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OSMOTIC FRAGILITY AND Na^+ -K^+ ATPase ACTIVITY OF ERYTHROCYTES OF HIV/AIDS PATIENTS

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A cross sectional study was carried out to investigate the osmotic fragility and Na^+-K^+ ATPase activity of the erythrocytes of HIV/AIDS patients. Whole blood was taken from subjects at the Human Virology Laboratory of the Nigerian Institute of Medical Research. Subjects were judged suitable for the various investigations by means of a questionnaire. The Genie II HIV diagnostic kit was used to confirm HIV positive status. HIV positive subjects were grouped into two: those receiving anti-retroviral therapy were referred to as the ARV group and those not receiving antiretroviral therapy were designated as non-ARV group. Each group was further sub-divided according to the Centers for Disease Control 1993 classification of HIV disease. HIV negative subjects must have tested no later than two months to the sample collection date and must not lead a high-risk lifestyle. Twenty microliters of whole blood were used for the erythrocytes osmotic fragility assay. One milliliter of whole blood was used to prepare the erythrocyte ghost membrane for the Na^+-K^+ ATPase activity assay. The two HIV positive groups showed significant increase in percentage haemolysis under osmotic stress at 0.65% saline. The ARV group had an average percentage haemolysis of 2.56 ± 0.81% while the non-ARV group had an average of 3.19 ± 1.11% compared to an average of 0.83 ± 0.36% for the control group ($p < 0.05$). A pattern observed in the result was an increase in activity with increasing severity of the HIV/AIDS disease. Data from the present study indicate that the osmotic fragility of erythrocytes was significantly potentiated, while Na^+-K^+ ATPase activity was not significantly altered ($p < 0.05$) in HIV/AIDS disease.

Key words: Osmotic fragility, Na^+ -K^+ ATPase activity, erythrocytes, HIV/AIDS

INTRODUCTION

Symptomatology of HIV/AIDS is very diverse. However, anaemia is one of the most universal clinical symptoms of the disease (1). The aetiology of anaemia in HIV disease has been extensively researched primarily from the physiological and pharmacological angles (2,3,4). Malnutrition in HIV/AIDS has also been widely reported (5,6). Biochemically, metallic cofactors and coenzymes obtained through the diet are critical to the proper functioning and integrity of the native conformations of biomolecules. Nutrient deficiencies can result in disruption of supramolecular structures like biomembranes. We therefore decided to investigate the osmotic fragility of erythrocytes of HIV/AIDS patients. However, since the Na^+-K^+ ATPase is the major transmembrane pump involved in regulating osmosis in the cell, we considered it pertinent to determine its activity in the erythrocytes of HIV/AIDS patients.

SUBJECTS AND METHODS

Study design

This was a cross sectional study involving 67 HIV seropositive subjects recruited and confirmed positive at the Nigerian Institute of Medical Research (NIMR), Human Virology laboratory in Lagos, Nigeria. Ten HIV seronegative subjects served as the control group. The subjects were representative of the geo-political, ethnic, economic, religious and education diversity of Nigeria. The purpose of the study was clearly explained to them. Consent was obtained and

counseling given before blood samples were taken. Participants who were on multivitamins or prolonged non-AIDS related treatment were excluded from the study. The age range was between 20 and 60 years. The HIV positive group was sub-divided into those who were on antiretroviral therapy (ARV) at the Institute and those who had not commenced any form of antiretroviral therapy (non-ARV).

The HIV disease-stage classification was according to the Centers for Disease Control revised 1993 classification for HIV infection among adolescents and adults (7). Subjects were deemed unsuitable for the study if they were on mineral supplements and/or cardiac glycosides. Some minerals have been reported to reverse osmotic fragility in erythrocytes and cardiac glycosides are specific inhibitors of Na^+-K^+ ATPase activity (8,9).

Blood collection

Blood samples were collected between 08:30h and 09:30h daily. Six milliliters of blood were collected by venous puncture into potassium EDTA bottles. Four ml were aliquoted for $CD4^+$ T-lymphocyte count within 6h of collection; 1 ml for erythrocyte ghost membrane preparation while the remaining 1 ml was used for osmotic fragility assay. All tests were conducted on the day of collection. Two ml of blood were taken from the control subject since $CD4^+$ counts were not conducted on them.

HIV confirmation

Subjects were screened for HIV status at various centers outside the Institute but confirmation was done at the Virology Laboratory with the Genie II HIV confirmation kit. $CD4^+$ counts were performed with the Dynabeads method.

Osmotic fragility assay

Osmotic fragility of the erythrocytes was determined by the method of March *et al* (10). Saline solution of concentrations 0.85%, 0.65%, 0.35%, and 0.10% were prepared. To each dilution series, 20 µL of freshly collected blood was added and all the tubes were shaken gently. They were incubated at 37°C for two hours after which they were centrifuged at 3,000 rpm in a refrigerated desk centrifuge and the absorbance of the supernatant measured at 583nm.The absorbance obtained at 0.85% was subtracted from absorbance at the other concentrations. The result at each concentration was then expressed as a percentage of the highest absorbance. This represents the degree of haemolysis.

Erythrocyte Ghost Membrane (EGM) Preparation

One ml of freshly collected whole blood was used for the EGM preparation. The whole blood was centrifuged at 3,000 rpm for 10 minutes. The plasma was removed to obtain the packed erythrocytes. The erythrocytes were washed twice in five times volume of isotonic buffer at 4,200 rpm for 20 minutes. The supernatant was decanted and the pelleted cells haemolysed in five times volume of hypotonic buffer and centrifuged at 4,200 rpm for 20 minutes. This was repeated four times and the supernatant decanted each time. The pink ghost was then washed five times in four times volume of washing buffer at 4 200 rpm for 20 minutes each. The supernatants were decanted. The entire washing process was done in Jouan CR3i refrigerated centrifuge at 4°C.

Determination of Na^+ -K^+ ATPase Activity

Na^+-K^+ ATPase activity was determined by a modification of the method of Bowler and Tirri (11). It was calculated as the difference between total ATPase activity and Quabain-inhibited activity. Total ATPase activity was assayed in an incubation medium consisting of 50mM Tris-HCl (pH 7.4), 120mM NaCl, 20mM KCl, 4mM $MgCl_2$, 240mM sucrose, 1mM EDTA and 3mM disodium ATP. 50 µL of EGM suspension were aliquoted into two tubes labeled 1 and 2. 100 µL of incubation medium were also added to each tube but 100 µL of 1mM Quabain solution was added to tube 2 only. The reaction mixtures were incubated at 37°C for 20 minutes. They were stopped by adding 100 µL of 1% SDS.

Assay for Inorganic Phosphate

Inorganic phosphate produced from the hydrolysis of ATP by ATPase was assayed by the method of Fiske-Subbarow (12). 2.5% ammonium molybdate in 0.9M H_2 SO4 was added to the EGM reaction mixture after it was stopped. To each tube was added 1 ml of ammonium molybdate and 9% ascorbate. The blue color that developed was read spectrophotometrically at 640nm.

Determination of Protein Concentration

The protein concentration of the EGM was determined according to Lowry *et al* (13), using bovine serum albumin as standard protein.

Statistical Analysis

Data obtained were analyzed by two-tailed Student's t-test (14). A p-value of < 0.05 was considered statistically significant. Calculations were done using Microsoft Excel 2000 statistical tools.

RESULTS

Sixty-seven HIV positive subjects were recruited for this study. During the process of HIV confirmatory tests, 2 subjects were found to be HIV negative and were screened out but one of them joined the control group. Also screened out were 9 subjects who were on cardiac glycosides (6 subjects) and mineral supplements (3 subjects). The remaining HIV positive subjects were grouped either as ARV (35 subjects) or non-ARV (21 subjects). At 0.65% hypotonic saline concentration, the erythrocytes of the non-ARV and ARV groups were found to have higher percentages of haemolysis than the control group (Table 1). Both groups showed statistically significant results ($p<0.05$).

The Na^+-K^+ ATPase activities for the non-ARV and ARV groups were higher than the activity of the control group (Table 2). The difference was however not statistically significant ($p<0.05$). On detailed investigation, the Na^+ -K^+ ATPase of non-ARV and ARV subjects with $CD4^+$ T-lymphocyte count of less than 200 cells/µL of blood showed higher activity than subjects with $CD4^+$ count of between 200-499 cells/µL (Table 3).

TABLE 1

Degree of Hemolysis of Erythrocytes of HIV-Negative (Control), Non-ARV, and ARV HIV/AIDS Patient in 0.65% Saline Solution[1]

Subject	Percent Hemolysis	n
Control	0.83±0.36	10
Non-ARV	3.19±1.11	21
ARV	2.56±0.81	35

[1]Value represent mean ± standard error of mean
Control = HIV negative subjects
Non-ARV = HIV positive patients not on antiretroviral therapy
ARV = HIV positive patients on antiretroviral therapy
n = number of subjects, $p<0.05$

TABLE 2

Na^+ - K^+ ATPase Activity of ARV, Non-ARV, and HIV Negative Subjects

Subject	Na^+ - K^+ ATPase Activity (nmol P_i/h/mg protein)	n
Control	2.22±0.81	10
Non-ARV	3.01±0.52	21
ARV	3.69±0.58	35

[1]Value represent mean ± standard error of mean
Control = HIV negative subjects
Non-ARV = HIV positive patients not on antiretroviral therapy
ARV = HIV positive patients on antiretroviral therapy
n = number of subjects, $p<0.05$

TABLE 3

Na^+ - K^+ ATPase Activity of ARV and ARV subjects based on $CD4^+$ Count Classification[1]

CD4+ Cell Count (cells/μl)	**Non-ARV**	**n**
<200	3.38±0.79	3.99±1.16
200 499	2.58±0.71	3.73±0.84
≥ 500	β	γ

[1]Value represent mean ± standard error of mean, expressed as nMol Pi/h/mg protein
α Three of ARV subjects did not have CD4+ count done
β There was no subject in this category
γ There was only one subject hence no statistical analysis could be done

DISCUSSION

The erythrocytes of the two groups of HIV positive subjects in this study showed significant susceptibility to osmotic stress. Those who had not commenced any form of antiretroviral treatment (the non-ARV group) had a greater degree of erythrocytes fragility than the group on antiretroviral drugs (the ARV group). Osmotic fragility had been associated with lower concentration of protein sulfhydryls in erythrocyte ghost membranes (8). Xia *et al* suggested in their report that an important function of zinc is to protect cysteine residues in critical plasma membrane proteins from auto-oxidation. Auto-oxidation of the cysteine residues will ultimately lead to a significant conformational change in these proteins, which may in turn cause structural fragility of the plasma membrane.

Micronutrient deficiency has been reported in AIDS patients (5). The deficient micronutrients include zinc, vitamin A, iron, iodine, and trace elements (6). These nutrients are essential as cofactors for the proper functioning and structural integrity of various biomolecules, especially proteins. Some act as antioxidants. Deficiency in a critical micronutrient can completely upset the homeostatic functioning of a cell or the entire organism. This deficiency could be due to reduced intake, increased utilization (15) or urinary excretion (16, 17). The report that reduced micronutrient intake may lead to nutritional deficiency lends credence to osmotic fragility of erythrocytes observed in the HIV positive subjects. Anorexia, nausea, vomiting, and diarrhea are conditions that can result in reduced nutrient intake, which leads to malnutrition. These secondary symptoms have been observed and reported in acute and late stage HIV disease (1).

It may seem surprising that patients who are undergoing antiretroviral therapy, and are showing significant improvement in health, should also be significantly susceptible to osmotic stress. These patients may have osmotically fragile cells also as a result of micronutrient deficiency. Stephensen *et al* (16) and Jordao *et al* (17) reported that deficiency might occur in HIV/AIDS as a result of increased urinary excretion. Antiretroviral therapy involves a cocktail of drugs taken under a strict regimen. In an attempt to detoxify and/or metabolize these drugs, the liver increases their water solubility (18). Ultimately there is an increase in urine production and a depletory loss of vital water-soluble nutrients like metallic ions may occur. The patients may therefore suffer from conditions like anemia. Zidovudine (AZT) therapy has been reported to be the commonest cause of anemia in HIV-infected persons (1, 4). This supports our findings of possibility of anemia in patients on antiretroviral therapy. Whereas previous reports have attributed this condition to marrow erythroid hypoplasia, aplasia, and megaloblastic maturation (19), we believe that from the present data and cited literatures, osmotic fragility resulting from micronutrient deficiency, is critical to the development of anemia in patients on antiretroviral therapy.

Na^+ -K^+ ATPase activities of the erythrocytes were found to be increased in non-ARV and ARV HIV positive patients compared to HIV negative patients, though they were not statistically significant in the present study. The increased activities may be a consequence of the osmotic fragility of the

plasma membrane of the erythrocytes above. The Na^+-K^+ ATPase pump is the primary mechanism by which the cell prevents lysis from osmotic stress (20). The activity of the pump increases when the cell is threatened by plasmolysis. The pump performs a continual surveillance role in maintaining normal cell volume. To obtain a detailed insight into these findings, the Na^+-K^+ ATPase activities were further analyzed based on the disease stage of the subjects. The criterion used for disease stage classification was the Centers for Disease Control (CDC) revised 1993 classification of HIV disease. The Na^+-K^+ ATPase activities increased with the degree of severity of the disease as measured by the $CD4^+$ counts. For both the ARV and non-ARV group, the average Na^+-K^+ ATPase activity of those with $CD4^+$ counts of less than 200 cells/μL of blood was higher than those with $CD4^+$ counts of between 200-499 cells. Further, the activity of the ATPase in the two $CD4^+$ count classification (200-499 and less than 200 cells/μL) were higher for the untreated HIV/AIDS subjects (non-ARV) than the treated subjects (ARV). Data showed that the Na^+-K^+ ATPase activity of HIV/AIDS subjects was slightly elevated with increased severity of the disease. This corroborates the preceding finding that the erythrocytes of HIV/AIDS persons are highly susceptible to osmotic stress and greater so when the disease is left untreated. The plasma membrane becomes highly porous to trans-membrane cationic movement with cations such as Na+ and K+ moving down their concentration gradients. In an attempt to reverse the resultant hypernatria of the intracellular fluids, the Na^+-K^+ ATPase activity is increased.

In conclusion, data obtained from the present study indicate that osmotic fragility of erythrocytes is significantly increased in HIV disease. The Na^+-K^+ ATPase activity of the erythrocytes is only marginally increased in an attempt by the cells to reverse the deleterious effects of osmotic fragility in HIV/AIDS disease.

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BLOOD CHEMISTRY AND PLATELET SEROTONIN UPTAKE AS ALTERNATIVE METHOD OF TRACKING HIV/AIDS

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A cross sectional study was conducted to investigate the blood chemistry and platelet serotonin uptake as alternative method of determining HIV disease stage in HIV/AIDS patients. Whole blood was taken from subjects at the Human Virology of the Nigerian Institute of Medical Research. Subjects were judged suitable for the various investigations by means of a questionnaire. The Genie II HIV diagnostic kit was used to confirm HIV positive status. HIV positive subjects were grouped in to two: those receiving antiretroviral therapy were referred to as the ARV group and those not on antiretroviral therapy were designated as non-ARV group. Each group was further sub-divided according to the Centers for Disease Control 1993 classification of HIV disease. HIV negative subjects must have been tested no later than two months to the sample collection date and must not lead a high-risk lifestyle. Serum was used to assay for blood chemistry activities with Randox analytical reagents. Blood platelets were prepared from one milliliter of whole blood and platelet serotonin uptake rates were determined. The serum glutamic-oxaloacetic transaminase (SGOT) of non-ARV subjects was the only blood chemistry parameter that showed any significant variation from normal ($p<0.05$). The mean activity of this enzyme was 28.4 ± 5.29 U/L compared to a normal value of 12 U/L. A disease stage-related variation was observed. Platelet serotonin uptake rates of the two HIV positive groups showed no significant difference with the HIV negative control. The data obtained showed that serum glutamic-oxaloacetic transaminase activity is significantly increased in HIV/AIDS patients in a manner that is disease stage related. However, serum glutamic-pyruvic transaminase, bilirubin, triglycerides, amylase, serum creatinine, and alkaline phosphatase showed no significant variation from normal values. Platelet serotonin uptake of HIV subjects was not significantly different from the control.

Key words: Blood chemistry, platelet serotonin uptake, HIV/AIDS

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a progressive disease that gradually, if untreated, overwhelms the immune systems of infected patients (1). Many rapid and easy-to-use diagnostic kits are available for easy detection. Antiretroviral treatment in most cases is dependent on the disease stage of the patient. This is determined primarily by the CD4+ T-lymphocyte counts (2). The testing kits for this count is relatively expensive, costing a minimum of five dollars per test. The equipment and reagent storage facilities required can also be quite expensive. Those factors make the procedure inaccessible to most Third World inhabitants who are bearing the brunt of the current global epidemic. We studied eight of the commonest and most routinely performed blood chemistry parameters and platelet serotonin uptake rates with a view of finding alternative methods of determining HIV disease stage. Blood chemistry analyses are regularly carried out to monitor patients' tolerance for drugs and as pathological markers. Serotonin uptake by platelets has been used as an indirect measure of the rate of serotonin intrasynaptic inactivation in depressed patients (3,4,5).

