be applied as drops and topical agent in the management of pseudomonas associated ear, eye and wound infections in these circumstances.

ACKNOWLEDGEMENT

The authors wish to thank the entire staff of the Genetics Laboratory of the Nigerian Institute for Medical Research (NIMR) and Mr. Adeleke from Pharmacognosy Department, College of Medicine University of Lagos for their technical assistance.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2004002/2437

BACTERIOLOGY AND ANTIMICROBIAL SUCEPTIBILITY PROFILE OF AGENTS OF OROFACIAL INFECTIONS IN NIGERIANS

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A prospective study to determine the pattern of microorganisms seen in orofacial infections as well as investigating the antimicrobial susceptibility profile of the isolates was undertaken. Specimens were obtained aseptically from 25 patients presenting with orofacial infections at the Department of Oral Surgery and Pathology, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. The specimens were transported in an anaerobically pre-reduced transport medium for processing in the laboratory. Isolation and identification were done employing standard bacteriological techniques. Antimicrobial susceptibility testing was performed by the disc diffusion method. All the 25 clinical samples obtained yielded growth of bacteria. Anaerobes were cultured from 24 (96%) specimens while 1 specimen yielded only aerobic isolates. Altogether, 44 bacterial isolates were obtained and 40 (91%) were anaerobes. Most of these anaerobes were Gram-negative rods and Gram-positive cocci. About 75-100% of the anaerobes were susceptible to commonly available antibiotics. Strikingly, sulphonamides demonstrated the weakest *in-vitro* activity against all isolates. The study revealed again the polymicrobial nature of orofacial infections as well as the predominance of anaerobes in the aetiology of these infections. Erythromycin and penicillin should be considered as frontline drugs in the treatment of mild orofacial infections while drugs like ciprofloxacin and clindamycin can be reserved for more severe and resistant infections.

INTRODUCTION

Bacterial infections are among the most commonly encountered problems in the maxillofacial surgical practice and previous reports from Nigeria showed that orofacial infections remain a major problem. This problem persists in spite of the availability of broad spectrum of potentially useful antibiotics (1-6).

The microbiology of orofacial infections has been studied widely and various forms of aerobic and anaerobic microorganisms reflective of normal oral flora have been isolated. *Streptococcus* and *Staphylococcus* species as well as the Gram-negative anaerobic bacilli namely *Prevotella, Porphyromonas, Fusobacteria* species and anaerobic cocci are among the prevalent organisms isolated in most studies (7, 8).

Treatment of acute orofacial infections would require the use of empiric antibiotic prescriptions. A clinician can rely on the knowledge of the likely microorganisms that may cause an infection in a particular site of the body and the nature of the antibiotic susceptibility pattern in the local environment of his practice as a guide to the rational choice of antibiotic therapy. In severe or recalcitrant forms of orofacial infections like necrotizing fasciitis, deep space infections and chronic osteomyelitis, cultural studies involving both aerobic and anaerobic bacteriology are however desirable to provide information on likely pathogenic organisms causing the disease and their antibiotic susceptibility pattern. This piece of information would guide the clinician to select the most appropriate antibiotics available for the treatment of these infections.

Anaerobic bacteriology unfortunately is expensive and requires special facilities and expertise to perform. It is not readily available in many hospitals in

the developing countries even in the referral centers. In spite of the high prevalence of orofacial infections in Nigeria, only one report (7) incorporated anaerobic bacteriology in the study of dentoalveolar abscess. There is therefore a paucity of information about the identities of the anaerobic organisms associated with orofacial infections in this environment. The present study examined the microbiology of different types of orofacial infections with the aim of providing information on the prevalent microorganisms isolated in these diseases. Antibiotic sensitivity patterns of these organisms were determined in order to provide a guide to clinicians for making rational decisions over the choice of antibiotics in the management of these infections.

PATIENTS AND METHODS

A prospective study of 25 patients aged 17-65 years (17 males and 8 females) with various forms of orofacial infections was carried out (19 odontogenic and 6 non-odontogenic infection). Table 1 gives a breakdown of the types and sources of these infections; chronic suppurative osteomyelitis (5), acute dentoalveolar abscess (5), buccal space abscess (4), lateral pharyngeal abscess (1), submandibular space abscess (3), submandibular space cellulitis (1) and chronic suppurative maxillary sinusitis (3). All the patients were seen at the Department of Oral Surgery and Pathology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. Specimens for bacteriological investigation were obtained aseptically through intact mucosa or skin. Abscesses were either aspirated with sterile syringes or swabbed during incision and drainage while bone or granulation tissues were surgically obtained through an intraoral incision in patients with chronic osteomyelitis. Prior to these procedures, the skin or mucosa was cleaned with 70% alcohol and isolated with sterile gauze. Specimens were collected into an anaerobically pre-reduced transport medium (Bionor, Norway) and sent to the laboratory immediately.

