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EVALUATION OF MEASLES VACCINE COLD CHAIN IN LAGOS STATE, NIGERIA

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Abstract

The National (level 1), State (L2), and Local government vaccine cold stores (L3) as well as some vaccination centres (L4) were physically inspected in Lagos State, Nigeria and the potency of the live-attenuated measles vaccine was tested. Both the L1 and L2 storage facilities were formally adequately equipped and maintained. This was also reflected in the potency of the vaccines. However, many vaccines at L1 were within weeks from expiration. Considerable problems with refrigeration and delayed forwarding became apparent at level L3 causing loss in potency both at L3 and L4: although, all L4 stores check-listed met all the EPI/NPI accreditation criteria, ¾ of the vaccines were sub-potent and this situation did not improve over the three year study period (1996-98). Time to expiration did not seem to be the main cause of loss of potency but rather poor and delayed handling. It is recommended that vaccines are moved more rapidly through the system and used well before expiration. Because of frequent power failures despite standby generators, we further recommend to include in the WHO criteria, book-keeping of periods of power failures, running time of generators and a complete recording of fuel consumption. Attitudes among vaccinating staff and handling of vaccines should also be improved by continued training.

Keywords: Measles vaccine; cold stores; potency

Running Title: Cold chain in Nigeria.

INTRODUCTION

Despite improved vaccine coverage, measles remains endemic in many African and other tropical developing countries with staggering morbidity and mortality rates (1-3). In these countries, the loss in vaccine (L2) and the downward distribution to all State-controlled Local Government Cold Stores (LGCS- L3), which in turn organise the distribution of vaccines to the various Vaccination Centres (VC, L4) operated under the responsibility of the Local Government Areas (LGA) of the State. The vaccination centres

(L4) are the point of care at which vaccines are administered to the recipients.

The maintenance of the cold chain at all levels of vaccine storage and distribution is obviously an essential and critical part of immunisation programmes and its regular follow-up is the key to a successful vaccination system. Therefore, potency testing of live viral vaccines is an important component for evaluating the cold chain on its various levels (8). This study evaluates the cold chain of the live attenuated measles vaccines at the different levels of vaccine storage and distribution throughout Lagos State, Nigeria.

MATERIALS AND METHODS

VACCINE COLD STORES

The following stores representing the different levels (L1-L4) of vaccine storage participated in this study: National Central Cold Store (NCCS) Oshodi (L1); the Lagos State Cold Store (LSCS) Ikeja (L2) and 5 Local Government Cold Stores (LGCS): Ikorodu, Kosofe-Ketu, Ajeromi-Ifeleodun, Eti-Osa and Alimosho LGAs of Lagos State (all L3). Primary Health Centres (PHC) which served as the Vaccination Centres (VC) (L4) were Palm Avenue PHC, Isolo Road PHC and Kajola PHC (in Mushin LGA), Saint Sabina PHC (in Agege LGA), Ikosi PHC (in Kosofe LGA), Layeni PHC (in Ajeromi-Ifeleodun LGA), Lagos Island LGA-staff clinic (in Lagos Island LGA), Ita-Elewa PHC (in Ikorodu LGA), Isheri-Olofin PHC (in Alimosho LGA) and Muri-Okunola PHC (in Eti-Osa LGA). The VC attended mainly to the needs of the middle and low socio-economic class of the population.

The cold chain facilities of most cold stores were physically inspected in 1998. The material checklist included, the cold room equipment, type of cold chain monitors, availability of sufficient deep freezers, refrigerators, ice packs, adequacy of electric power supply, availability and functionality of standby generators as power supply alternatives to the National Electric Power Authority (NEPA). Assessment was done using the standard questionnaires of the Expanded Programme on Immunisation/National Programme for Immunisation (EPI/NPI) (10).