SUBJECTS AND METHODS

Study design

This was a cross sectional study involving 67 HIV seropositive subjects recruited and confirmed positive at the Nigerian Institute of Medical Research (NIMR) Human Virology Laboratory in Lagos, Nigeria. Ten HIV seronegative persons served as the control group. The subjects were representative of the geo-political, ethnic, economic, religious and educational diversity of Nigeria. The purpose of the study was clearly explained to them. Consent was obtained and counseling given before blood samples were taken. Participants who were on multivitamins or prolonged non-AIDS related treatment were excluded from the study. The age range was between 20 and 60 years. The HIV positive group was sub-divided into two: those who were on antiretroviral therapy (ARV) at the Institute and those who had not commenced any form of antiretroviral therapy (non-ARV). The HIV disease-stage classification was according to the Centers for Disease Control revised 1993 classification for HIV infection among adolescents and adults (2).

Blood collection

Blood samples were collected between 08:30h and 09:30h. Six ml of blood were collected by venous puncture. Five ml were put into plain bottles for blood chemistry analyses while the remaining 1 ml was put into potassium EDTA bottles for platelet preparation. Another 4 ml were collected for CD4+ T-lymphocyte count which was done within 6 h of sample collection. Sera and platelets were prepared within 3h of sample collection and the assays done within 24h.

HIV confirmation

Subjects were screened for HIV status at various centers outside the Institute but confirmation was done at the Human Virology laboratory with the Genie II HIV confirmation kit. CD4+ counts were performed with the Dynabeads method.

Determination of Blood Chemistry/Enzyme Activities

The chemical analysis of total bilirubin, direct bilirubin, triglyceride, amylase and creatinine levels in the sera of the HIV/AIDS patients were determined using the Synchron CX5 automated spectrophotometer (Beckman, Switzerland). The activities of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase and alkaline phosphatase in HIV/AIDS patients were also carried out using the Synchron CX5 automated spectrophotometer.

Determination of Platelet Serotonin Uptake

Platelets were prepared from the collected blood according to the method of Oxenkrug (4) as modified by Ebuehi and Akinwande (5). Platelet viability was confirmed using the method of Oxenkrug (4). Briefly, 8 tubes containing 1 ml of platelet-rich plasma each were placed in a shaking water bath at 37^{0}C for 5 min. 0.4ml of 4 x 10^{-6} M serotonin creatinine sulphate was added to each of the first five tubes and 0.4 ml of 0.9% NaCl to each of the other three tubes. Incubation was stopped 5 min after addition of serotonin and the tubes were transferred into an ice-bath. Platelets were then prepared using the method of Oxenkrug (4). The isolated platelets were suspended in 2.5 ml of 0.4M $HClO_4$ and then centrifuged at 1500g for 15 min. To 2 ml of the resulting supernatant (containing the discharge serotonin in 0.4M $HClO_4$) was added

0.5 ml concentrated HCl. The concentration of platelet serotonin was determined spectrophotometrically. Protein concentration in platelet-rich plasma and in the discharged serotonin, were determined by the method of Lowry *et al* (6). The difference between serotonin concentration of platelets in the five experimental tubes and in the three control tubes was taken as serotonin uptake by platelets within 5 min of incubation. The rate of serotonin uptake was expressed per mg protein.

Statistical Analysis

Data obtained were analyzed by two-tailed student's t-test (7). A p-value of <0.05 was considered statistically significant. Calculations were done using Microsoft Excel 2000 statistical tools.

RESULTS

Of the eight blood chemistry parameters investigated, only the serum glutamic-oxaloacetic transaminase (SGOT) activity of the non-ARV group gave a significantly ($p<0.05$) higher value than the normal. SGOT activity for these patients had a mean of 28.4 U/L as compared to the normal value of less than or equal to 12 U/L (Table 1).

The two $CD4^+$ count classification for which subjects were avaliable in the non-ARV group also showed sigificantly higher SGOT activity values than the normal. Those with a $CD4^+$ count of less than 200 cells/μL had SGOT activity of 34.76 U/L while those with a $CD4^+$ counts of between 200-499 cells/μL had activity value of 21.06 U/L. These values showed significant difference at $p<0.05$ (Table 2). The platelet serotonin uptake rate of HIV positive subjects showed no significant difference from the HIV negative subjects (Table 3).

TABLE 1

Blood Chemistry Parameters of Non-ARV and ARV HIV Positive subjects[1]

Blood Chemistry Parameter	Normal Serum Value	Non-ARV Subject	ARV Subject
Serum glutamic-oxaloacetic transaminase (U/L)	≤12	28.4±5.29	13.65±1.95
Serum glutamic-pyruvic transaminase (U/L)	≤12	15.25±4.68	9.25±1.74
Total bilirubin (mg/dl)	≤1	0.58±0.05	0.52±0.05
Direct bilirubin (mg/dl)	≤0.25	0.24±0.04	0.26±0.04
Triglyceride (mg/dl)	60-150	81.86±9.39	81.09±8.81
Amylase (U/l)	≤52	27.39±4.23	27.35±3.15
Creatinine (mg/dl)	≤1.3	1.01±0.06	1.02±0.04
Alkaline phosphatase (U/l)	9-35	31.18±5.06	40.04±7.01

[1]Values represent mean ± standard error of mean. Source of normal serum levels of parameters: Randox Laboratory Manuals.

TABLE 2

Serum Glutamic – Oxaloacetic Activity of Non-ARV Subjects Classified according to $CD4^+$ Counts[1].

$CD4^+$ Count (cells/μl)	SGOT (U/l)
<200	34.76±8.95
200-499	21.06±3.83
≥500	-*

[1]Values represent mean ± standard error of mean

**There was only one patient in this category*

TABLE 3

Platelet Serotonin Uptake of HIV Positive and HIV Negative Subjects[1].

Subject	Uptake Rate (nMol serotonin/mg proten/5min)
HIV positive	2.00±0.76
HIV negative	2.21±0.52

[1]Values represent mean ± standard error of mean

DISCUSSION

Serum glutamic -oxaloacetic transaminase (SGOT) activity of non-ARV group was significantly raised above the normal value. Laboratory data have shown elevated levels of muscle enzymes, including creatine kinase, aldolase and SGOT in HIV disease (8). These findings are typical of polymyositis. Inflammatory muscle disease has been reported to occur often in persons with HIV disease than in the general population (9). In some patients polymyositis is the initial manifestation of HIV infection (10).

Polymyositis may be diagnosed months before the onsets of AIDS. Patients usually complain of progressive proximal muscle weakness involving upper and lower extremities, with increasing difficulty rising from a chair, walking upstairs and using arms for any length of time.

Detailed analysis of the SGOT values for non-ARV subjects showed a relationship with the HIV disease stage as determined by the $CD4^+$ count. SGOT is a serum enzyme that is used in the clinical assay of the liver damage.

In HIV/AIDS patients, SGOT, along with other blood chemistry parameters studied in this work, is normally used to monitor the reaction of patients to antiretroviral drugs. From the present findings, SGOT may be used, along with other tests, as an early detection method for AIDS. As the enzyme activity was found to also be disease-stage related, it could also be used to follow the progress or stage of the disease in patients who have been diagnosed as AIDS patients.

The other blood chemistry parameters studied showed no significant difference with the normal values. Sinicco *et al* (11) report that hepatic enzymes might be elevated to as much as ten times normal in acute hepatitis in HIV disease, but return to normal within six weeks. Lebovics *et al* (12) and Schneiderman *et al* (13) also reported that neither the pattern nor the extent of elevation of these serum enzymes correlates with specific findings in the liver. SGOT levels seem to correlate with the severity of HIV disease. This suggests that SGOT could be used as a rapid indicator of HIV disease stage, which could aid patient monitoring in rural and field settings.

The platelet serotonin uptake observed from HIV positive subjects showed no significant difference with the serotonin uptake of HIV negative subjects. These findings suggest that HIV/AIDS does not affect platelet serotonin uptake and rate of intrasynaptic inactivation. Serotonin uptake by blood platelets has been used as an indirect measure of the rate of serotonin intrasynaptic inactivation in depressed patients (3,5) and in the rats (14). A possible explanation for the lack of significance in the results obtained in this study for platelet serotonin uptake may be that most of the HIV positive subjects were getting confirmation of the HIV status for the first time. Follow-up study of the post-confirmation depression might reveal more interesting facts.

In conclusion, serum glutamic-oxaloacetic transaminase (SGOT) activity holds promise as a tracking method for HIV disease progression in patients with HIV/AIDS. However, larger study will be required to confirm this.

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HUMAN BITE INJURIES IN THE ERA OF AIDS: A REVIEW

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The risk of human immunodeficiency virus infection (HIV) transmission following human bite is important to many groups of people. Meanwhile, the pandemic of HIV/AIDS continues unabated, with perhaps more than 3 million new infections last year alone. A review of the literature concerning human bite injuries and HIV was performed to examine current opinion regarding the transmission of HIV via this route. It is concluded that human bite contaminated with infected blood carry a small, but definite, risk of transmitting this important life-threatening disease.

Key words: HIV, Human bite, Risk

INTRODUCTION

Human bite wounds are underestimated and under treated. Since the recovery of HIV was accomplished from the saliva of infected persons, a great deal of attention has focused on the risk of transmitting this disease through contaminated human bites (1). Some of this interest has rightly focused on the risk of transmission among playmates by the bites of infected toddlers in day care centers (2). It is estimated that by end of the year 2003 close to 50 million people worldwide had become infected with HIV, the majority of them in sub-Saharan Africa. In the majority of cases, the disease is transmitted during intimate sexual relationships, parenteral exposure to contaminated blood or blood products and from mother to child during the perinatal period. Other methods of transmission are via donated organs or semen and the sharing of contaminated hypodermic needles.

The objective of this study was to review the evidence for transmission of HIV by human bites. The materials used were journal articles and edited conference papers that were identified by computer searching, bibliographies, and consultation with experts.

INCIDENCE OF HUMAN BITES

The true incidence of human bites might always remain unknown because most of the victims have minor injuries and never seek medical help. In the United States of America, where 1 % of all emergency consultations are due to bite from various animals, human bites are the third commonest source of the injuries (3). Only a few reports exist that strongly link HIV transmissions with human bite injuries (4, 5, 6, 7). To date, there is only one case in which scientists have proved conclusively by molecular techniques that the virus in the assailant and his victim were very closely related strains (8). Richman and Richman had evaluated the cumulative published data on the risk of HIV infection following human bites

and concluded that it is in the region of 0.3 – 0.5% (1).

SPECIAL GROUPS AT RISK

Pretty *et al* enumerated the groups that appear to be at special risk for transmission of HIV/AIDS (3). The first are those likely to be bitten as an occupational risk such as police officers and institutional staff, the victims and perpetrators of crimes involving biting, both attack and defense situation; and physicians who treat such patients. Bites commonly occur also in the young ages. Analysis of accident logs in child day care centers indicated that biting is common, especially among toddlers 13 to 30 months of age (2). Such bites may be inflicted during altercations or play with the attendant risk of HIV transmission if there is blood-to-blood contact involving HIV-positive child.

TRANSMISSION OF HIV

There had been reports in which HIV appeared to have been transmitted by a bite. Though HIV has been recovered from the saliva of AIDS patients, transmission through saliva in the absence of contaminated blood is considered a very unlikely event (4). HIV is infrequently isolated from saliva and it even appears that saliva has inhibitory effect on the infectivity of the virus. It has not been possible to prove that the bite of an AIDS patient alone could result in transmission of the disease in the cases available in the medical literature (5,6,7). These reports describe the presence of blood and severe injury with extensive torn and damaged tissue. In at least two of the cases above, there was proof of extensive tissue tearing and the presence of blood during the incidences, but corroborative scientific evidence was lacking (4, 6).

To examine the relative risk of transmission through bites and scratches, Tsoukas *et al* (8) studied 30 health workers who had been traumatized by an aggressive AIDS patient. This patient frequently bit others, his mouth full of saliva and blood. He was HIV antibody and antigen positive. HIV was recovered from his peripheral blood lymphocytes. After 2.5 years of follow-up, all traumatized personnel were clinically normal, no HIV was cultured from their blood, and all were HIV antibody and p24 antigen negative. They concluded that the risk of HIV transmission through this route must be very low. However, Brazilian scientists have presented evidence that confirm for the first time that the bite of a HIV-positive individual, which had broken the skin had actually transmitted the disease (9). The case involved a 31-year old male who was suffering from AIDS-related brain disorder who bit his mother on her hand during a seizure resulting in seroconversion several weeks later. DNA sequencing and phylogenetic analysis of the material from the proviral DNA acquired from both individuals indicated only an insignificant difference between the viral strains. These results support the epidemiological findings that the woman's infection was acquired from her infected son.

CONCLUSION

We conclude that the transmission of the HIV through human bite is biologically proven but remains unlikely epidemiologically. The presence of blood in the saliva may heighten the risk significantly. Exposure to HIV-contaminated blood from the bite of another human being is one of the multiple, unusual routes by which disease transmission could occur. Because the total numbers of

infected persons continues to rise, even more people will bear risk for infection through this route in future. Nonetheless, the consistent conclusion of many studies and reviews is that there is no evidence for HIV transmission that could be directly attributed to exposure to uncontaminated saliva.

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SCHISTOSOMAL APPENDICITIS IN A SLIDING HERNIA (CASE REPORT)

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We report a rare case of a forty-seven year old Nigeria male with schistosomal appendicitis in a sliding hernia. The clinical and pathological features of the case are discussed, followed by a review of the literature. It is concluded that a high index of suspicion is necessary to diagnose unusual presentations of schistosomiasis in an endemic area such as Nigeria to facilitate early diagnosis and adequate treatment.

INTRODUCTION

The vermiform appendix may be found abnormally in the sac of 1-2% of inguinal hernias (1). *Schistosoma haematobium* infection usually affects the urinary tract and rectum, but in endemic areas, it is not uncommonly found in the appendix (2). We report a sliding right inguinal hernia that contained an appendix with histological evidence of intense *Schistosoma haematobium* ovideposition. The case is presented to highlight the importance of early anti-schistosomal treatment.

CASE REPORT

J. A., a 47-year-old male farmer, was admitted for recurrent right inguinal swelling of seven-year duration. Examination revealed a middle-aged man in good general condition with an oval right inguino-scrotal swelling. He also had a reducible non-tender umbilical swelling. He was operated under general anaesthesia and the findings were a thick-walled hernia sac containing bowel and appendix with the caecum adherent to the posterior wall of the sac. Herniorrhaphy and incidental appendicectomy were performed. Post-operatively, he developed scrotal haematoma that necessitated exploration, evacuation as well as right orchidectomy and scrotoplasty. He was discharged in good condition but has defaulted in follow-up. The histological examination of the appendix showed mucosal atrophy, submucosal fibrosis and numerous ova of schistosoma within the lamina propria and the muscular layers. An acid-fast stain confirmed *Schistosomal haematobium* species (acid fast negative ova shell). The intensity of the infestation of the appendix was found to be grade IV corresponding to more than 10 eggs per high power field according to the method of Gelfand *et al* (high power field = x 40 objective, x 8 eye piece) (3). No ova of schistosoma were found in the urine or stool.

DISCUSSION

Schistosomiasis, a water-borne infection is one of the most wide spread parasitic disease in the world and about 300 million people are affected. It occurs frequently in Nigeria (4) and has been observed in the Sokoto area (5). Schistosomal appendicitis could be the first manifestation of infestation

of the pelvic or abdominal structures and it implies that the disease may develop later in other organs. An interesting dimension in this patient is that the infested organ was found as content of a sliding hernia. The discovery of an appendix within a hernia sac is quite fortuitous. Indeed, Claudius Amyand of St. George Hospital, in what is generally regarded as the first successful appendicectomy, removed the appendix of an eleven-year old boy, whose hernia operation had revealed an appendix in the sac (6). In the normal anatomic state, the organ would be pointing to the pelvic brim and in this position it is prone to being a content of right inguinal hernia (1). Such a herniated appendix may be mistaken for an inguinal lymphadenitis. An abscess resulting from it may also be erroneously incised resulting in a faecal fistula (1). It should be emphasized that the histological diagnosis of schistosomal appendicitis merit prompt antischistosomal therapy (2) and this aspect of treatment must not be overlooked, particularly in those communities where the disease still poses a major challenge.

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ONCHOCERCIASIS – A PUBLIC HEALTH PERSPECTIVE

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Onchocerciasis is a chronic parasitic disease with a wide range of cutaneous and ocular manifestations. It is caused by the tissue nematode, *Onchocerca volvulus*, and it is transmitted by the bite of a female black fly, *Simulium damnosum*. Onchocerciasis is a serious public health and socio-economic problem with 95% of all cases being found in Africa south of the Sahara. The WHO Expert Committee has estimated that over 80 million people are at the risk of infection worldwide, some 18 million infected, and 1 million people visually impaired of which some 340,000 are blind. Nigeria is highly endemic for this disease, to the extent that 40% of all cases worldwide are believed to occur in the country. The prevalence of blindness in villages near to fast flowing rivers may reach 15%, often, affecting males (of working age, perhaps 30-40 years old) more frequently than females. In spite of these ravaging consequences of this disease however, remarkable successes have been achieved by the control effort of the Onchocerciasis Control Programme (OCP), which uses chemical and biological larvicides with low environmental impact to kill black fly larvae flies. Other methods of effecting Onchocerciasis control include: (i) Reducing the number of bites by the *Simultum* fly on man; (ii) Killing the microfilariae with microfilaricides; and (iii) Killing the adult worms. The social and economic consequences of the disease in Nigeria and other African countries are huge, with considerable human suffering. It thus demands unrelenting intensive and concerted effort at the international, national and community levels, making optimal use of the identified modes of control for effective control of this disease which has serious public health and economic consequences.