Isolation and identification of organism

Specimens were collected into transport media (Bionor, Norway) and stored at 4°C and processed within 2 hours. Specimens were cultured on Nutrient agar (Oxoid, England) containing 6% whole blood incubated aerobically at 37°C, Cooked meat broth (Oxoid, England), Nutrient agar containing 6% lysed human time-expired blood and 0.5 mg/ml Vitamin K (Roche, Nigeria) and fastidious anaerobe agar (Techlab, USA), prepared according to the manufacturer's instructions and incubated anaerobically. Anaerobic incubation took place at 37°C in anaerobic jars in an atmosphere of 1% O_2/8%CO_2 generated using commercial gas-generating kits (BBL, Cockleysville, USA) in accordance with manufacturers' instructions. Plates were incubated at 37°C for 48-72 hours (for aerobic cultures) and 3-7 days (for anaerobic cultures). Colonies appearing on either plate were streaked unto fresh plates and incubated for 48 hours to 2 weeks. Gram-negative rods were identified using the API 20 E system (Biomerieux, France). All other isolates were identified by conventional biochemical tests (9). Isolates were maintained by cryopreservation using the medium of Gibson and Khoury (10).

Antibiotic sensitivity testing

Antibiotic sensitivity testing was conducted by the disc diffusion method (11). The test medium was Iso-sensitest agar (Oxoid, England) supplemented with whole blood for streptococci and lysed blood with vitamin K for anaerobes. Commercially available antibiotic disks were used and interpretation of inhibition zone was in accordance with manufactures instructions (AB Biodisk, Sweden). *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* NCTC 10418 were used as controls.

RESULTS

All the 25 clinical samples obtained yielded growth of bacteria. Forty-four bacterial isolates were obtained. Anaerobes were cultured from 24 (96%) specimens and this accounted for 40 (91%) of the number of organisms isolated. Mixed anaerobic/aerobic growth was obtained from 2 (8%) specimens while anaerobes were exclusively cultured from 22 (88%) specimens. One case of submandibular space cellulitis yielded aerobe only (Table 1). Gram-negative anaerobic cocci were the commonest bacteria isolated, predominantly *Prevotella melaninogenicus* (14), *Porphyromonas gingivalis* (8), *Prevotella denticola* (5), and *Peptostreptococcus spp* (6). While *Streptococcus spp* (3) and *Staphylococcus aureus* (1) were the aerobic species isolated.

Table 2 shows the antibiotic profile of the anaerobic and streptococcal isolates. Majority of these organisms were susceptible (75-100%) to the commonly available antibiotics, trimethoprim, chloramphenicol, tetracycline and erythromycin. Clindamycin and ciprofloxacin also displayed excellent *in-vitro* activity against the anaerobic isolates. The least susceptibility to penicillin V was observed in *Peptostreptococcus anaerobius* (33.3%) otherwise, this drug displayed good *in-vitro* activity against the anaerobic bacteria (75-100%).

Streptococcus species were completely susceptible (100%) to trimethoprim, ciprofloxacin, chloramphenicol and erythromycin. The sulphonamides demonstrated the weakest *in-vitro* activity against the aerobic and anaerobic bacterial isolated in this study. Susceptibility testing for *Staphylococcus aureus* was not done.

Table 1: Classification of orofacial infection and the bacterial isolates

Type/source of infection	No of cases	Anaerobic organism	No of isolate	Aerobic organism	No of isolate
Chronic suppurative osteomyelitis (Chronic periodontitis)	5	*P. melaninogenica*	1		
		P. denticola	2		
		P. gingivalis	2		
		P. anaerobius	1		
		P. prevotii	1		
Buccal space abscess (Pulpitis)	4	*P. melaninogenica*	2		
		P. denticola	1		
		P. gingivalis	1		
		P. anaerobius	2		
		P. magnus	1		
		P. productus	1		
		F. nucleatum	1		
		P. intermedia	1		
Lateral pharyngeal space abscess (Pulpitis)	1	*P. melaninogenica*	1	*Strept. spp*	1
		A. viscosus	1		
Acute dentoalveolar abscess (Pulpitis)	5	*P. endodontalis*	1		
		P. melaninogenica	4		
		P. gingivalis	1		
		P. intermedia	1		
		P. Productus	1		
Canine fossa abscess (Pulpitis)	4	*P. gingivalis*	3		
		P. melaninogenica	1		
		P. denticola	1		
		P. productus	1		
Submandibular space abscess (unknown)	3	*P. melaninogenica*		*Strept. spp*	1
		P. denticola			
		P. Gingivalis			
Submandibular space cellulites (unknown)	1			*Staph. aureus*	1
				Strept. spp	1
Chronic suppurative maxillary sinusitis (unknown)	2	*P. gingivalis*	1		
		P. melaninogenica	2		