Key words: Onchocerciasis, Public Health, Control

INTRODUCTION

Onchocerciasis is a chronic parasitic disease with a wide range of cutaneous and ocular manifestations. It is caused by the tissue nematode, *Onchocerca volvulus*, and it is transmitted by the bite of a female black fly, *Simulium damnosum*. The black fly breeds in rivers, with a preference for turbulent and highly oxygenated waters. Thus there is a high prevalence of disease in communities located near rivers and turbulent streams. Two basic ecology-related clinical and epidemiological varieties exist in Nigeria: the savannah and the rain forest belt types of onchocerciasis. The rain forest belt variety that causes little blindness had not received much public health attention till lately. However, recent studies have shown dermatological and psychosocial consequences, which have serious public health implications (1). Genetic research on *Onchocerca volvulus* has shown that there is intra-and inter-strain variation in polymorphic microsatellite loci between savannah and forest strains of the parasite, suggesting that different genetic constitutions are associated with the different clinical manifestations (2).

LIFE CYCLE OF ONCHOCERCA VOLVULUS

When a person is already infected by the worm and is bitten by the *Simulium* fly, the small embryo worms (microfilariae) present in the skin of the infected person enter the body of the fly. There they pass through the gut wall and travel to the thoracic muscles. Further development takes place and after about 7 days, the larvae move to the head of the fly,

ready to be transmitted to the next human host when the fly requires another blood meal. In this way, the microfilariae of *O. volvulus* are transmitted to another person. Microfilariae do sometimes appear in the blood and are also found in the eye in heavy infections. Worms normally take about a year to mature and for the female to start producing microfilariae. At this time, symptoms first appear in the form of itching and microfilariae can be found in the skin at about the same time. One female worm can produce 0.5-1 million microfilariae in 1 year. Thus, the cycle of transmission from person to person continues.

EPIDEMIOLOGY OF ONCHOCERCIASIS

Onchocerciasis is a serious public health and socio-economic problem found in about 27 countries in sub-Saharan Africa, parts of Latin America and in the Arabian Peninsula. The WHO Expert Committee has estimated that over 80 million people are at risk of infection worldwide, some 18 million infected, and 1 million people visually impaired of which some 340,000 are blind (3). Ninety-five percent (95%) of all cases of onchocerciasis are found in Africa south of the Sahara, in a wide belt stretching from West to East (4). The period of greatest transmission is in the rainy season coinciding, as expected with the period of maximal *Simulium* breeding.

The social and economic consequences of the disease are huge, with considerable human suffering. The prevalence of blindness in villages near fast flowing rivers may reach 15%, often affecting males (working age, perhaps 30-40 years old) more frequently than females, but this probably an occupational hazard. The blindness is usually bilateral, the victim gradually loses the visual field in each eye, and vision becomes severely impaired leading to blindness (5).

ONCHOCERCIASIS AS A PUBLIC HEALTH PROBLEM IN AFRICA

Onchocerciasis, popularly known as **River blindness disease** has been recognized as a public health problem in Nigeria since 1906. Nigeria is highly endemic for this disease to the extent that 40% of the world cases are believed to exist in the country. The manufacturer of the effective drug Mectizan, i.e. Merck, Sharp and Dohme, has promised to make the drug available to endemic countries free of charge for as long as it is required. However, the distribution cost to the rural endemic areas estimated at $ 0.1 per dose, is being financed jointly by the 3 levels of government and participating non-government development organization (NGDOS), involved in the control programme. The National Onchocerciasis Control Programme (NOCP) of the Federal Ministry of Health coordinates the control activities of all partners involved. The National Mectizan Treatment project was launched in 1991 at Minna, Niger state (6).

Many communities are endemic for onchocerciasis in Nigeria. These communities are usually those situated within 10km distance from a major river and its tributaries, and prevalence levels of 30-80% have been reported in them (7). The affected rural population predominantly engages in peasant farming while those who are closer to the river are also involved in fishing. Infection occurred mostly in the older age group 40 years and above, and farmers are proportionately more affected than the other occupational groups.

A prevalence survey of visual loss among 1,625 individuals in an onchocerciasis endemic community conducted in Sierra

Leone found that cataract and onchocerciasis were the major causes of visual loss in this population and more than half of the ocular morbidity was preventable or treatable by public measures or curative medicine (8). However, in our 5- year review of skin-snip results at the Medical Out-Patient laboratory of the University College Hospital, Ibadan, Nigeria, between 1.1% and 2.6% of the total number of patients sent for skin-snip examination yearly were found to be positive for onchocercal microfilaria (unpublished data). These relatively low figures may be largely due to the fact that treatment and control of onchocerciasis has been divulged and entrusted in the hands of members of the affected communities. A community now directs and takes charge of the responsibility that all it members get treated annually. Hence, few cases get reported to the health facility.

In a study of the perceptions of villagers on onchocerciasis conducted in a community in Plateau state, Nigeria, villagers in endemic areas were found to be aware of the nuisance of black fly bites but the majority of them lacked the aetiological knowledge of onchocercal lesion. Hence disease management is misdirected towards consulting the oracle and appeasing the gods (9). In another study conducted in Nebbi District, North-western Uganda, which involved the use of focus group discussion and semi structured interviews designed to explore the experiences, meanings and illness-related coping strategies employed by the community, the results indicated that onchocerciasis is considered to be mysterious elusive disease which cannot be treated by local herbs. The disease is often mistaken for measles and leprosy. Persons who suffer onchocerciasis believed that the cause of the disease is the small black fly, dirty water or rivers. However, non-infected individuals believed that the condition is caused by poor personal and environmental hygiene, and personal contact with persons affected by onchocerciasis. Affected people recommended public education to control the disease while non-affected people recommended the avoidance of personal contact with affected people, ensuring hygiene and improvement of environmental sanitation and the nutritional status of the community. The belief systems of the community are probably responsible for the discriminatory practices of the people against those affected by the condition (10).

In an epidemiological study on onchocerciasis in the lower Jos Plateau in Nigeria (11), a 7-month old baby delivered by a mother suffering from onchocerciasis showed early clinical signs of the disease; pruritus was present all over the body. The infant's skin snip on incubation revealed microfilariae of *Onchocerca volvulus*. Also, one in 20 babies born in heavily infected areas have microfilariae in the skin soon after birth due to transplacental transmission of microfilariae when the adults may become established in newborn infants (4).

CLINICAL MANIFESTATIONS

The microfilariae of *Onchocerca volvulus* have a particular predilection for the skin and the eyes of the infected person, but may also give rise to musculoskeletal pain interpreted as 'rheumatism' and probably including epilepsy and growth retardation. **Dermatological manifestations** include generalized or localized body itching, acute papular onchodermatitis (APOD), chronic

papular onchodermatitis (CPOD), atrophy of the skin, depigmentation of the skin (leopard skin), and thickened and rough skin (lizard skin). Other lesions associated with onchocercal skin disease are subcutaneous nodule, lymphadenopathy, hanging groin and lympho-edema. **Ocular manifestations** that have been associated with the onchocerciasis include iridocyclitis, punctuate keratitis, sclerosing keratitis, anterior uveitis, choroido-retinitis, pupillary abnormalities and its most dreaded complication blindness (12).

The pathology is thought to be due to the cumulative effects of cellular inflammatory response to the immobile and dead microfilariae in the skin and eyes (13). Recent reports however, indicate that the first step towards the development of blindness is due to certain *Onchocerca volvulus* proteins, which are implicated in the growth of new blood vessels in the eye, and that the proteins are neither immunogens nor mitogens, thus ruling out an immunological mechanism. In addition, the role of eosinophil eotaxin (a type of cytokine) in diethylcarbamazine (DEC)-induced skin lesions has been confirmed (2).

IgG antibodies are also known to play a major role in protective immunity to *O. volvulus* with infected individuals having paradoxically high IgG4 level and individual with putative protective immunity having significantly lower worm specific antibodies than infected subjects (14). Increased IgG4 levels were correlated with clinical severity and microfilarial load. IgG4 may be induced by worm or microfilarial antigens and may contribute to the immunopathology of disease by blocking microfilarial clearance and destruction.

DIAGNOSIS

Clinical features

The symptoms and signs of onchocerciasis; onchodermatitis, signs of scratching, depigmentation of the skin, 'lizard' skin and subcutaneous nodules, are often sufficient to make a certain diagnosis.

Skin-snip examination

Definitive diagnosis is made by demonstrating microfilariae of *O. volvulus* in the skin snips or at times, conjunctival snips. While the skin snip method has been useful both in diagnosis and in monitoring of infection, the test is insensitive when ivermectin has reduced the microfilaria burden. In addition, skin snip testing also fails to detect prepatent infections.

Serological tests

The success of ivermectin treatment in onchocercal infection has given further impetus to the development of serological tests. Bradley (15) has used a cocktail of three recombinant proteins, which achieved a sensitivity of 96% and a specificity of 100%. This could be useful for seroepidemiological studies detecting prepatent infection in control areas. The IgG subclass response to the recombinant antigen cocktail showed that the IgG1 response declined after interruption of transmission but the IgG4 response remained raised even eight years after transmission had stopped (16). An assay based on the polymerase chain reaction has also been developed that uses primers and probes specific to *Onchocerca volvulus* and achieved a sensitivity of 100% (17).

Other methods of diagnosis

Presence of microfilariae in the anterior chamber or in the cornea may be seen using the slit-lamp microscope. Biopsy of subcutaneous nodules can reveal adult worms at histology. Positive mazzotti test; severe body itching as a result of dead and dying microfilariae following the use of diethylcarbamazine (banoside), may occur.

A recently introduced epidemiological tool is the Rapid Epidemiological Assessment (REA), which is used to determine the endemicity levels of onchocerciasis. The prevalence of nodules is the simplest, most acceptable, non-invasive and reasonably reliable method of REA. It involves determining the prevalence of nodules in a random sample of 30-50 adult males over 20 years of age, who have been resident in the community concerned for at least 10 years. The percentage prevalence in the community is 5 times the number of nodule carries (18).

MISDIAGNOSIS

Onchocerca nodules present in the forehead, eyelid, brow and post auricular areas may be clinically misdiagnosed as dermoid cyst, fibroma, cancer, fibroadenoma and tuberculosis.

CONTROL OF ONHOCERCIASIS

Onchocerciasis control can be effected in four ways;

(i.) Inhibiting the development of the *Simulium* flies. This can be achieved through insecticide spraying by aircraft to stop vector breeding and removal of obstacles that creates water turbulence such as rocks or man-made structures. (ii). Reducing the number of bites by the *Simulium* fly on man through wearing clothing that covers most of the skin surface, or communities relocating from sites near the breeding area of *Simulium* fly. (iii). Killing the microfilariae with microfilaricides. (iv.) Killing the adult worms by removal of the subcutaneous nodules (nodulectomy) or chemotherapy with macrofilaricides.

Drug treatment and control of onchocerciasis have been unsatisfactory in the past. The available drugs, diethylcarbamazine citrate (DEC) and suramin are too toxic for mass distribution, while nodulectomy is ineffective and impractical in Africa (14). Mectizan (Ivermectin) is an effective microfilaricide with presumed macrofilarial effect suitable for large-scale treatment.

ONCHOCERCIASIS CONTROL PROGRAMMES

Because there is no effective macrofilaricide, vector control to prevent the transmission of the parasite is an important available control method. This has been most effectively carried out in West Africa countries by the huge **Onchocerciasis Control Programme (OCP)**. The **OCP** in West Africa was launched in 1974. It uses chemical and biological larvicides with low environmental impact to kill black fly larvae and its aim is to control blinding onchocerciasis, which is caused by the savannah strain of *Onchocerca volvulus* transmitted by the *Simulium damnosum* complex. It covers virtually every river where the flies are found in West African countries (over 1.2 million square kilometers), serving a population of 25 million. This project has had good result over the last 20 years in West Africa. Some areas are now completely free of onchocerciasis transmission. Fertile tracts of land that had to be abandoned because of the ravages of the disease and the flies have been resettled and are contributing to the financial well being of host countries.

Since the adult female *Onchocerca volvulus* worm has an average life span of 11 years in its human host, it was estimated that 14 years of spraying would be required to eliminate the adult worm reservoir in humans. The possibilities of re-invasion by black flies when spraying is discontinued, the tendency to development of resistance to the larvicides by *Simulium* larvae, and the huge expense of $20-30 million per year, are weaknesses of this particular approach. The timely discovery that ivermectin, a drug used in veterinary practice, is a highly effective microfilaricide had enabled a dual approach to be taken in onchocerciasis control.

A similar multinational multi agency partnership programme for onchocerciasis control i.e. Onchocerciasis Elimination Programme for the Americas (OEPA) was later launched in the Americas in 1993. Since it's inception, the OEPA has provided more than $ 2 million in financial, managerial, and technical assistance to stimulate and/or support programs in Brazil, Columbia, Ecuador, Guatemala, Mexico, and Venezuela, so as to take full advantage of the Merck donation.

Building upon the success of the Onchocerciasis Control Programme in West Africa, which is on the verge of eliminating the disease from 11 West African countries, a new programme, **African Programme for Onchocerciasis Control (APOC)** was created in 1997 to establish sustainable control in the remaining 19 countries in Africa, where the disease is still a public health problem. Whereas the OCP used vector control, the new programme will control the disease in the non-OCP countries by establishing community-directed treatment programmes with the drug ivermectin, supplemented with vector eradication in a few isolated foci.

Ivermectin (Mectizan)

Ivermectin kills the microfilariae of the worm *O. volvulus* but typically with only mild reactions, unlike diethylcarbamazine. Of particular significance is the knowledge that ocular damage does not routinely occur. Ivermectin is providing real hope for the future in the treatment and control of onchocerciasis. Ivermectin is being donated free of charge by Merck, Sharp and Dohme. The main logistical problem in treating population with ivermectin is the provision of manpower and facilities to effectively deliver the drug to communities in need. Dose finding studies on ivermectin have confirmed that a single oral dose of 150 mg/kg body weight once a year significantly reduced and maintained the skin microfilarial counts at low levels over a period of one year following treatment (19). Ivermectin is being considered as a follow-up intervention after the OCP is disbanded through a process called "devolution".

CONCLUSION

Although onchocerciasis is not a life-threatening disease, it has severe public health and economic repercussion, as family providers are often stricken with blindness and other chronic effects of the disease, Vector control has been beneficial in highly endemic areas in which breeding sites are vulnerable to insecticide spraying. In view of the successes attained so far by the use of ivermectin, an effective microfilaricide, and the control effort of the OCP in West Africa, and the OEPA in the Americas and considering the magnitude of human suffering being impacted by this preventable disease, continued intensive and concerted effort is therefore needed at

International, National and Community levels in collaboration with non-governmental development organizations, making optimal use of the identified modes of control to ensure a sustainable and effective control of this disease which has serious public health and economic consequences. Also, epidemiological surveys in onchocerciasis endemic areas further emphasizes the need for intensive public health education programmes to be targeted at onchocerciasis endemic communities as a means of facilitating infection control, as well as to correct the discriminatory practices of the people against those affected by the condition.

To control onchocerciasis as a public health problem, ivermectin needs to be given at least once per year to the population of all seriously affected communities. It is known that ivermectin treatment is exceptionally popular among endemic populations and since these communities effectively take responsibility for their own treatment, it should be possible therefore to control onchocerciasis at minimal cost, with active community involvement. Community-directed treatment with ivermectin may also provide an important entry point for other community-directed health interventions and thus help to develop a practical basis for strengthening primary health care in the various affected communities in Africa.

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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF STREPTOCCOCUS PNEUMONIAE IN ILORIN, NIGERIA

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Antimicrobial resistance is an increasing problem, particularly among previously sensitive *Streptococcus pneumoniae*. The emergence of wide spread resistance to antimicrobial agents complicates therapy of infections caused by these organisms. Between January and December 2002, one hundred and fifty-eight isolates of *Streptococcus pneumoniae* at the microbiology laboratory of the University of Ilorin Teaching were studied; in order to determine their antimicrobial susceptibility patterns. All the isolates were recovered from clinical samples and identified by their alpha-haemolytic reaction on sheep blood agar, bile solubility and their sensitivity to optochin. Susceptibility testing was carried out using the stokes-disc diffusion method. Majority of the *Streptococcus pneumoniae* isolates (78.4%) were recovered from the cerebrospinal fluids, 18 (11.3%) from sputum, 14 (9%) from throat swab and 2 (1.3%) from eye swab. Eight three percent of the isolates were resistant to penicillin G and 12.7% were resistant to more than three antibiotics. The isolates were largely sensitive to the third generation cephalosporins and quinolones. The study has shown that penicillins are no longer useful for the treatment of infections caused by *Streptococcus pneumoniae* in this centre. The cephalosporins and quinolones however remained effective and are therefore recommended.

Key words; *Streptococcus pneumoniae*, Susceptibility, Antimicrobial

INTRODUCTION

The genus streptococcus belongs to the non-spore forming aerobic to facultatively anaerobic bacteria. They are Gram positive, occurring in pairs or in chains (1). *Streptococcus pneumoniae* can induce a diverse spectrum of diseases associated with considerable morbidity and mortality. Pneumococci are the leading cause of community- acquired pneumonia and a very frequent cause of otitis media, sinustis and meningitis (2). In the past, most pneumococcal strains were susceptible to penicillin with minimum inhibitory concentration (MIC) less than 0.06 µg/ml (3), allowing most physicians to treat persons who had severe infection with penicillin alone and without testing for resistance. However resistance to penicillin and other antimicrobial agents has evolved and spread rapidly (4,5,6).