P = Prevotella, Porphyromonas, Peptostreptococcus, F = Fusobacteria, A = Actinomyces
Staph = Staphylococcus, Strept = Streptococcus

Table 2: Antibiotic profile of anaerobic/streptococcal isolates

Organism/Antibiotic	Trimeth (%)	Sulph (%)	Pen V (%)	Cipro (%)	Tet (%)	Chl (%)	Ery (%)	Clind (%)
P. melaninogenica (13)	10 (76.9)	3 (23.1)	10 (76.9)	13 (100)	12 (92.3)	13 (100)	12 (92.3)	13 (100)
P. gingivalis (8)	6 (75)	0 (0)	6 (75)	7 (87.5)	7 (87.5)	7 (87.5)	7 (87.5)	8 (100)
P. intermedia (2)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
P. denticola (4)	3 (75)	4 (100)	3 (75)	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)
P. endodontalis (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
F. nucleatum (2)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	1 (100)	1 (50)	2 (100)
P. anaerobius (3)	3 (100)	0 (0)	1 (33.3)	3 (100)	3 (100)	2 (100)	3 (100)	3 (100)
P. prevotii (1)	1 (100)	0 (o)	1 (100)	1 (100)	1 (100)	3 (100)	1 (100)	1 (100)
P. productus (2)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	1 (100)	2 (100)	2 (100)
P. magnus (1)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)
A. viscosus (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-
Streptococcus spp. (3)	3 (100)	1 (33.3)	2 (66.7)	3 (100)	2 (66.7)	3 (100)	3 (100)	2 (66.7)

Trimeth = Trimethoprim, Sulp = Sulphonamide, Pen V = Penicillin V, Cipro = Ciprofloxacin, Tet = Tetracycline, Chl = Chloramphenicol, Ery = Erythromycin, Clind = Clindamicin

DISCUSSION

The result of this study demonstrates again the polymicrobial nature of orofacial infections as well as the predominance of anaerobic bacteria in the pathogenesis of these infections. In the present study, the Gram-negative rods and the anaerobic cocci were the commonest anaerobic bacteria isolated. They are *Prevotella, Porphyromonas, Fusobacterium* and *Peptostreptococcus* species. This observation is consistent with result from most studies on orofacial infections (7, 12, 13, 14). *Streptococcus* species are common oral commensals and are frequently isolated in odontogenic infections (7, 14). These organisms were isolated mainly from the odontogenic infections in the present study. *Staphylococcus aureus* found in the nares, perineum and skin. It is not a normal flora of the oral cavity but may be an important pathogenic organism in suppurative non-odontogenic infections of the head and neck region (8). The only *Staphylococcus aureus* isolated in this study was obtained from a case of non-odontogenic submandibular cellulitis.

Resistance to older cheaper antibiotics is becoming increasingly common in Nigeria. In this study, however, it was observed that most of commonly available antibiotics, erythromycin, penicillin V, tetracycline, trimethoprim and chloramphenicol demonstrated very good *in-vitro* activities against most of the anaerobic bacteria. Erythromycin and ciprofloxacin also displayed excellent *in-vitro* activities against the streptococcal isolates. Erythromycin and penicillin V are not only very effective within the environment of the study, but are both cheap and readily available. They should therefore be considered as frontline drugs in the treatment of mild forms of orofacial infections in Nigeria. This will permit the conservation of ciprofloxacin and clindamycin for the management of more complex forms of orofacial infections and other resistant infections. It is important to realize that the successful management of orofacial infections depends on the removal

of sources of infection, establishment of prompt and adequate surgical drainage and the institution of appropriate antibiotic therapy. Antibiotic therapy alone is not a substitute for surgery.

ACKNOWLEDGEMENT

This work was funded by grants from the International Program in the Chemical Sciences (NIGOI) and Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife. We thank Techlab, USA for the gift of Fastidious Anaerobe agar.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003042/2438

DETECTION OF HAEMAGGLUTINATION–INHIBITION ANTIBODIES AGAINST HUMAN H_1N_1 STRAINS OF INFLUENZA A VIRUSES IN SWINE IN IBADAN, NIGERIA

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A survey of haemagglutination inhibition (HI) antibodies against influenza A virus was carried out on pigs sera collected at Bodija abattoir, Ibadan between December, 2001 and August 2002. Out of the 107 sera tested, 101 (94.39%) had HI antibodies to influenza A (H_1N_1) human strain while the remaining 6 (5.61%) were negative. The result of this work indicates that H_1N_1 influenza viruses are currently circulating among the pigs slaughtered in Bodija abattoir. The public health implications in terms of possible transmission contact with the pigs are discussed. The result of the HA titres obtained with turkey red blood cells (RBC) compared to that of guinea pig RBC indicated that the H_1N_1 stains of influenza A viruses have greater avidity for turkey RBC than those of guinea pigs. This is equally of diagnostic importance

INTRODUCTION

Influenza is an acute, highly contagious and febrile respiratory disease associable with the destruction of cell lining the upper respiratory tract, trachea and bronchi. (1, 2). Influenza viruses belong to the family orthomyxoviridae and are classified as A, B, or C based on the antigenic differences in their nucleoprotein (NP) and matrix (M) protein. These three types have been shown to cause true influenza in man (3).

Influenza A viruses are widespread in nature infecting a wide range of host species including man, horses, swine, birds and mammals (4). According to Murphy and Webster (5), Influenza B and C viruses infect only human hosts. However, Adeniji *et al* (6) reported the detection of haemagglutination inhibition (HI) antibodies to B/Victoria/2/87 strain of influenza in pigs in Nigeria. This study was carried out to detect the presence of HI antibodies against human strain of H_1N_1 influenza virus in pigs' sera and to establish the zoonotic nature of the infection between humans and swine.

MATERIALS AND METHODS

One hundred and seven blood samples were collected from slaughtered pigs at the Bodija Municipal Abattoir, Ibadan between December 2001 and August 2002. The sera from these samples were stored at -20°C until tested. The virus antigen influenza A (H_1N_1) strain and its positive control antiserum were collected from the Department of Virology, College of Medicine, University of Ibadan. The haemagglutination antibody (HA) test was carried out using the standard method as previously described (6). The use of 1% turkeys' red blood cells (RBC) and 1% guinea pig RBC as indicator for the test was also compared. Haemagglutination–inhibition (HI) test was then carried out using the positive control antiserum and the test serum samples.

RESULTS

Out of the 107 sera samples tested, a total of 101 (94.39%) had HI antibodies to the influenza H_1N_1 strain. The HI titre values obtained ranged between 4 and 4096. The HI titre value of 63 (58.88%) of the tested samples ranged from 4 – 32, 37 (34.58%)

ranged from 64 – 256 while the remaining 7 (6.54%) ranged from 512 – 4096. Four HA units with 1% turkey RBC was 1024 while 4 HA units with 1% guinea pig RBC was 256. The presence of HI antibodies in the tested samples however indicated current circulation of the H_1N_1 viruses in the pigs in Ibadan.

DISCUSSION

Surveillance can assist in identifying the transmission pathways of influenza viruses through sero-surveys and it is an important component of influenza monitoring activities. One hundred and one (94.39%) of the 107 sera tested were positive for HI antibodies. The result indicates that there is a continuous and high activity of H_1N_1 strain of influenza A virus among pigs in Ibadan. This implies a prevalence of influenza A virus infection among the pig population in Ibadan. Since there was no record that these pigs were vaccinated, the antibody response could only have been due to natural exposure to a wild strain of the virus.

The result of this study conform to the work of Easterday (1) who found that influenza A viruses infect pigs, horses, seals, birds and humans. It also corroborates the findings of other workers who reported high prevalence of antibodies to A/Mississippi/1/85 (H_3N_2), A/Victoria/3/75 (H_3N_2), A/Chile/83 (H_1N_1) strains of influenza A viruses in horses and pigs (7). The HA result showed a very high titre of the virus antigen, indicating a high potency of the virus antigen with high avidity for turkey RBC compared to guinea pig RBC. This is of diagnostic importance particularly in influenza outbreaks involving human and swine host species. The result of this work also has implication for possible transmission to humans who are in close contact with the pigs. This is of significant public health importance as pigs and chickens have been hitherto incriminated in playing some major roles in the epidemiology of influenza viruses in Nigeria (8). Detection of HI antibodies against human H_1N_1 strain of influenza A viruses in swine indicates the presence of the virus in Ibadan. In order to confirm this and to establish other types or subtypes present in Ibadan and other parts of the country, further work needs to be carried out especially in the isolation and characterization of the virus. Vaccination of the pigs is also recommended due to the zoonotic nature of the infection.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2004 ISSN 1595-689X VOL 5 NO 3
AJCEM/2003041/2439