The problem of increasing resistance among previously sensitive bacteria species to common antimicrobial agent has become a worldwide phenomenon (7). Penicillin resistance in *Streptococcus pneumoniae* was first reported in Australia in 1967 (8), in New Guinea in 1969 (9), in South Africa in 1977 (10), and since then in many countries throughout Africa, Asia and Europe (11). Countries like Spain, Hungary and Iceland are notorious for harboring penicillin resistant pneumococci with very high MIC to β-lactam compounds. The link between penicillin resistance in pneumococci and high levels of antibiotic consumption are also very clear in these countries (5,11). Pneumococcal resistance to β-lactam antibiotic occurs due to structural alterations in the penicillin binding proteins (PBPs). Though typically, resistance to

β-lactam antibiotics by most organisms is due to the production of β-lactamase enzyme, which is able to hydrolyze penicillin compounds, resistance in *Streptococcus pneumoniae* to penicillin and other β-lactams is due to the expression of low affinity PBPs and not β-lactamase production. In these resistant isolates, there has been a reduction in the affinity of at least three of the five high molecular weight PBPs found in this organism.

The present study was carried out to determine the antimicrobial susceptibility patterns of *Streptococcus pneumoniae* in this environment, because of its increasing resistance to commonly used antibiotics.

MATERIALS AND METHODS

This study was carried out between January and December 2002 at the University of Ilorin Teaching Hospital. All *Streptococcus pneumoniae* isolates from clinical specimens such as cerebrospinal fluid, sputum and swabs were included in the study. A total of 158 isolates were characterized by standard bacteriological technique (1). First, all streptococci provisionally identified by the alpha haemolysis on blood agar were sub-cultured on to sheep blood agar. A 6-mm size filter paper disc impregnated with 5 μg optochin was placed on the blood agar and incubated aerobically at 35°C for 18-24 hours. Growth around the disc with zone diameter inhibition greater or equal to 10mm shows that the isolate is susceptible to optochin and presumed to be *Streptococcus pneumoniae*. The viridians streptococci are resistant to optochin and grow to the edge of the optochin disc or gives zone diameter less than 10mm. All the *Streptococcus pneumoniae* isolates also had positive bile solubility test. The 158 confirmed *Streptococcus pneumoniae* isolates were tested against the following antibiotics using the Stoke's disc diffusion method (12); Ampicillin (10 μg), penicillin G (1unit), Erythromycin (10 μg), Ciprofloxacin (5 μg), Ofloxacin (5 μg), Ceftriaxone (30 μg) and Ceftazidime (30 μg). *Streptococcus pneumoniae* NCTC 10319 and a viridian *Streptococcus* NCTC 10712 served as control organisms.

RESULTS

A total of 158 *Streptococcus pneumoniae* were isolated during the period of study from different clinical specimens. One hundred and twenty four isolates (78.4%) were recovered from the cerebrospinal fluids, 18 (11.3%) from sputum, 14 (9%) from throat swab and 2 (1.3%) from eye swabs (Table 1). All were associated with clinical infections.

The antibiotic susceptibility pattern is as shown in Table 2. A total of 131 (83%) isolates were resistant to penicillin G, 117 (73.8%) to ampicillin and 89 (56.6%) to erythromycin. Seventy (44.3%) of all the isolates were resistant to erythromycin and penicillin and 20 (12.7%) were resistant to more than 3 antibiotic groups i.e. resistant to erythromycin, ampicillin/penicillin, cephalosporins and quinolones. The isolates were largely susceptible to the cephalosporins; 83% to ceftazidime, 82% to cefuroxime, 72% to ceftriaxone and the quinolones; 80% susceptible to ciprofloxacin and 77.2% to ofloxacin.

TABLE 1:

Distribution of *Streptococcus pneumoniae* isolates in the different clinical specimens at UITH Ilorin.

Specimen type	No of isolates	Percentage
CSF	124	78.4
Sputum	18	11.3
Eye swab	2	1.3
Throat swab	14	9.0
Total	158	100

CSF = Cerebrospinal fluid

TABLE 2:

In vitro antibiotic susceptibility patterns of *Streptococcus pneumoniae* isolates at UITH Ilorin.

Antibiotics	No sensitive (%)	No resistant (%)
Ceftazidime	131(83)	27(17)
Cefurozime	130(82)	28(18)
Ceftriazone	114(72)	44(28)
Ciprofloxacin	126(80)	32(20)
Ofloxacin	122(77.2)	36(22.8)
Erythromycin	69(43.4)	89(56.6)
Ampicillin	41(26.2)	117(73.8)
Penicillin G	27(17)	131(83)

DISCUSSION

Antimicrobial resistance is an increasing problem particularly in *Streptococcus pneumoniae*. In the past, *Streptococcus pneumoniae* was almost uniformly susceptible to penicillin, allowing most infection to be treated with penicillin thus making the susceptibility testing of pneumococci unnecessary. Bacteria resistance has been reported to almost every antibiotic currently available. Many bacteria now exhibit simultaneous resistance to two or more antibiotics. Pneumococcus resistance may occur alone or in combination with resistance to other antimicrobial agents (11,13).

In this study, the pneumococcal isolates were largely resistance to penicillins. This correlates with the findings of other workers (14,15). No doubt penicillin are the most widely used antibiotics in the developing countries. The greater the quantity of drug used and the longer the drug have been in use, the more likely it is that strains resistant to the antibiotics will develop and spread (13).

Forty four point three percent of the isolates were also resistant to both penicillin and erythromycin, while 12.7% were resistant to more than three antibiotic groups. This shows the gradual increase in the level of resistance of the isolates in agreement with the trend worldwide (11). Contrary to other reports (11, 13, 14) however, the cephalosporin resistance rate in this study was low. This is probably as a result of the fact that these drugs are expensive and less abused in this environment. Previous studies in this centre (16, 17) have shown many pathogens to be relatively sensitive to cephalosporins.

In the first line empiric treatment of infections due to *Streptococcus pneumoniae* in our environment, penicillin will no longer be advocated. We recommend the use of second or third generation cephalosporins or the fluoroquinolones, where indicated, in the empiric treatment of serious infection due to *Streptococcus pneumoniae*. Reducing the impact of drug resistance in *Streptococcus pneumoniae* may be achieved through policy

that serves to reduce indiscriminate antibiotic use in the community and increase understanding of other factors that contribute to the development of resistance such as under dosage, which is a common practise in our communities.

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PLASMID PROFILES OF KLEBSIELLA ISOLATES IN ILORIN, NIGERIA

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Antibiotic resistant organisms are most common in locations where antibiotics are in great use. This accounts for the fact that hospitals harbor many antibiotic resistant bacteria. It is not surprising that antibiotic-resistant organisms are more common in certain parts of the world, particularly in developing countries, which probably results from the over use of antibiotics. Many of this resistance in bacteria are mediated by plasmids. This study was carried out to identify factors responsible for poor clinical outcome in Klebsiella infections due to antibiotic resistance, and to detect the type of plasmids harbored by various strains of Klebsiella. Three hundred *Klebsiella spp.* were isolated from various clinical samples at the University of Ilorin Teaching Hospital and biochemically characterized. Five species were identified based on biochemical characteristics; *K. pneumoniae, K. rhinoscleromatis, K. ozaenae, K. planticola* and *K. oxytoca*. Plasmid was extracted and analyzed by Birnboim and Doly method. 55 (18.3%) had plasmids of different molecular weight with sizes ranging between 1.1 and 8.0 kb. Species that harbor plasmids are *K. pneumoniae* and *K. oxytoca*. It appears that plasmid is naturally occurring in some strains, but the incidence of plasmid is probably higher in areas where antibiotics are readily available to the general populace.

INTRODUCTION

Klebsiella is a genus of the family Enterobacteriaceae. Members of this genus are defined as Gram negative, non motile, aerobic to facultatively anaerobic bacilli which are catalase positive and oxidase negative (1). Enterobacteriaceae are a major component of the normal intestinal flora, but are relatively uncommon in other body sites (2). They are major cause of nosocomial infections, and may account for 80% of clinically significant isolates of Gram negative bacilli in clinical microbiology laboratories and 50% of all clinically significant isolates (3,4,5). Klebsiella are isolated in many types of human infections such as abscesses, pneumonia, meningitis, septicaemia, intestinal and urinary tract infections. Hospitalized patients often become colonized with *Klebsiella spp.* and among the Enterobacteriaceae, are a major cause of nosocomial infections (4). Nosocomial *K. pneumoniae* infection is associated with a high mortality in both neonates and adults and antimicrobial treatments of the infection has been complicated by the emergence of multi-resistant strains (6).

Plasmids are circular extra chromosomal genetic elements that may encode a variety of supplementary genetic information including the information for self-transfer to other cells by conjugation and such properties as resistance to antibiotics (7). Plasmids carrying resistance factors are distributed through nearly all genera of medically important bacteria, with notable exceptions of *Neisseria meningitidis* and *Streptococcus pneumoniae* (8), and can be transferred via conjugation even between members of different species or genera (9). Multiply resistant *Klebsiella spp.* have been reported, and about 15-30% of Klebsiella are also resistant to broad-spectrum

cephalosporins by the production of R-plasmid encoded beta lactamases (10).

The present study was carried out to identify factors responsible for the poor clinical outcome in Klebsiella infections due to antibiotic resistance, and to detect the types of plasmids harboured by various strains of Klebsiella. We believe this can be used as an epidemiological tool in the control of infection during outbreaks caused by Klebsiella species within the hospital.

MATERIALS AND METHODS

All Klebsiella isolates from clinical specimens such as blood, urine, wound swabs pus, and aspirates between 1st January 2000 and 31st June 2000 were included in the study. Three hundred isolates of Klebsiella were examined by standard bacteriological techniques. All organisms provisionally identified as Klebsiella were first sub-cultured on to Blood and MacConkey agar plates and were incubated aerobically at 37°C. The organisms that conformed to the genus were further tested biochemically to differentiate them into species. The tests were; glucose fermentation for acid and gas production, lactose fermentation for acid production, dulcitol fermentation and glucose fermentation at 5°C. The other tests were Methyl Red (MR) and Voges Proskauer (VP) reaction (Table 1).

Plasmid was extracted using the modified Birnboim and Doly method (11). This is a mini scale isolation procedure based on the differential behaviour of closed circular, open circular and linear DNA under alkaline condition. The high alkalinity brings about the separation of the complimentary strands of DNA. Plasmid is then removed from the supernatant by ethanol precipitation. Electrophoresis of the extracted plasmid DNA was carried out in 0.7-1% agarose gels in Tris borate buffer with a bromophenol blue tracking dye inside a horizontal slab gel apparatus. The gel was run for 4-5 h at 5v/cm constant voltage and then stained by immersing in water containing ethidium bromide (0.5 µg/ml) for 45 minutes at room temperature. The stained gel was visualized with a short wave ultra violet light transilluminator and the photograph of the plasmid bands on gel was taken using type 667 films on a Polaroid camera. *Escherichia coli* V517 was used as a control. This contained eight plasmid bands of different molecular weight. The relative mobility was calculated by measuring the distance of each band from the origin to the end point of electrophoresis.

RESULTS

Three hundred isolates were biochemically confirmed to be *Klebsiella spp.* Five species were identified based on biochemical characteristics; *Klebsiella pneumoniae,* 150 (50%), *K. oxytoca* 90 (30%), *K. rhinoscleromatis* 30 (10%), *K. planticola* 15 (5%) and *K. ozaenae* 15 (5%) (Table 2). Of the 300 isolates analyzed, 55 (18.33%) had plasmids of different molecular weights. The plasmid sizes ranged between 1.1 and 8.0 kilobases as shown in the plates. The calculation was done by finding the relative mobility of each gel (x), which was inputted into the equation; Y= -2.15 X 1.47 where Y is the log of molecular weight of the plasmids. Species that harboured plasmids are *K. pneumoniae* 25 (45.6%), *K. oxytoca* 20 (36.4%), *K. planticola* 5 (9%) and *K. rhinoscleromatis* 5 (9%). Most of these strains were from urine and wound swabs. Multiple plasmids occurred mainly in *K. pneumoniae* as shown in the

plates. Plates 1 and 2 show the various plasmid bands (Lane 1-11). The control used was *E. coli* v517 with eight plasmid bands of different molecular weights.

Most of the isolates in plates 1 are *K. pneumoniae* with multiple plasmid bands, while the isolates in plate 2 belong to other species with single plasmid band.

TABLE 1: Biochemical characterization of Klebsiella species

KLEBSIELLA SPP	LAC	DUC'	GAS IN GLU	GLU5°C	MR	VP
Pneumoniae	+	+	+	-	+	-
Oxytoca	+	+	+	-	-	+
Ozaenae	+		+	-	+	-
Planticola	+	+	+	+	-	+
Rhinoscleromatis	-	-	-	-	+	-

TABLE 2: Percentage distribution of Klebsiella species isolated

SPECIES	NUMBER OF ISOLATES	PERCENTAGE
K.pneumoniae	25	45.5%
K. oxytoca	20	36.4%
K. planticola	5	9.0%
K. rhinoscleromati	5	9.0%

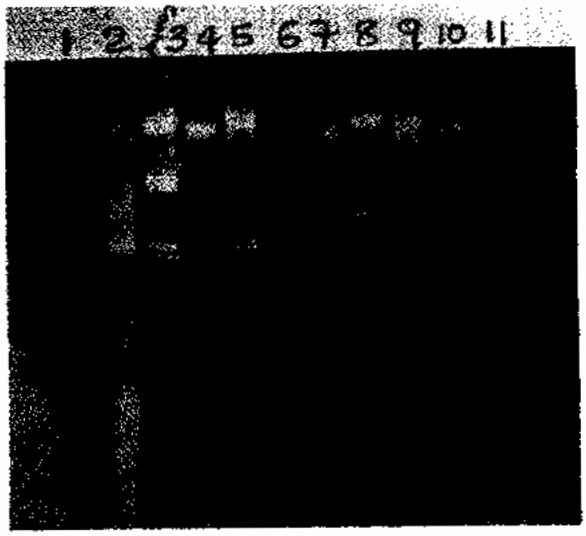

PLATE 1:- Plasmid fingerprints of *Klebsiella pneumoniae* lane 1 – 11 with *Escherithia coli* V 517 as control and having eight plasmid bands. Lanes 2, 4, 5, 7, 9 and 11 have plasmids of about 8 kilobases, Lanes 2, 4, 5, 7, 9 and 11 have various plasmids lower than 8 kilobases.

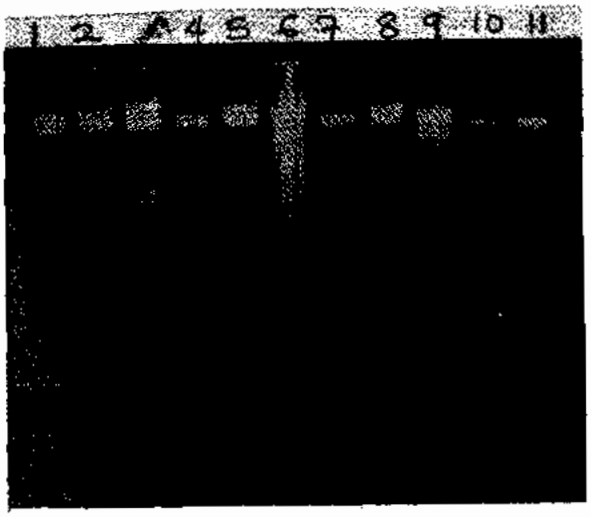

PLATE 2:- Plasmid fingerprints of *Klebsiella oxytoca*. Lane 1 – 11 with *E. coli* V 517 as control and having eight plasmid bands. Lanes 2 has plasmid of about 8 kilobases, while lanes 8, 9, and 11 have plasmids lower than 8 kilobases.

DISCUSSION

Five species of Klebsiella were found in this study; *K. pneumoniae, K. oxytoca, K. rhinoscleromatis, K. planticola* and *K. ozaenae.* A similar study in Lagos in 1998 found only four species (12). It is noteworthy however that *K. pneumoniae* was found to be the predominant specie in both studies, closely followed by *K. oxytoca.* In another study done in Lagos in 1985, over 90% of the clinical isolates of Klebsiella were *K. pneumoniae* (13) as against the 50% obtained in this study. From the above studies it can be observed that the most frequently isolated strains from clinical samples in Nigeria is *K. pneumoniae,* which is a recognized pathogen. It accounts for large number of hospital and community acquired infections involving the urinary tract, blood and lungs (14).

Fifty-five (18.55%) of the Klebsiella isolates harboured plasmids, majority of which were found in *K. pneumoniae* and *K. oxytoca.* Most of the plasmids were of low molecular weight ranging between 1.1 and 8.0 kb. Plasmids of lower molecular weight can usually be found in multiple copies in a single bacterium. In some studies done elsewhere, the presence of plasmids of lower molecular weight have been described as not related to bacteria resistance (15). Another factor that may be responsible in those without plasmids is the presence of β-lactamase production, which has been reported in *Klebsiella spp.* (16). Staphylococci often have resistance determinants distributed on several small plasmids (17). Instability of these small plasmids apparently accounts for the variable antibiogram observed in isolates derived from a single colony of cultures obtained at different intervals from a single point.

From the plasmid fingerprints, some of the plasmids were shown to be related. Although multiple plasmids were observed in this study, the percentage was much lower than 50%. It is contrary to previous report (18) where a plasmid value of about 90% was isolated in some Gram negative clinical isolates of *Neisseria gonorrhoea, Campylobacter jejuni, Escherichia coli, Shigella dysenteriae* and *Salmonella spp,* although *Klebsiella spp.* was not included in the study. It appears that plasmid occurs naturally in some strains of *E. coli,* but the incidence of plasmid is probably higher in countries where antibiotics are readily available to the general populace. A serious outbreak of Klebsiella infections in a neurosurgical unit was only brought under control when prophylactic ampicillin, which was routinely given to surgical patients, was stopped (19).

This study further highlights the need for proper antibiotic control. In the absence of antibiotic use or prescription, many of the bacteria strains spontaneously loose their plasmids. Antibiotics are probably overused for prophylactic purposes, and this has contributed to the development and spread of antibiotic resistance in bacteria strains.