SEMINAL FLUID ANALYSIS AND BIOPHYSICAL PROFILE: FINDINGS AND RELEVANCE IN INFERTILE MALES IN ILORIN, NIGERIA

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To determine if there was a bearing of body mass index (BMI) on male infertility, a cross-sectional study of males of infertile couples, attending our infertility clinic was carried out. Apart from BMI determination, the semen of these men were analyzed to ascertain their spermogram. Out of 47 men involved in the study, 66% (31) were below 40 years of age. Seventeen (36.2%) of these were between 30-39 years. About 49% of the study subjects had oligospermia, while 23.4% were azoospermic. Those subjects between 30-39 years had the worse spermogram. Thirty-two (68.1%) and 34(72.3%) of the whole population had good spermatozoa motility and morphology respectively. Poor BMI, whether low or elevated, affected the semen quality. In conclusion, infertile males should be encouraged to seek help early. Attending clinician should pay attention to their past or present genital infections and the biophysical parameters.

INTRODUCTION

Despite the assumption that Africans are characterized by high fertility, the magnitude of infertility and reproductive failure in Africa, continuously receives the attention of many scientists (1). This is so because most traditional African cultures place high social premium on fertility, hence childlessness is a major personal tragedy and humiliation for the individual concerned (2). Women are usually made to bear such personal tragedy as they are commonly, though wrongly, blamed for infertility. This is in spite of the fact that male factor, has been shown to be responsible for up to 50% of cases of infertility (3). In Nigeria, the prevalence of male infertility has been suggested to be on the increase (4, 5). At the same time, obesity said to be on the increase in developing countries (6), is associated with increased lipoperoxidation (7) and decreased sperm quality (8).

The aim of this study was to evaluate the seminal fluid and body mass indices of infertile men in our centre. This was to ascertain if there was any relationship between them vis-à-vis fecundity.

MATERIALS AND METHODS

This cross-sectional study was carried out between December 2002 and May 2003, at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The subjects were the husbands of women undergoing investigation for infertility at the infertility clinic of the hospital. These men were adequately briefed about the study and their consents obtained. The semens of the subjects were collected after three to five days abstinence from sexual intercourse. We particularly advised semen collection after masturbation, within the hospital where possible. For those who opted to collect semen at home, they were told to ensure that the sample gets to the hospital within one hour. As much as possible, coitus interruptus was discouraged.

Samples were collected into clean wide-bore container, devoid of any chemical remnant or detergent. Semen analyses were done immediately on receipt of samples by the same person, based on the commonly

used criteria in our environment (9). The weight and height of each subject was measured and recorded, from which the body mass index (BMI) was determined (10).

RESULT

Forty-seven subjects were recruited into the study. The mean age of the subjects was 36.2 years and ranging from 24 - 55 years. Sixty-six percent of the subjects were below 40 years of age, 10.6% were above 50 years and 23.4% were between 40-49 years. Seventeen (36.2%) of those less than 40 years, were aged 30-39 years (Table 1).

Seventy two percent of the study population had abnormal spermogram. Those with oligospermia constituted 48.9% while the azoospermics were 23.4% (Table 2). With regard to motility, 32 (68.1%) subjects had more than 60% actively motile spermatozoa while 31.9% had less than 60% motility. Also, 34 (72.3%) subjects had more than 60% normal spermatozoa morphology and 13 (27.7%) had less than 60% normal morphology.

The subjects who were in the age group 20-29 years had the highest mean spermatozoa count of 37.23 million/ml. The least count of 11.8 million/ml was recorded in the age group 30-39 years. Similarly, the highest mean motility (63.93%) was noticed in the 20-29 years age group, while the least motility of 49.38% was seen in the 30-39 years age group (Table 3).

Poor BMI, whether low or elevated, was associated with abnormal sperm count and motility. Those with normal BMI (20-24 kg/m^2) had the highest mean sperm count of 39.35 million/ml and the highest mean sperm motility was observed also in this 20-24kg/m^2 BMI group (Table 4).

Table 1: Age distribution of infertile males in Ilorin

Age group (Years)	Frequency	(%)
20-29	14	29.8
30-39	17	36.2
40-49	11	23.4
50-59	5	10.6

Table 2: Percentage distribution of sperm count among infertile males in Ilorin

Sperm count (millions/ml)	Frequency	Percentage (%)
0	11	23.4
<20	23	48.9
20-39	6	12.8
40-59	3	6.4
60-79	2	4.3
80	2	4.3

Table 3: Age frequency and the corresponding distribution of sperm count sperm motility and BMI, in the different age group

Age group (Years)	Frequency (%)	Mean count (million/ml)	SD	Mean motility (%)	SD	BMI (kg/m²)	SD
20-29	14(29.8)	37.23	87.70	63.93	35.42	23.26	4.94
30-39	17(36.2)	11.18	16.92	49.38	42.30	25.72	5.12
40-49	11(23.4)	23.37	25.41	65.46	33.50	24.11	3.06
50-59	5(10.6)	12.87	16.47	59.00	38.31	23.16	4.69
			P=0.4637		**P=0.5648**		**P=0.4615**

Table4: Effects of BMI on sperm counts and motility

BMI (Kg/m²)	Mean count (millions/ml)	SD	% mean motility	SD
15-19	4.5	5.55	27.50	42.63
20-24	39.35	73.85	74.47	27.23
25-29	14.27	23.35	54.41	38.12
30-34	3.00	4.24	42.50	60.10
> 35	0.175	0.141	55.00	42.43
		P=0.0932		**P=0.0756**

DISCUSSION

Out of over ninety women seen in the clinic, and whose husbands were invited, only forty-seven showed up and were finally recruited for the study. Even though men contribute between 30-50% of the causes of infertility (3, 11), the society is yet to fully appreciate this. The community need to be properly educated on this, as the longer the couples remain infertile, the worse their chance for an effective treatment (12). Particularly, this delay in seeking help is not good for men. It has been shown that semen qualities deteriorate by as much as 3% per year (13). This is significant given that most of the men in our study were between 30-39 years of age. The impression thereof is that our men marry lately or seek help lately in the hospital. It is possible that these factors add up to contribute to the high percentage (72%) of abnormal spermogram among our subjects. Earlier on, a 40-50% prevalence of abnormal spermogram was reported in males of infertile union in Nigeria (14).

This study showed that 23.4% of our subjects were azoospermic, as against 35% seen in a more specific male infertility clinic in Ibadan, Nigeria (15). The prevalence of azoospermia in our study and that of Ibadan is worrisome. Two important causes of azoospermia are failure of spermatogenesis and bilateral ductal obstruction (16). Ojengbede *et al* (17) are of the opinion that rather than bilateral ductal obstruction, azoospermia in Nigeria is more likely to be due to failure of spermatogenesis. This is because most of the azoospermics in their study (17) had prior sexually transmitted diseases which have been linked to seminiferous tubular damage and infertility (16, 18).

In our study population, asthenozoospermia and teratozoospermia were not serious problems. About 70% of the determined spermogram had good motility

and morphology. This is good, because even those with oligospermia have a good chance of achieving pregnancy, especially in absence of sub-fertility of the partner (9, 11). The subjects in the age bracket 30-39 years had the least sperm count and motility. One contributor to this scenario is thought to be infection as this has been linked to infertility (5, 16, 17, 18).

Added to the possibility of infection in our patients, BMI abnormalities also contribute significantly to the problem. Low sperm count and poor spermatozoa motility were associated with abnormal BMI in the study. Though not statistically significant, we observe that the highest mean BMI was recorded in the 30-39 years group, which recorded the worst spermogram. Elevated BMI is associated with increased lipoperoxidation and generation of reactive oxygen species-ROS (7). Studies have shown that 40-88% of infertile patients have high levels of ROS (19), and this can cause low sperm counts, defective sperm structure, reduced motility and ability of sperm to penetrate the oocyst or egg cell (20).

CONCLUSION

It is imperative from the foregoing that the infertile male should be investigated early and thoroughly evaluated like the females. The evaluation of the infertile male should be rapid, if possible, non-invasive and cost effective. It is essential that the right diagnosis is made on time, as this is a very important part of the management of male infertility (21). In our environment, attention should be paid to infection, whether residual or ongoing, and the biophysical parameters of concerned individual should be evaluated and managed as necessary.

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