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COMPARATIVE STUDY ON SPECIFIC AND EARLY DETECTION OF PULMONARY MYCOBACTERIA COMPLEX USING SMEAR AND CULTURE METHOD AND SEROLOGICAL PATHOZYME EIA KITS

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The objective of this study was to compare the sensitivity and specificity of smear and culture methods with rapid serological EIA myco kits manufactured by Omega diagnostics, for the early detection of *Mycobacterium tuberculosis* (MTB) complex. Sera from various categories of smear and culture results were compared with the result of 38KDa, 16KDa and purified protein for IgA, IgM and IgG antibodies with sensitivity of 4%, 24% and 76%, respectively and with specificity of100% for IgG in Smear and Culture Positive (S^+C^+) category. The sensitivity of the test improved to a level of 80% for IgG + IgA without affecting the specificity. A combination of IgG + IgA and IgM further improved the sensitivity to 88% but reduced the specificity to 91%. Amongst the S^-C^+ and S^-C^- 64% and 14.7% were positive for IgG respectively. The predictive value of the kit using S^+C^+ subject was 96%. For all culture positivity (n=78), there was 2.6%, 33.3% and 71.8% sensitivity for IgA, IgM and IgG respectively. IgA +IgG and IgA + IgM + IgG combination gave 74.4% and 84.6% sensitivity respectively with the same level of specificity. Fifty-five percent of culture positive subjects were found to be MTB complex positive by routine biochemical tests, while 40% through PATHOZYME TB COMPLEX PLUS kit (high positive (H^+) values). When high positivity is combined with low positivity of the same kit ($H^+ + L^+$), 65% of the isolates were found to be MTB complex. Our study showed 88% sensitivity and 91% specificity for combined IgA + IgM + IgG antibodies recorded for MTB (S^+C^+ group) and 85% sensitivity and 91% specificity for all culture positives. Our study has demonstrated that the myco kits and TB complex plus kit produced by Omega Diagnostics are a good tool for specific, early and rapid identification of active tuberculosis for both diagnostic and epidemiological purposes.

Key words: Tuberculosis, diagnosis, comparative, specificity, sensitivity, culture and serological technique.

INTRODUCTION

Globally, pulmonary tuberculosis (TB) is the leading infectious cause of death, killing 3 million people every year (1). An increase in both pulmonary and extra pulmonary TB is taking place in both developed and developing areas of the world, complicated by AIDS pandemic and multi drug resistant species (2). In Nigeria, the tuberculosis situation has worsened in the past few years (3) and more than 80% of all cases of tuberculosis in Nigeria are of the pulmonary form (4). From the public health point of view, there is a definite need for a specific, sensitive and simple means of performing cost-effective and rapid diagnostic tests for pulmonary tuberculosis.

The existing specific and highly sensitive methods of diagnosis lack rapidity. For example, the culture method that takes between 4-8 weeks for a meaningful result to emerge. The radiological method is rapid but lacks specificity and the PCR method is specific and sensitive but rather expensive for routine diagnosis. Again, earlier attempts to develop serodiagnostic tests for pulmonary and extra-pulmonary tuberculosis with crude *Mycobacterium tuberculosis* antigens such as whole culture filtrate, purified protein derivative (PPD) and sonicates in ELISA had limitations. They either lacked adequate sensitivity or specificity or both. Therefore, a search for the identification of a highly specific

and rapid serological method for diagnosis of this ancient killer of man becomes imperative.

Omega's PATHOZYME TB EIAS utilize the immunodominant and highly specific 38KDa antigen. In addition, PATHOZYME MYCO utilizes a highly immunogenic lipopolysaccharide antigen (LPS). The MYCO range includes 3 individual kits for detection of IgG, IgA and IgM antibodies produced in response to infection by bacteria belonging to the genus mycobacterium. PATHOZYME TB COMPLEX plus contains microplates, which are coated with the 38KDa and another highly specific 16KDa antigen. The assay detects IgG antibodies specific to *Mycobacterium tuberculosis* complex namely; *M. tuberculosis, M. africanum* and *M. bovis,* which are largely implicated in bronchopulmonary tuberculosis in Nigeria and the world at large.

The goal of this study was to conduct a comparative analysis of diagnosis of *Mycobacterium tuberculosis* complex using conventional smear and culture method and rapid serological PATHOZYME EIA kit method manufactured by OMEGA Diagnostics, which are believed to be reproducible and specific.

MATERIALS AND METHOD

Study center/population:

The study was carried out in Lagos, Nigeria. One hundred and sixty two samples were collected from new subjects (15 years and above) presenting with bronchopulmonary disorders at Yaba Chest Clinics and Central Public Health Laboratory, Yaba, Lagos. Twenty-two (15 years and above), previously BCG vaccinated normal healthy individuals (NHI) serve as the control group. The study was carried out between October 2000 and August 2001. The samples were processed in the TB reference laboratory of Nigerian Institute of Medical Research Yaba, Lagos, Nigeria. The 162 patients were pre-counseled and their informed consent obtained and a repeated early morning sputa and single venous blood sample were obtained from each subject.

Microscopy/Culture

The sputum samples were decontaminated and concentrated by the modified Petroff method (5), and screened for the presence of acid-fast bacilli (AFB) by both ZiehlNeelsen (ZN) smear microscopy technique and by culture on 2 Lowenstein Jensen slopes. Growth was confirmed by culture smear demonstrating AFB. Four biochemical analyses were conducted on the isolates.

Serological procedure

The venous blood was put in clean new non-anticoagulated bottles. The blood was left to clot for one hour, the serum removed and subsequently stored at – 4°C until processed. The Serological procedures were conducted according to the manufacturer's technique. Both the reagents and the sera were brought to room temperature and 1/100 dilution of the sera used. The optical density (OD) were read at 450 nm and blanked on air with basic EIA reader (Genscreen Bio-Tek instruments).

Calculation and interpretation of results

Assay validations were shown to comply with the manufacturers standard. The OD of IgM low positive control, and IgG, IgA 2u/ml controls were > 0.20. (Manufacturer's standard). The control sera were each plotted on the semilog graph sheet provided and various computations and extrapolations were done according to the manufacturers protocol guide as follows;

Sero-units:	IgG	IgA
Negative Results	<400u/ml	>300u/ml
Low positive	400u/ml- 900u/ml	300u/ml-600u/ml
Positive Result	>900u/ml	>600u/ml

For IgM, cut off is OD of the average low positive control (LPC); Negative result is OD less than the OD of the low positive control, Low positive result is OD between the of LPC and (OD of the LPC X 1.5), and Positive result is an OD greater than the OD of LPC X 1.5.

RESULTS

Sputum and serum samples were collected from a total of 184 subjects, 102 (55.4%) male and 82 (44.6%) females, aged 15 years and above. One hundred and sixty two (88%) were patients presented at the Yaba Chest Clinic and Central Public Health Laboratory Yaba, with bronchopulmonary infections and 22 (12%) were previously BCG vaccinated normal healthy individuals.

Table 1 shows the serum antibody levels of isotypes IgG, IgA and IgM and TB complex plus to the purified antigen determined in subjects with new active smear and culture positive (S^+C^+) (n=50), smear positive and culture negative (S^+C^-) (n=16), smear negative and culture positive (S^-C^+) (n=28), smear negative and culture negative (S^-C^-) (n=68) subjects and normal healthy individuals (n=22).

Table 2 shows the positivity rate in the polar groups S^+C^+ and NHI. Thirty-eight out of 50 patients were positive for IgG antibodies giving a sensitivity of 76%. All the 22 NHI control sera were negative for IgG antibodies making TB IgG 100% specific. The positivity rates for other classes were low, for IgA (4%) and IgM (24%) antibodies, although there was specificity of 100% and 91% respectively. It was further observed that some of the sera, which were negative for IgG antibodies, were positive for IgA and/or IgM antibodies. When we combine the sera positive for IgA in those positive for IgG, 2 additional sera were included with none positive from the healthy normal individuals, maintaining a specificity of 100%, but very low sensitivity of 4% for IgA. Additional 4 sera were equally positive for IgM alone, when combined with those also positive for IgM in the IgG positive group, 12 patients became positive with a sensitivity of only 24%. Two normal healthy individuals were positive for IgM, reducing the specificity to 91%.

When we combine positivity for IgG and IgA (IgG + IgA), an increase in sensitivity to 80% (40/50) was recorded with 100% specificity. A combination of IgG + IgA +IgM, gave 4 additional positive results increasing the overall sensitivity of the test to 88% but reducing the specificity to 91%.

The overall predictive value of the positive results for IgG +IgA + IgM for the category S^+C^+ was 96%. It was observed that when the High positive (H^+) and Low positive (L^+) were combined for S^+C^+ sera, sensitivity of 100%, 36% and 60% were obtained for IgG, IgA and IgM respectively (Table 1). Amongst the S^+C^- (n=16), no High positivity was recorded for IgG, but 6 (37.5%) had Low positivity, while in S^-C^+ group (n=28), 18 (64.3%) had H^+ and 10 (35.7%) L^+ results. For S^-C^- clinical TB bronchopulmonary disease subjects (n=68), 10 (14.7%) had H^+ and 8 (11.8%) had L^+ results for IgG.

Table 3 demonstrates the positivity of all culture positive subjects (S^+C^+ + S^-C^+) (n=78). It showed that 66 (84.6%) were detectable serologically using the kits with 71.8% IgG sensitivity. For TB complex plus, which utilizes 38KDa and 16KDa antibodies specific for MTB complex, 22(28.2%) were H^+

from all culture positive subjects ($S^+C^+ + S^-C^+$), while 36 (46.2%) were positive when H^+ and L^+ were combined. This showed that 22 of 78 by high positivity (H^+) and 36 of 78 by high positivity plus low positivity ($H^+ + L^+$) were *M. tuberculosis, M. bovis or M. africanum.*

From culture morphology, period of growth, pigmentation and biochemical analysis, 55% of the isolates studied were found to be MTB complex.

TABLE 1: EIA positivity is sera of the subjects; smear and culture categories

Isotype:		**Smear and Culture category:**				**NHI**
		S^+C^+ [n=50] n(%)	**S^+C^- [n=16] n(%)**	**S^-C^+ [n=28] n(%)**	**S^-C^- [n=68] n(%)**	**[n=22] n(%)**
	H^+	38(76)	0(0)	18(64.3)	10(14.7)	0(0)
	L^+	12(24)	6(37.5)	10(35.7)	8(11.8)	0(0)
IgG	$L^+ + H^+$	50(100)	6(37.5)	28(100)	18(26.5)	0(0)
	N	0(0)	10(62.5)	0(0)	50(73.5)	22(100)
	H^+	2(4)	2(12.5)	0(0)	4(5.9)	0(0)
	L^+	16(32)	0(0)	2(7.1)	6(8.8)	0(0)
IgA	$L^+ + H^+$	18(36)	2(12.5)	2(7.1)	10(14.7)	0(0)
	N	32(64)	14(87.5)	26(92.9)	58(85.3)	22(100)
	H^+	19(36)	4(25)	6(21.4)	12(17.6)	2(9.1)
	L^+	12(24)	0(0)	12(42.9)	6(8.9)	2(9.1)
IgM	$L^+ + H^+$	30(60)	4(25)	18(64.3)	18(26.5)	4(18.5)
	N	20(40)	12(75)	10(35.7)	50(73.5)	18(81.8)
	H^+	14(28)	0(0)	8(28.6)	4(5.9)	0(0)
TB COMPLEX	L^+	8(16)	4(25)	6(21.4)	12(17.5)	0(0)
plus	$L^+ + H^+$	22(44)	4(25)	14(50)	16(23.5)	0(0)
	N	28(56)	12(75)	14(50)	52(76.5)	22(100)

Key

S^+C^+ = Smear and culture positive; S^+C^- =Smear positive and culture negative

S^-C^+ = Smear negative and culture positive; S^-C^- = Smear and culture negative

NHI = Normal Healthy individuals; H^+ = High positive; L^+ = Low Positive; N = Negative

TABLE 2: Kits Positivity in the Polar Groups; High Positive Group for S^+C^+

Isotype	S^+C^+ [n=50] n^+	%SEN	NHI [n=22] n^+	%SP
IgG	38	76	0	100
IgA	2	4	0	100
IgM	12	24	2	90.9
IgG + IgA	40	80	0	100
IgG+IgA+IgM	44	88	2	90.9

KEY: S^+C^+= Smear and culture positive; SEN = Sensitivity; SP = Specificity; n^+ = Number positive

Assay Validation: IgA and IgG 2U/ml control OD, must be > 0.2. Result: IgA = .239, IgG = .317

For IgM low positive control OD, must be > 0.2. Result; = .283

TABLE 3:

Positivity in the Polar Group Positive S^+C^+ and S^-C^+ (all culture positive groups)

ISOTYPE	ALL POSITIVE GROUP (S^+C^+ and S^-C^+) (n=78)	%SEN	%SP
IgG	56	71.8	100
IgA	2	2.6	100
IgM	26	33.3	90.9
IgG + IgA	58	74.4	100
IgG+IgA+IgM	66	84.6	90.9

DISCUSSION

The lack of adequate sensitive and specific early diagnosis of MTB has been one of the major problems in containing the spread of the disease. Existing methods of TB identification have various shortcomings; Acid Fast Bacilli (AFB) smear microscopy, requires the highest number of AFB in the sputum, requires at least 3 visits to a health facility by the patient and is labour intensive, time consuming and misdiagnosis is apparent. It has 50% sensitivity, but may be as low as 30%, and 98% specificity (2). Infact, World Health Organization in 1998 declared that bacteriological culture of tuberculosis is notoriously slow, difficult and expensive and the WHO guide concentrates on the exact

procedures and precautions needed to prevent errors and ensure reliable results.

In laboratories, increase pressure of work per day may not enable enough time to properly read each slide, therefore, the sensitivity is reduced to 25-35%. However, the culture method is still the most sensitive and specific for the identification of MTB, although results are delayed for weeks or months. Largely speaking, smear negative subjects are risks to the entire population. The disadvantages of microscopic based MTB diagnosis methods motivated early serological studies by Nassau *et al* in 1976 (6). Daniel and Anderson (7) reported 72% and 94% sensitivity and specificity respectively of species specific antigen 5 of *M. tuberculosis*, which was later proven to be the same as 38KDa antigen (8). The 38KDa antigen isolated by different methods has been used in diagnostic tests (mainly ELISA) with varying outcomes. As antigen 5 by Nassau *et al*, showed high specificity in all the studies conducted in the world (88-98%), while sensitivity ranged from 49% to 89% (8, 9,10,11, 12).

The 38KDa antigen used in the Omega Myco and TB plates was similar to those reported, in its ability to detect smear positive cases. In all the cited studies, antibody of class IgG alone had been measured except for study by UmaDevi *et al* in 2001 (8), which added IgA and IgM. This was after Omega diagnostics had produced their Myco kits. The scientific reason behind the combination of these antibodies was the fact that some sera that were negative for IgG were still positive for IgA and IgM antibodies. Generally, IgM detects more recent infection and this was indicated on the high level of IgM antibody detected from S^-C^+ subjects (n=28), but the significance of the use of IgA and IgM is questionable since Lagos, Nigeria is an endemic area for tuberculosis and many adults used for the study may have been exposed and infected with the bacilli.

The differences between our results and lower sensitivity reported by some others may be explained by the mode of preparation of the antigen reference. For instance, Omega used a recombinant 38KDa protein and a highly purified antigen derived from *Myco bacterium tuberculosis*, while UmaDevi *et al* used a 2-D preparative electrophoresis for the purification of their 38KDa antigens. The addition of 16KDa antigen for TB complex plus made the kit species specific. For Myco complex, sensitivity was shown for smear-positive and culture negative (S^+C^-) cases (n=16) and also with S^-C^- subjects, which had no way of correlating with the existing standard i.e. culture positively, otherwise, the high specificity and sensitivity recorded by these kits would have been a panacea to all important early and specific diagnosis of MTB.

From the result, 76% all S^+C^- subjects were of high positivity category, the remaining 24% were of low positivity. Since none of the S^+C^+ subjects were negative, we suggest that cases of low positivity, which presents with all symptoms of bronchopulmonary tuberculosis be regarded as positive for MTB. We therefore recommend a further addition of kits for measuring circulating immune complex (CIC) of anti-38KDa antibodies which would greatly improve the sensitivity and specificity of the kits and may help to buttress results acceptance for S^-C^+ cases as reported by UmaDevi *et al* (8), who reported that 45 of 55 S^-C^+ subjects were positive for CIC antibodies plus serum IgG, while 44 of 64 S^-C^- were

positive. An addition of a kit to measure anti-38KDa circulatory immune antibodies will equally help reduce false positives and hence improve sensitivity and specificity.

Our study has demonstrated that the Myco kits and TB complex plus kit produced by Omega Diagnostics are a good tool for specific early and rapid identification of active tuberculosis for both diagnostic and epidemiological purposes.

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METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES IN ILORIN, NIGERIA

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Nosocomial infections caused by methicillin-resistant strains of *Staphylococcus aureus* often pose therapeutic dilemma to the clinicians because of the multi resistant nature of these strains of *Staphylococcus aureus*. Outbreaks of both nosocomial and community acquired infections are also frequent and difficult to control. This study determined the prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) at the University of Ilorin Teaching Hospital, between January and December 2001. The methicillin disc diffusion method for the detection of methicillin resistance and the Kirby-Bauer disc diffusion for antibiotic susceptibility tests, were used. The MRSA prevalence rate was 34.7% (51/147) of all *Staphylococcus aureus* isolates. Forty-five isolates were associated with infections and 6 were colonizing strains. Thirty-six (70.6%) were hospital (nosocomial) acquired while 15 (29.4%) were community-acquired. Forty-eight patients have received antibiotics previously including 30 who had received multiple antibiotics. Skin and soft tissues were sites of infections in 36 cases and surgical, emergency and intensive care units accounted for 31 isolates. All MRSA isolates were resistant to more than two antibiotics but remained largely susceptible to third generation cephalosporins, macrolides and quinolones and all were sensitive to vancomycin. We recommend the use of third generation cephalosporins and quinolones where indicated, in the treatment of serious MRSA infections in this environment. Control of the spread of MRSA in this hospital must include reinforcement of appropriate use of antibiotics, hand washing and laboratory surveillance for MRSA, particularly in the surgical wards and intensive care units, in order to identify sources of outbreaks.

Keywords: Methicillin-resistant, Staphylococcus aureus, Ilorin.

INTRODUCTION

In recent times, strains of methicillin resistant *Staphylococcus aureus* (MRSA) have become a source of great concern to Clinical Microbiologists because these strains are now established nosocomial pathogens in health care facilities worldwide (1). These strains which appeared in Europe soon after the introduction of methicillin for β-lactam resistant *Staphylococcus aureus* in 1960 (2), and in the United States a decade later (3), were initially associated with sporadic outbreaks in hospitals, and generally sensitive to other antibiotics. However since the 1970s, these strains have been replaced by multiply resistant MRSA, which are now endemic in hospitals and are difficult to eradicate (4).

World wide, MRSA comprise up to 50% of *Staphylococcus aureus* isolates in hospitals (5), and is involved in surgical wound infections, nosocomial pneumonias, bacteraemias and other forms of infections (6-9). Treatment options have been severely restricted to potentially toxic antimicrobials, leading to increase mortality, morbidity and costs (10).

In view of the hetero-resistant nature of most strains of MRSA, routine microbiology laboratories may fail to detect MRSA if sensitivity testing is done at incubation temperatures greater than 35°C and if agar plates are read before 24 hours incubation (4). In spite of this, the overall incidence of MRSA isolation has gradually increased in many developed countries to present levels of about 30% in Spain, France and Italy (11) and up to 54% in Japan (12). In Africa, where routine laboratory testing may particularly fail to detect MRSA strain, it is not surprising that few hospitals report MRSA infection (13-16). In this institution, there is no routine screening for methicillin resistance among clinical isolates of *Staphylococcus aureus*. Previous studies in this centre (17, 18) have implicated *S. aureus* to be predominant Gram positive pathogen involved in nosocomial wound infections, burns, bacteraemia and septicaemia in all age group.

It is therefore appropriate to determine the contribution of MRSA strains to these infections and determine their susceptibility pattern. These would serve as guides to therapeutic approach and in formulation of control policy for MRSA in this environment.

MATERIALS AND METHODS

Patients

Hospitalized patients of all age groups and sexes in different wards and intensive care units with clinical features suggestive of sepsis were recruited into the study. Also outpatients attending the hospital general outpatient department for the first time on account of sepsis or sepsis-related conditions and with no previous hospitalization in the last six months were included in the study.

Specimens/Isolation

Routine clinical specimens made up of sputum, urine, swabs, pus/aspirates, blood and cerebro-spinal fluids were aseptically collected, transported and processed in the microbiology laboratory of the University of Ilorin Teaching Hospital according to standard techniques (19). Colonies of *Staphylococcus aureus* were identified on culture media by a combination of morphology, Gram reaction, positive catalase, tube coagulase and deoxyribonuclease tests.

Methicillin resistance screening

The methicillin disc diffusion test was employed (20). A sample of the test isolates and control strains were grown overnight at 35°C in nutrient broth. The overnight cultures were diluted with sterile saline (0.85% NaCl) in bijou bottles and their turbidity compared to 0.5 McFarland standard using white paper as background. The inocula were then spread with sterile cotton wool swab on Mueller Hinton agar (Oxoid, England) supplemented with 2% NaCl. 5 µg methicillin discs (Oxoid, England) were applied onto the plates with a sterile forcep. The agar plates were incubated for full 24 hours at 35°C aerobically and then inspected for growth. The diameter of zone of inhibition of each isolate was measured using a calibrated ruler and sensitivity or resistance estimated by comparing with the zone diameter interpretive standard (5). Isolates with zone diameter less than 14 mm to methicillin were considered methicillin resistant strains (5).

Susceptibility to other antimicrobials was carried out using Kirby Bauer disc diffusion method (21). The following single-paper discs were used; Chloramphenicol (30 µg), Erythromycin (15 µg), Tetracycline (30 µg),

Cefotaxime (30 µg), Ciprofloxacin (30 µg), Cotrimoxazole (1.25,23.75 µg), Vancomycin (30 µg), and Penicillin (1 mu). Zone diameter for sensitivity (S) of MRSA for each antibiotic was as defined by the National Committee for Clinical Laboratory Standards (5); Gentamicin (>15 mm), Chloramphenicol (>18 mm), Tetracycline (>19 mm), Cotrimoxazole (>16 mm), Vancomycin (>12 mm), Penicillin (>29 mm), Erythromycin (>23 mm), and Cefotaxime (>23 mm).

Patients bio-data

Where MRSA was isolated, full patient bio-data such as age, sex, types of infection, date of admission, length of admission, history of underlying diseases, operations and procedures, movement of patients in the hospital, previous hospitalization, antimicrobial therapy and outcome of infections were obtained from the request forms and case files. Using standard clinical and microbiologic criteria (22), MRSA was ascertained as infecting or colonizing strain. Where there was clinical evidence of infection, the MRSA was considered as infecting strain and where there was no clinical evidence to suggest the MRSA isolated was responsible for infection or when a strain was isolated from a site not involved in infection, the strain was considered a colonizer.

RESULTS

A total of 147 *Staphylococcus aureus* strains were isolated and characterized from clinical specimens during the study period, out of which 51 were methicillin resistant, giving an MRSA prevalence rate of 34.7%. Thirty-six of the 51 MRSA (70.6%) were acquired in the hospital (nosocomial) while 15 (29.5%) were acquired in the community. All 36 nosocomial strains were associated with clinical infections while 6 of the 15 community strains were colonizers. Surgical wards accounted for 24 of 36 (66.7%) nosocomial isolates, followed by ICU (19.4%), medical wards (8.3%) and emergency unit (5.6%). (Tables 1 and 2)

All the nosocomial strains were isolated from patients who had been hospitalized for at least one week for either illness initially unrelated to MRSA infections or had undergone surgery. All the community-acquired strains were isolated from patients attending the hospital general outpatients for the first time and had no previous hospitalization in the last six months before presentation. Skin and soft tissues were the commonest sites of infection, accounting for 80% of all infections in this study and infections followed surgery or trauma. Other sites of infection were the middle ear in 4 patients, bone in 3 patients and urinary tract in 2 patients (Table 3). The colonizing strains were all community acquired and were recovered in the urinary tract in one patient and upper respiratory tract in five patients.

Forty-eight (48) patients representing 94.1% of patients had used antibiotics within one month before MRSA was isolated. Thirty patients (58.8%) had used 3 or more different antibiotic types, 12 patients (23.5%) had used 2 while 2 patients (11.8%) had used 1 antibiotic previously. Only 3 patients claimed not to have used antibiotics at least six months before MRSA was isolated (Table 3). Ampicillin/cloxacillin was the commonest drug used by patients (37/51), followed by tetracycline (30/51), cotrimoxazole (20/51), chloramphenicol (17/51), quinolones (3/51) and others not specified (10/51).

The antibiotic susceptibility pattern of MRSA is as shown in Table 4. All isolates were sensitive to vancomycin (30 µg) and also moderately sensitive to cefotaxime (82.4%), erythromycin (76.5%) and ciprofloxacin (76.5%).

Fifty two point nine percent of the isolates were susceptible to gentamicin but the isolates were generally resistant to cotrimoxazole, chloramphenicol, penicillin, and tetracycline with 52.9%, 94.1% and 100% of isolates respectively resistant to these agents.

TABLE 1: Distribution of Methicillin-Resistant S.aureus isolates at UITH Ilorin

MRSA	Hospital acquired (%)	Community (%)	
Infecting strain	36(100)	9(60)	45(88.2)
Colonizing strain	0	6(40)	6(11.8)
TOTAL	**36(70.6)**	**15(29.4)**	**51**

TABLE 2: Ward Distribution of isolates at UITH Ilorin

Ward/Unit	No of isolates
Orthpaedic (W2)	10
General Surgery (W5)	14
ICU	7
Emergency (A/E, EPU)	2
Medical (W4, W6, W7)	3
GOPD	15
Total	**51**

ICU – Intensive Care Unit, W – Ward, EPU – Emergency Paediatric Unit

A/E – Accident and Emergency, GOPD – General Outpatient Dept.

Table 3: Characteristics of MRSA isolates at UITH Ilorin

1.	**Types/Sites of infection**	**Number (%)**
	i. Post operative skin/soft tissue infection	36(80)
	ii. Otitis media	4(8.9)
	iii. Osteomyelitis	3(6.7)
	iv. Unitary tract infection.	2(4.4)
2.	**Colonizing strain**	
	i. Upper respiratory tract	5(83.3)
	ii. Urinary tract	1(16.7)
3.	**Previous use of antibiotics**	
	i. 1 antibiotic	6(11)
	ii. 2 antibiotics	12(23.8)
	iii. > 3 antibiotics	30(58.8)
	iv. None.	3(5.9)
4.	**Types of antibiotics**	
	i. Ampicilin/Cloxacillin	37
	ii. Tetracycline	30
	iii. Cotrimozale	20
	iv. Chloramphenicol	17
	v. Quinolones	3
	vi. Others (not specified)	10
	vii. > 3 antibiotic types	30

Table 4: Antibiotic susceptibility pattern of MRSA isolates at UITH Ilorin

Antibiotic types	Number sensitive (%)	Number resistant (%)
Penicillin (1 mega unit)	3(5.9)	48(94.1)
Gentamicin (10μg)	27(52.9)	24(47.1)
Chloramphenicol (30μg)	3(5.9)	48(94.1)
Erythromycin (15μg)	39(76.5)	12(23.5)
Tetracycline (30μg)	0(0)	51(100)
Cefotaxime (30μg)	42(82.4)	9(17.6))
Ciprofloxacin (30μg)	39(76.1)	12(3.5)
Cotrimoxazole (1.25/23.75μg)	24(47.1)	27(52.9)
Vancomycin (30μg)	51(100)	0(0)

DISCUSSION

The MRSA prevalence of 34.7% (51/147) of *Staphylococcus aureus* isolates in this hospital is comparable to the prevalence reported by Bello *et al* in Jos (23), Durmaz *et al* in Turkey (24), Voss and Doebbeling in France, Italy and Spain (11), but less than the prevalence reported by Okésola *et al* in Ibadan (13), Kesah *et al* in Lagos (14), Sow *et al* in Dakar (16), Loureiro *et al* in Rio de Janeiro (25), and Lotsu *et al* in Japan (12). The prevalence is also higher than that reported in two Somalian hospitals among nasal carriers by Nur *et al* (15), in general hospital, Port of Spain among clinical isolates by Swanston (26), in Saudi University hospital by Bukharie and Abdelhadi (27), and in Scandinavian hospitals (28). The occurrence and frequenty of MRSA is known to vary geographically from hospital to hospital and over time depending on the level of infection control practices and antibiotic consumption rates (29). In the United States hospitals, prevalence rates reported vary between 10 and 50% (28).

Twenty nine point four percent (15/51) of the MRSA isolates in the study were community acquired. This is comparable to what obtains in some places (4,30), where these strains contribute significantly to the total MRSA problem. Community acquired MRSA infection is common among narcotic drug addicts (30). However, in all the patients with community acquired infection and colonization in this study none had history of narcotic abuse. Thirty-six of the 45 MRSA infecting strains were hospital acquired and 80% of the infection they caused occurred on the skin and soft tissues following surgery, 8.9% in the middle ear, 6.7% in the bone and 4.4% in the urinary tract. This is in conformity with reports elsewhere (25,, 30). *Staphylococcus aureus* is a normal flora of the skin in most people and expectedly can contaminate wounds or any breach in the integuments to set up a focus of infection. Six of the 15 community acquired MRSA strains were harboured by patients in their respiratory and urinary tracts. These strains were isolated in the course of investigations for suspected pulmonary tuberculosis in 5 of the patients and urinary tract infection in 1 patient, but were found not to be involved in clinical disease. Anterior nares, skin and upper respiratory tracts are known common sites of colonization by MRSA (26, 28).

Staphylococcus aureus is usually found in surgical, intensive and emergency care services and it is therefore not surprising that most (91.7%) of the MRSA isolates in this study were located in these service areas where antibiotic usage is greatest. All but 3 of the patients with MRSA infection have been exposed to antibiotics previously, which included ampicillin/cloxacillin, tetracycline, chloramphenicol and cotrimoxazole and 30 (58.8%) have used 3 or more (multiple) different antibiotic types before developing infection.

All the MRSA isolates were resistant to tetracycline and all but 2 were resistant to chloramphenicol and penicillin, 3 most commonly abused drugs in our environment. However, MRSA isolates here remained largely sensitive to the third generation cephalosporins, macrolides and quinolones. This is at variance with what obtains in some places (11, 24, 26). This observation can be explained by the fact that these drugs are expensive and therefore not readily available and affordable by majority of people here, who

are of low socioeconomic class. A previous study in this hospital showed *Staphylococcus aureus* isolated from wound infections to be generally sensitive to these agents (17). It is however interesting to note the relatively high sensitivity of MRSA in this study to gentamicin (52.9% of isolates sensitive), which though at variance with the trend world-wide, may represent a phenotype of MRSA (GS-MRSA) similar to the ones reported recently in French hospitals (31). Southern blot hybridization with *mec A* and *aa6'-aph2'* gene-specific DNA probes would have confirmed the presence of methicillin resistance gene and the lack of the bifunctional 2' aminoglycoside phosporylase-6-aminoglycoside acetytransferase gene (*aac6' – aph2'*) responsible for gentamicin, tobramycin and amikacin cross-resistance in these strains. Unfortunately there is no such facility in this environment.

All the MRSA isolates in this study were sensitive to vancomycin. This is in agreement with reports elsewhere in this country (13, 14), and in other places (11, 23, 31). Vancomycin has remained the drug of choice for treating serious MRSA infections such as endocarditis and septicaemia for over 40 years (10). This glycopeptide is relatively toxic and needs to be administered by intravenous infusion. There are no published reports on vancomycin usage in our environment and hence resistance is not expected. However Japanese and American researchers have isolated MRSA with reduced susceptibility to vancomycin from patients with MRSA endocarditis who were on intravenous vancomycin therapy (33). It is only a matter of time before vancomycin resistant MRSA becomes another significant epidemiologic problem.

The methicillin disk diffusion susceptibility test to detect methicillin resistance used in this study has been standardized (20). In this study, Mueller-Hinton agar was supplemented with 2% NaCl, 5 μg methicillin disc was used, and plates were incubated for full 24 hours at 35°C. Isolates that initially appeared sensitive when plain Mueller-Hinton agar was used became resistant when supplemented with 2% NaCl. The mechanism of methicillin resistance is complex and still unclear (34). The chromosomal *mec A* gene in MRSA is believed to encode an abnormal penicillin binding protein (PBP 2a) with reduced binding affinity to penicillin and related β-lactam compounds. Expression of the resistance gene is increased in the presence of 2-4% NaCl, pH more than 5.2, incubating temperature of 30-35°C and the presence of β-lactam agents in the medium (34). This study has confirmed some of these assertions.

CONCLUSION/RECOMMENDATION

The prevalence of MRSA in this institution is relatively high. It is therefore imperative to establish a guideline for MRSA detection and control in the hospital by; daily monitoring of clinical laboratory for MRSA isolates, a programme of monthly prospective culture surveillance of inpatients believed to be at high risk for acquisition of MRSA, re-identification of previously colonized or infected patients at the time of subsequent readmission into the hospital, screening of hospital personnel when an outbreak is suspected, routine hand washing by hospital personnel and policy regulation antibiotic prescription and usage. With the unavailability of vancomycin in our environment coupled with its toxicity, we recommend the use of

third generation cephalosporins such as ceftazidime, cefotaxime or ceftriaxone as well as the fluoroquinolones where indicated, in the treatment of serious MRSA infections in this locality.

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ANTIBIOTIC SUSCEPTIBILITY PATTERN AND MULTIPLE ANTIBIOTIC RESISTANCE INDEX OF PSEUDOMONAS AERUGINOSA URINE ISOLATES FROM A UNIVERSITY TEACHING HOSPITAL

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Urine samples submitted to the Medical Microbiology diagnostic laboratory of the Ahmadu Bello University Teaching Hospital, Zaria, were routinely screened for *Pseudomonas aeruginosa* over a three-month period with 13/150 (8.67%) of the pathogenic bacteria isolated positively identified. All the isolates were resistant to the cheap, commonly available antibiotics; rifampicin, ampicillin/cloxacillin, erythromycin, chloramphenicol and ampicillin but were uniformly susceptible to ciprofloxacin. The high prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance for continuous tracking of antibiotic resistance trends among all clinically relevant isolates and introduction of effective interventions to reduce multidrug resistance in such pathogens.

Key words: *Pseudomonas aeruginosa*, antibiotic susceptibility, multiple antibiotic resistance, urinary tract infection

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen, which is highly resistant to antibiotic therapy (1). Previously, *Pseudomonas aeruginosa* was rarely considered as a real pathogen (2), perhaps because despite abundant opportunities to spread, its ability to survive in almost any environment, its innate resistance to many antibiotics or disinfectants and its array of putative virulence factors, *Pseudomonas aeruginosa* rarely causes community acquired infections in immunocompetent individuals (3).

Nowadays, however, it is among the most common pathogens involved in nosocomial infections (4), and has been described as a lethal pathogen with 34% of bacteraemia mortality attributable to it, a crude mortality of 50% in the bacteraemic neutropaenic host and an overall mortality of 45% and 69% respectively, in bacteraemic nosocomial pneumonia and pneumonia in mechanically ventilated patients (2).

Pseudomonas aeruginosa has also been isolated as a pathogen responsible for urinary tract infections (UTIs) representing 10.7% of isolates found exclusively in nosocomial UTIs (5), 3.5% in intensive care units, 35.6% in other hospital units and 27.7% in out-patients and general practice (6).

It has been noted that antimicrobial resistance is a global concern (7). There is need for accurate and up-to-date information regarding the frequency of resistance, resistance trends and comprehensive comparison of various antimicrobial agents tested against different pathogens. Moreover, the case with which resistance develops to traditionally used anti-pseudomonads (due to

mutation, acquisition of plasmids, possession of intrinsic resistance factors) has increased dramatically in recent years. The possession of efflux pump systems capable of conferring resistance to a wide range of unrelated classes of antimicrobial agents has also been demonstrated in *Pseudomonas aeruginosa* (1).

There is therefore the need for antimicrobial sensitivity testing to be done routinely and accurately as guide to clinical judgments in the chemotherapy of *Pseudomonas aeruginosa* infections. In this paper, we report the results of antibiotic susceptibility and multiple antibiotic resistance (MAR) index of *Pseudomonas aeruginosa* isolates obtained from urine samples in the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

MATERIALS AND METHODS

Isolation and Characterization

Urine samples submitted to the Medical Microbiology diagnostic laboratory of the Ahmadu Bello University Teaching Hospital, Zaria were routinely screened for *Pseudomonas aeruginosa* over a three-month period. Samples inoculated into sterile peptone water were grown overnight at 37°C and subcultures made into appropriate selective and diagnostic media. All isolates which produced colonies that were non-lactose fermenting, colourless or with shades of greenish pigments on MacConkey or Nutrient agars, oxidase positive, and Gram-negative rods, were subcultured on Pseudosel® agar. Isolates which were able to grow on this agar producing greenish or brownish colonies with foul smelling odour, and failed to ferment any of glucose, lactose, arabinose, sucrose, mannitol and xylose; but possessed such biochemical characters as determined with Kligler iron agar, ability to utilize urea, citrate as well as positive catalase test were identified as *Pseudomonas aeruginosa* (8,9).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was determined using the agar diffusion plate method as described by the National Committee for Clinical Laboratory Standards (10). The antibiotics used were; ampicillin 30 µg, rifampicin 10 µg; ampicillin/cloxacillin 30 µg, ciprofloxacin 10 µg, gentamicin 10 µg, streptomycin 30 µg, erythromycin 30 µg and chloramphenicol 20 µg. *Pseudomonas aeruginosa* NCTC 10662 served as control.

Determination of MAR index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (11,12).

RESULTS

A total of 213 urine samples were screened within the study period with 150 pathogenic bacteria isolated. Thirteen of the 150 pathogenic bacteria isolated were identified as *Pseudomonas aeruginosa* giving a positive rate of 8.6%. All the *Pseudomonas aeruginosa* isolates were resistant to rifampicin, ampicillin / cloxacillin, erythromycin, chloramphenicol and ampicillin. However, 77%, 92.3% and 100% were susceptible to streptomycin, gentamicin and ciprofloxacin respectively (Fig.1). All the isolates were found to be multi-resistant with MAR index of at least 0.625 (Table 1). Three distinct resistant patterns were identified and presented in Table 2.

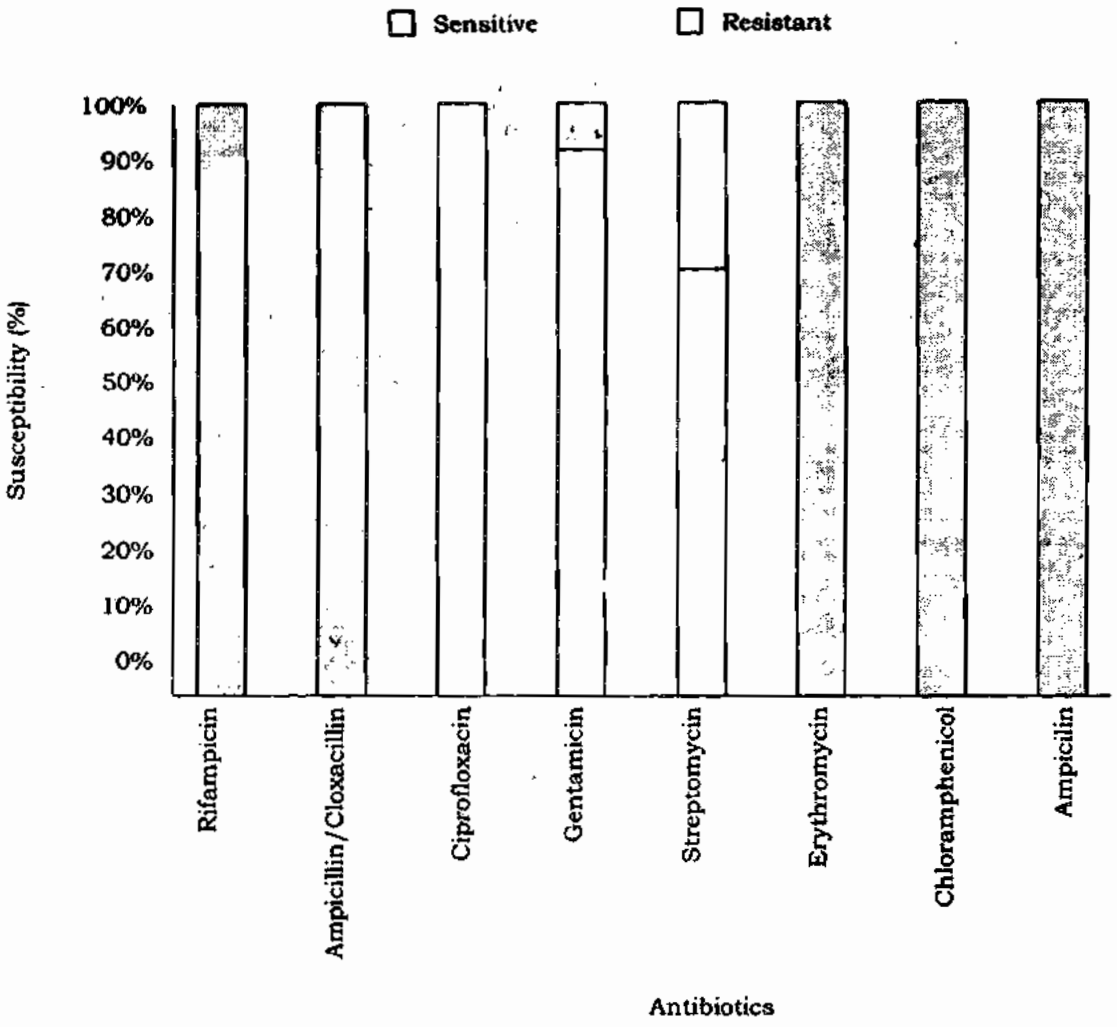

Fig. 1: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates

Table1: Multiple antibiotic resistance (MAR) index of *Pseudomonas aeruginosa* isolates

MAR Index	No of isolates	%
0.625	10	76.923
0.75	2	15.385
0.875	1	7.629
TOTAL	**13**	**100**

Table 2: Resistance patterns in *Pseudomonas aeruginosa* isolates

Resistance Pattern	%
Rif^R, Apx^R, $Gent^R$, $Strep^R$, $Eryth^R$, Chl^R, Amp^R,	7.692
Rif^R, Apx^R, $Eryth^R$, Chl^R, Amp^R,	76.923
Rif^R, Apx^R, $Strep^R$, $Eryth^R$, Chl^R, Amp^R,	15.385
TOTAL	**100**

DISCUSSION

Previous studies in this environment have confirmed urine to be the leading source of *Pseudomonas aeruginosa* in hospital settings (13). This observation was attributed to the fact that most patients going for surgery tend to get catheterized, a procedure which has been shown to create inherent risks for infections (14). A prevalence rate 8.67% was observed for the isolation of *Pseudomonas aeruginosa* as a urinary pathogen in this study. This was higher than an earlier report of 6.4% prevalence in blood cultures (15), but agrees with 14.4% (all laboratory specimens) of Oduyebo, *et al* (16), and the 10.5% of Olayinka (13). The seeming fluctuation in prevalence rates in these studies was probably due to the differences in sample size and the durations of sampling.

About 7.7% of *Pseudomonas aeruginosa* isolates studied in this work were found to be resistant to gentamicin. This is significant because gentamicin is traditionally considered in this environment as the first line drug against Gram-negative bacilli in the hospital setting (16). The level of resistance in this study to streptomycin (another aminoglycoside) is 23%. There is little conclusive information on the mechanism of *Pseudomonas aeruginosa* resistance to aminoglycosides; however, the observed resistance to the penicillins (ampicillin 100%, ampicillin/cloxacillin 100%) in this study may not be unconnected with the well-known fact that *Pseudomonas aeruginosa* is intrinsically resistant to the penicillins and other antimicrobial agents (17).

The *Pseudomonas aeruginosa* isolates were uniformly susceptible to ciprofloxacin (a fluoroquinolone) underlying the need for the rational use of antibiotics, as it is known that new and costly antibiotics are less available for abuse/misuse (16). The multiple antibiotic resistance (MAR) index gives an indirect suggestion of the probable source(s) of an organism. According to previous workers, MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used (11,12), and as evident in Table 1, all of the isolates have MAR index far greater than 0.2 and are resistant to at least five antibiotics (Table 2).

Some of the mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa* have been well studied (4,6). Some of the inherent resistance factors are impermeability, multi-drug efflux pump systems and a chromosomal *AmpC*-lactamase. Resistance to β-lactams and aminoglycosides can also arise from the acquisition of plasmids, transposons or integrons encoding β-lactamases and aminoglycoside-modifying enzymes (6). In addition to the constitutive low level of susceptibility of this organism to antimicrobial agents, new mechanisms of resistance have been identified, which include the production of β-lactamases (18). In the present study, 38.46% of *Pseudomonas aeruginosa* isolates produced β-lactamases. The significance of this observation derives from the knowledge that some of these enzymes can hydrolyze β-lactam agents (4).

At present, there are no strict rules concerning antibiotic prescriptions in this hospital, but in view of the fact that concerns are mounting about the spread of antimicrobial-resistant strains of microorganisms the world over, continued surveillance of antibiotic-resistance profile and

the effective communication of same to all involved in the use of antibiotics appears mandatory. Similarly, the high prevalence of multidrug-resistant strains of *Pseudomonas aeruginosa* in this study underscores the need for effective control measures in this environment.

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MEASLES IN ILORIN: AN EPIDEMIC IN THE MIDDLE OF ERADICATION PLANS

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Forty-one cases of measles were seen within 3 months period in Emergency Paediatric Unit (EPU) of the University of Ilorin Teaching Hospital (UITH) as against a recent report from the same center, which reported 52 cases over a 12 months period. More of the patients in this series needed hospitalization. Males were more affected, 17.1% were infants while 12.2% were older than 10 years. Coliforms were isolated from 40% of the positive blood cultures. Pneumonia was the leading complication and sultamycillin was the commonly used antibiotics. The mean duration of hospital stay was 6.3 days (SD=2.9) and case fatality was 14.6%. This pattern in the middle belt region of Nigeria represents an epidemic. This occurring at a time when a global eradication is being planned and anticipated, calls for a re-appraisal of the dynamics and logistics of the Expanded Programme on Immunization (EPI)

Keywords: Measles; Epidemics; Global Eradication.

INTRODUCTION

During the early 1980s, in the aftermath of smallpox eradication, some public health officials and scientists proposed that effort be directed towards global eradication of measles. However, few years after, the herd immunity required to interrupt measles virus transmission was much higher than obtained in most communities and prospect for eradication receded (1,2). The major obstacle to its eradication were the contagiousness of measles, the lack of a vaccine that is effective among children aged less than 9 months and the incorrect perception that measles is a mild illness (3).

Since the inception of Expanded Programme on Immunization (EPI) in 1974 the number of cases and deaths attributed to measles have declined substantially from an estimated 100 million cases and 5.8 million deaths in 1980 to an estimated 44 million cases and 1.1 million deaths in 1995. In 1974, only 5% of world's children aged 12-23 months had been vaccinated against measles. By the middle of the 1980s, measles vaccination coverage among children of the same age group in developing countries had reached 42%. From 1985 through 1990, measles cases reported worldwide declined by 56% because of heightened efforts by governments and donor agencies (4). Despite these achievements, measles remain one of the leading causes of childhood mortality in developing countries, especially among the under 5 years old children from places of low vaccination coverage (5). Even countries that have recorded several years of low incidence frequently have experienced large measles outbreaks (6,7).

There had been anecdotal reports and personal communication that measles may be occurring in epidemic manner in other parts of Nigeria, which have not been previously documented in Ilorin, Nigeria. Ilorin, the capital of Kwara State has better immunization coverage when compared with

other parts of Nigeria. The State has won the national awards for leading in immunization coverage in the Poliomyelitis Eradication and if such performance would be extrapolated for other antigens, then one expect measles coverage to be optimal in Kwara State as well. I therefore, decided to study the occurrence of new cases (incidence), of measles prospectively in UITH Ilorin.

METHODS

Site

The University of Ilorin Teaching Hospital (UITH) is a referral center, which serves both as teaching institutions as well as general hospital in Kwara State. The hospital is made up of two wings namely general hospital wing where emergency paediatrics unit is located and maternity wing. The maternity wing consists of the Obstetrics and Gynaecology department and the Neonatal Intensive Care Unit. The hospital is centrally located within the middle belt zone of the country and serves patients from sub-urban and rural areas in at least six other states of Ekiti, Osun,. Kogi, Oyo, Niger and Sokoto states. Ilorin being a state capital is multiethnic and multinational in composition.

Patient recruitment and logistic

All children presenting in the Emergency Paediatrics Unit (EPU) of the hospital within 3 consecutive months period (January 1st–March 31st 2000) who satisfied the World Health Organization (WHO) case definition for measles were recruited and prospectively studied. No viral studies were done for lack of facilities. The age, sex, weight, presenting clinical and immunization status were recorded. All the patients were managed in a standard way. Those with complications were on admission while others were managed on out patient basis. Standard care consisted appropriate antibiotics, calamine lotion, adequate fluids and /or food, Vitamin A administration on days 1, 3 and 14. Patent on admission were reviewed during ward round twice daily (morning and evening ward rounds) and patients on out patient basis were seen every 72 hours.

RESULT

During the study period, a total number of 306 patients were admitted, 178(58.2%) were male while 128 (41.8%) were females. Within this study period, 41 cases of measles were seen out of which 22 (53.7%) were admitted. This constituted 7.2% of the total admission over the period.

The sex, age and admission status distribution of patients with measles were as shown on Table 1. Seven (17.1%) were infants, 26 (63.4%) were between 1 and 5 years old. The under-5 year old constituted 80.5% while 3 (7.3%) were 6-10 years of age and 5(12.2%) were above 10 years of age. Twenty-seven (65.9%) were males while 14 (34.1%) were females and 22 (53.7%) were admitted while 19 (46.3%) were treated on out patient basis.

All patients presented with fever (100%) while other presenting features were as shown on Tables II. Skin rash was seen in 23 (56.1%), cough in 17 (41.5%), diarrhoea in 12 (29.3%), eye discharge in 8 (19.5%), catarrh in 17 (17.1%), vomiting in 6 (14.6%), breathlessness in 5 (12.2%) while convulsion, restlessness, mouth ulcers, refusal of feeds and submental lymphadenopathy were present in 1 (2.4%) patient each.

The complications were as shown in Table III. Bronchopneumonia was seen in 18 (43.9%), diarrhoea disease in 12 (29.3%),

purulent conjunctivitis in 8 (19.5%), pharyngitis in 3 (7.3%), malnutrition (Kwashiokor), convulsion and mouth ulcers in 1 (2.4%) patient each.

The variety of generic antibiotics used in the cases of measles was as presented in Table IV. Sultamycillin was used in 22 (53.7%), Gentamycin in 19 (46.3%) patients while Ampicillin and Cloxacillin were used in 7 (17.1%) patients each. Chloramphenicol was used topically in 3 (7.3%) patients, crystalline penicillin in 2 (4.8%) patients while erythromycin was given to 1 (2.4%) patient.

Seventeen patients (41.5%) had blood culture done and isolates were grown in 5 (12.2%). The percentage culture yield was 29.4%. The organisms isolated were Coliforms 2 (40%), *Staphylococcus aureus* 1 (20%), viridian streptococcus 1 (20%) and Acinetobacter 1 (20%).

The mean duration of fever, the only constant symptom before presentation, was 6.3 days (SD = 2.9). The case fatality was 14.6% as 6 patients with measles died of one complication or another.

Table 1: Distribution of measles case by age, sex and admission study

PARAMETER	NUMBER	PERCENTAGE (%)
AGE (YEARS)		
>1	**7**	**17.1**
1 – 5	**26**	**63.4**
6 – 10	**3**	7.3
>10	**5**	12.2
Sex		
Males	**27**	65.9
Females	**14**	34.1
Admission Status		
Admitted	**22**	53.7
Not Admitted	**19**	46.3

Table 2: Presentation of 41 patients with Measles

PRESENTATION	NUMBER	PERCENTAGE (%)
Fever	41	100
Rash (skin)	23	56.1
Cough	17	41.5
Diarrhoea	12	29.3
Eye discharge	8	19.5
Catarrh	7	17.1
Vomiting	6	14.6
Breathlessness	5	12.2
Mouth ulcers	1	2.4
Convulsion	1	2.4
Restlessness	1	2.4
Refusal of feed	1	2.4
Submental Lymphadenopathy	1	2.4

Table 3: Complications in 41patients with Measles

COMPLICATION	NUMBER	PERCENTAGE (%)
Brochopneumonia	18	43.9
Pharyngitis	3	7.3
Malnutrition	1	2.4
Diarrhea disease	12	29.3
Convulsion	1	2.4
Mouth ulcers	1	2.4
Purulent conjunctivitis	8	19.5

DISCUSSION

The incidence of measles had remarkably gone down worldwide until recently when an upsurge was been noticed (6,8). In Nigeria, there have been anecdotal reports of probable epidemics in certain states of the country earlier on (9). This has not been previously documented in Ilorin. In a most recent report by Ojuawo *et al* (10), 52 patients were seen over a 12 months period showing a remarkable variation to this report where 41 cases were seen within just 3 months period. Incidences of 1.3% in Ojuawo's report and 7.2% in this report were incomparable. Also in Benin, Nigeria, 68 cases were reported over a 3-year period (1). Report in this study probably qualifies for an epidemic.

The age distribution was comparable to the previous study in the same centre (8). Infants constituted 17.1% of all the patients. A significant number of the patients were also older children. This is in contradistinction to other studies in Nigeria (12,13). The need for admission in this series was much more or higher than the previous study in Ilorin or other centers in Nigeria (8, 10 –13). This might suggest that the cases in this series were more severe and probably have higher incidence of measles complications.

The variety of bacteria isolates grown in 5 patients form the baseline report from Ilorin. Except for Acinetobacter, all isolates were not unusual agents. The choice of antibiotics in treating bacterial infections complicating measles reflected on Table IV is also a foremost report from Ilorin. However, the antibiotics were within the usual range in other reports. Sultamycillin was mostly used probably because of its broader spectrum of activities against both Gram-positive and Gram-negative organisms. The mean duration of the most constant symptom, fever of 6.3 days, shows that majority of parents still do not consider fever as a major problem and hence the delay in seeking medical helps. Some might also have sought medical helps but with a missed diagnosis and later came to the emergency room when it became obvious that the first medication would not work. The 14.6% case fatality was remarkably higher than previous report from our center and from Benin, Nigeria. This might be due to severity of the illness and the heavy patients load that over stretch our facility.

With the present status of measles in Ilorin and occurrence of epidemics involving

older patients, a constant surveillance is advised. Also a re-immunization with measles vaccine should be considered. The sources of our vaccine must be carefully chosen and cold chain meticulously maintained for potency preservation. Also, with the occurrence of measles in children below 9 months, there is need to consider the use of Edmondson-Zegreb vaccine, which could be used in spite of the presence of the maternal immunity in Nigeria.

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AWARENESS AND COMPLIANCE OF ANTITETANUS IMMUNZATION AMONG ADULT FEMALES IN A TERTIARY INSTITUTION IN NIGERIA

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The purpose of this study was to investigate the awareness level and the compliance to anti-tetanus immunization among adult females in an urban community in South West of Nigeria. The rationale for the study was informed by the fact that high incidence of tetanus infections and deaths are still being reported from our clinics regularly. A total of 394 female workers and students of LAUTECH University and its Teaching Hospital participated in the cross-sectional survey. They were selected using the stratified sampling procedure. A pre-tested structure but open-ended questionnaire was administered on the respondents. The result of the analysis showed a high level of awareness (69. 8%) of anti-tetanus immunization among the respondents and a high significance association was found between the level of awareness and respondent's profession (X= 7. 65; p < 0. 0011). Majority of the respondents (56.1%) took their last dose of anti-tetanus immunization during their last pregnancies. More than one third (37.2%) of the 148 respondents who ever gate birth took only one dose or none during their last pregnancy, thus putting into question the immunological status of those mothers and the children they gave birth to then. It was concluded that despite the high level of awareness among the respondents, compliance was quite low. It is recommended that government should provide logistic supports to make immunization programme accessible on a sustainable basis to everybody in the country. Activities should be put in place that will promote behavioural change in women so that they can go for anti-tetanus immunization.

Keywords: **Antitetanus immunization, adult females; awareness; Compliance.**

INTRODUCTION

Tetanus is a serious disease with high mortality rate. Globally, the disease kills more than 450,000 infants each year and nearly 40,000. mothers die from tetanus infection acquired during delivery (1, 2). Maternal tetanus is caused by contamination from tetanus spores through puncture wounds and is linked with abortions and deliveries that are unsafe or unclean. Symptoms are similar to those of neonatal tetanus, including tight jaw, stiff neck and body muscles, difficult swallowing and spasms. Cases occur between 2 and 21 days after the injury; most occur within 14 days (3). Tetanus is very difficult to treat but can be prevented easily by vaccination especially if adequate number of doses are given to confer the required level of immunity.

Several studies have demonstrated that a large percentage of people are inadequately immunized against tetanus (4). A study carried out in Turkey to quantify anti-toxoid IgG antibody in blood sera in different age groups revealed a decline of tetanus IgG antibody with age (5). Another study that was undertaken to determine the serological response in children aged 1-15 years immunized with diphtheria-pertusis-tetanus vaccine (DPT) alone or with tetanus toxoid (TT) booster dose under the Expanded Programme on Immunization (EPI) in Dar es Salaam and Bagamoyo, Tanzania concluded that the current DPT immunization schedule provides adequate tetanus immunity

for children under 5, but however, about half of the older children had no protection against tetanus (6). A community-based study in Rivers state of Nigeria to determine the status of tetanus toxoid immunization in parturient women revealed that among the women surveyed, only 41.2% had complete (two doses of tetanus toxoid) immunization, while 58.8% had partial or no coverage status (7). In 1996, the Advisory Committee on Immunization practices (ACIP), and the American Academy of Family Physicians (AAFP), and the American Medical Association recommended a well-child office visit at age 11 to 12 years to check vaccination status. They recommended that vaccination status should be assessed for vaccine preventable diseases including diphtheria-tetanus (DT) toxoid not given in the past years (8).

A total of 2,945 cases of tetanus and 1,871 cases of neonatal tetanus were reported in Nigeria by the World Health Organization (WHO) in 1996 and 1998 respectively (9). The TT2 Coverage in Nigeria in 1998 was 29% (9). Tetanus infections and deaths from the disease are still being reported in our clinics regularly. The rising incidence of emerging and reemerging infectious diseases also remain a matter of great concern (10). In line with the above, this study was therefore carried out to assess the level of awareness and compliance of anti-tetanus immunization among a cross-section of adult females in South West of Nigeria.

MATERIALS AND METHODS

The subjects for this cross-sectional survey were adult Nigerian females. They were students of the Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, the LAUTECH Teaching Hospital, Osogbo both in South West Nigeria. Others were drawn from the school of nursing and among the health workers (nurses and doctors) of the LAUTECH Teaching Hospital. A pre-tested open-ended self-administered questionnaire was used for the study, which was carried out during the last quarter of 2002. The questionnaire contained questions on the background characteristics of respondents, their awareness of anti-tetanus immunization, if they had ever taken the immunization, how many doses of anti-tetanus immunization they have received and reason for taken them. About 450 questionnaire forms which was about 20% above the sample size estimated (to allow for non-response and incomplete filling of the questionnaire) were distributed to the female students and workers in the study area using the stratified random sampling method. The survey covered female students in the humanities, engineering, medical faculties; the female staff of the teaching hospital and students in the institution's nursing school. A total of 394 adult female respondents filled the questionnaire adequately enough for analysis. The percentages with the anti-tetanus immunization status were calculated as described by the WHO EPI coverage survey (11). Computer using the EPI-INFO version 5.0 statistical package (12) was used for data analysis. The Chi-square test was used to test level of association and significant value was pre-set at 0.05.

RESULTS

Table 1 presents information on some selected background characteristics of the 394 adult female respondents. Majority of the respondents are between 20 and 29 years of age (69.0%), while very few 3.6% and 5.0% were below 20 years and above 40 years of age

respectively. There were more (71.3%) respondents of the Yoruba ethnic group than the other ethnic groups (28.7%). Most of the respondents (62.7%) were Christian, 32.5% were Muslim and the rest 4.8% belong to other religions. Of the respondents, 56.6% were single and 40.4% were married. More than three quarter (78.9%) of the respondents had post secondary education, 14.2% had secondary education while only 6.9% had less than secondary education. About 48.2% of the respondents can be classified as health professionals, while 51.8% were non-health professionals.

Table 2 contains information about the level of awareness and compliance with the anti-tetanus immunization among the respondents. A total of 275 (69.8%) out of the 294 adult female respondents reported that they were quite aware of one type or the other of the anti-tetanus immunization programmes. However, only 97 (24.6%) of the respondents have received more than two doses of tetanus immunization in adulthood (apart from childhood immunization), which are considered enough to confer some immunity on the recipient. Of the respondents, 37.6% had been pregnant and given birth to a child before. (Table1)

Table 3 shows that there is no significant difference between respondents' educational status and level of awareness of anti-tetanus immunization ($p > 0.24$); while Table 4 shows a highly significant association ($p < 0.001$) between respondents' occupation/profession and awareness of anti-tetanus immunization. Table 5 shows that majority (41.4%) of the respondents are aware of the 2 doses of anti-tetanus immunization for pregnant women; 33.1% are aware of the TT1-TT5 immunization schedule for women of childbearing age; while only 13.2% are aware of the anti-tetanus booster dose for secondary school students. Table 6 shows that majority (56.1%) of the respondents who ever took anti-tetanus in adulthood did so during their pregnancies; 21.2% got the immunization post injury; 10.6% took it as booster dose while in secondary school and only 7.7% took it as part of pre-employment medical test. More than half (51.2%) of the respondents who ever received anti-tetanus immunization in adulthood took the last dose over a year ago, while 46% took the immunization during the last one-year (Table7). Of the 148 respondents who over gave live birth, 54.0% received two doses of TT during the last pregnancy; 8.8% received more than 2 doses; while 37.2% did not receive or received only one dose of TT during their last pregnancy (Table 8).

Table 1: Selected Background Characteristics of Respondents

Variables		Frequency [N]	Total [394 [%]
Age group [in yrs]	15-19	14	3.6
	20-24	135	34.3
	25-29	137	34.7
	30-34	59	15.0
	35-39	29	7.4
	40+	20	5.0
Marital status	Single	223	56.6
	Divorced/Widowed	12	3.0
	Married	159	40.4
Ethnic group	Yoruba	281	71.3
	Ibo	67	17.3
	Hausa	25	6.3
	Others	21	5.3
Religion	Christianity	247	62.7
	Muslim	128	32.5
	Others	19	4.8
Educational status	No formal schooling	5	1.3
	Primary	22	5.6
	Secondary	56	14.2
	Post secondary	311	78.9
Occupational/ Professional status	Nursing/Medical Students	128	32.5
	Health workers	62	15.7
	Other students	146	37.1
	Other non-health Workers	58	14.7
Ever given birth To a child before	Yes	148	37.6
	No	246	62.4

Table 2: Distribution of respondents by level of awareness and compliance with the anti-tetanus immunization programme.

Variable		Frequency	Total [394]
Awareness			
Antitetanus Immunization	Yes	275	69.8
	No	119	30.2
Compliance with			
Antitetanus Immunization [No. of doses TT. received]	Received 2+ doses	97	24.6
	Rec. only 2 doses	87	22.6
	Rec. only one doses	62	15.7
	Never received	148	37.6

Table. 3. Distribution of respondents by level of education and awareness of Anti-tetanus immunization programme

Educational status	Awareness		Total
	Yes [%]	No [%]	N [%]
Primary or no formal Education	16 [59.3]	11 [40.7]	27 [100.0]
Secondary and post Secondary education	257 [70.0]	110 [30.0]	367 [100.0]
Total	275 [69.8]	119 [30.2]	394 [100.0]

$X^2 = 1.37; p>0.24.$

Table 4. Distribution of respondents by occupational/professional status and awareness of anti-tetanus immunization.

Occupation/Profession	Awareness		Total
	Yes [%]	No [%]	N [%]
Health professional	120 [63.2]	70 [36.8]	190 [100.0]
Non-health professionals	155 [76.0]	49 [24.0]	204 [100.0]
Total	275 [69.8]	119 [30.2]	394 [100.0]

$X_2 = 7.65; p<0.001.$

Table 5. Distribution of the type of anti-tetanus immunization schedule that the respondents were aware of

Type of anti-tetanus immunization schedule	F [N]	[%]
DPT1-DPT3 at birth	53	12.3
Booster dose of TT immunization in the Secondary School	57	13.2
2 doses of TT immunization given during pregnancy	179	41.4
TT1-TT5 immunization schedule for woman of childbearing age	143	33.1
Total	432	100.0

Table of Multiple Responses.

Table 6. Reasons for taken anti-tetanus immunization the last time

Reasons	F [N]	[%]
Booster dose taken at secondary School	26	10.6
Post-injury prophylaxis	52	21.2
Part of pre-employment medical examination	19	7.7
During the last pregnancy	138	56.1
Cannot remember	11	4.4
Total	246	100.0

Table 7. Distribution of the period when respondents received the last dose of Anti-tetanus immunization

Period	F[N]	[%]
About 3 months ago	54	22.0
About 6 month ago	29	11.8
About 1 year ago	30	12.2
Over 1 year ago	126	51.2
Cannot remember when last	7	2.8
Total	246	100.0

Table 8. Distribution of anti-tetanus status during pregnancy

No of doses of TT immunization received during last pregnancy	F [N]	[%]
1 dose	45	30.4
2 doses	80	54.0
More than 2 doses	13	8.8
None	10	6.8
Total	148	100.0

DISCUSSION

The results of the present survey have shown a high level of awareness (69.8%) of anti-tetanus immunization among the women surveyed. This high level of awareness could be due to the high educational standard of the respondents who have mostly (93.1%) attained secondary school or post secondary school education. This further confirms the work of other workers of the overriding influence of female education over other strategies used in health care delivery (7,13). However, compliance with this programme was quite low among respondents, as only 46.7% of the respondents had received two or more doses of Tetanus Toxoid (TT) in adulthood, which are considered enough to confer immunity on the recipient for about 5 to 10 years. This was quite close to what was obtained in the study of Abuwa, *et al* (7). In this survey 53.3% could be said to have partial or no immunity against tetanus.

The study showed a highly significant association between respondents' occupation/profession and awareness ($p < 0.001$). This is not unexpected since a tertiary health institution is a place where both health workers and various groups of health students must have regularly received information about anti-tetanus immunization programme. Also they must have been involved in the dissemination of Information, Education and Communication (IEC) materials about it to patients and the public at large. Of the various anti-tetanus immunization schedules, majority (41.4%) of the respondents were aware of the usual 2 doses of tetanus toxoid immunization for pregnant women. This seems to be more popular among the parturient women. Only 33.1% were aware of the TT1-TT5 immunization schedule for women of childbearing age. This is quite worrisome since this is a major strategy that has since been formulated by WHO and already adopted by the Nigerian government since the early nineties to achieve worldwide elimination of maternal and neonatal tetanus as a public health problem by the year 2005 (14).

Most of the respondents (62.4%) in this survey had one time or the other received anti-tetanus immunization in adulthood. Of this number, 56.1% received the last dose during their last pregnancies. This further confirms its popularity and possible accessibility during antenatal clinics among the women surveyed as against the 21.2% that took it after sustaining an injury. Some respondents see no need for the immunization since they have not sustained any injury or were never pregnant to have a child. The survey also showed that, of the 148 of the respondents that ever gave birth to a child, 62.8% received at least 2 doses of TT immunization during their last pregnancies. This however, is more than what was reported in the survey in River States (7). The higher coverage in this study may possibly be due to the fact that the survey was conducted among fairly more educated women who are residing in an urban community where affordability, availability and accessibility to the vaccine is better than what was obtained in the earlier study which was conducted in semi-urban and rural areas. Unfortunately too, more than one third (37.2%) of those who had delivered a baby before did not take TT, and if they did, they received only one dose of TT immunization during their last pregnancy. This could not confer any immunity against tetanus on the mothers nor the children. This is quite

disturbing but it could be a reason for the continuing high prevalence of cases of maternal and neonatal morbidity and mortality from tetanus infection in our environment and the third world as documented by earlier reports (1, 2, 15).

This survey has shown a high level of awareness of anti-tetanus immunization programme among the adult females surveyed but a relatively low level of compliance as only less than half of those surveyed ever took the immunization in adulthood. It also revealed that some women even in the urban community do not receive anti-tetanus immunization during pregnancy.

It is therefore strongly recommended that government should refocus its attention on the immunization programmes in the country in terms of media health programme announcement; adequate logistic and material supports to the programme and this must be on a continuous basis. Sustainability in programme execution has always been a problem at all levels of health care delivery system in Nigeria.

The introduction of an appropriately targeted immunization for example to the students of the Universal Basic Education scheme will undoubtedly increase anti-tetanus immunization coverage and raise the herd immunity against tetanus infection in the country. There should be continuing health education for women and the IEC materials judiciously used during health education talks. It is expected that this will ultimately promote behavioural change in our women to go for anti-tetanus immunization that will lead to achievement of high herd immunity and eradication of tetanus. The health workers also require training and retraining to upgrade their capacity building, which will enable them provide qualitative immunization services.

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