2.2 Collection of vaccine samples and potency testing

From 1996 through 1998, a total of 56 vaccine samples were collected from 7 cold stores (L1-L3) and 9 vaccination centres in Lagos State. All the vaccines were of the lyophilised type (10 doses per

vial; Edmonston-Zagreb strain, Pasteur Merieux) and were delivered by UNICEF to the L1 store. At the vaccination centers (L4) aliquots (0.5ml) of the reconstituted vaccines were collected into sterile vials when ready for administration to children. Aliquoted samples were transported to the laboratory in well-insulated cold-boxes and either immediately titrated for potency or stored at -70°C until titration within 24 hours. The vaccine manufacturer, lot and batch numbers, date of collection and the expiry date were recorded for each sample. The 50% Cell Culture Infectious Dose (CCID_{50}) per human dose was determined according to WHO guidelines on quadruplicate wells on confluent monolayers of B95a cells (1×10^5 cells/ml) in 24-well Costar® tissue culture plates (11). The CCID_{50} was calculated as described by Reed & Muench (12).

RESULTS

ASSESSMENT OF COLD STORES

On physical inspection of the NCCS (L1) and the LSCS (L2), all criteria of the EPI/NPI accreditation checklist for a National and State cold store were met. In accordance with WHO recommendation, all vaccines showed expiry dates; both L1 and L2 had adequate refrigerated main and sub-stores with functioning standby generators to ensure uninterrupted power supply; requisition forms and functioning refrigerated vans for transportation of vaccines to L3 stores were also available. In addition, criteria of *Table 1* were fulfilled as applicable. Of the five L3 stores inspected, only two (Ikorodu- and Eti-Osa LGCS) met all EPI/NPI cold chain accreditation criteria; the others satisfied 69 – 81% of the criteria

applicable to L3 stores. *Table 1* shows that proper storage of diluents was the most frequently encountered problem among the L3 stores inspected. The 5 vaccination centres checklisted met all EPI/NPI accreditation criteria applicable to L4 stores: availability of adequate thermometers, cold boxes, ice packs, vaccine carriers, strict adherence to vaccine expiry dates, standard keeping of vaccine ledgers and records.

VACCINE POTENCY

Only 29 (52.7%) of the 55 vaccines titrated from L1 – L4 met the WHO recommended virus titre of $3.0 \pm 0.5 \log_{10}$ (i.e. ± 1000 infectious viral particles per human dose) for a potent measles vaccine and one vaccine vial was contaminated at source. *Figure 1* shows that both the percent of potent vials as well as the CCID₅₀ decreases as the vaccine were handed down from L1 to L4 and that this decrease is particularly strong for L4.

Overall, only 8 (25.8%) of the 31 vaccine vials titrated from the L4 met the WHO requirement. Expired vaccines were only found in one of the L3-stores (Ajeromi-Ifelodun LGCS) but only one of the three expired vaccine samples was sub-potent. In 1996, none of the 6 titrated measles vaccines collected at the three L4 facilities met the WHO recommended virus titre. In 1997, only 7 (31.8%) of the 22 vaccine vials collected from L4 met this WHO standard, although the three vaccines tested the same year from L1 were potent. In 1998, vaccine potency did not improve at the L4 level (1/4) but at the L3 level, 12/15 vaccine vials and 1/1 at the L2 level met the potency criteria. The vaccines titrated from the L1 and L2 facilities over a 2-year period had titres ≥ 3.0 . *Figure 2* shows that

many vaccines (31/56) of all levels were within 14 weeks from expiration dates. The median time to expiration of vaccine vials and batches were 6 and 16 weeks respectively at L1/L2 with some within 1-2 months from expiry. At L3 median time to expiration was 3 weeks including some vaccines, which were expired. On average vaccines became sub-potent around the expiration date at L3, and much earlier at L4.

Table f

EPI/NPI accreditation criteria met by L1-L3 cold stores in Lagos State, Nigeria

Storage Level	1 Adequacy of fridge for vaccine storage	2 Normal fridge t°	3 Diluent in fridge	4 Freezer for ice-packs only	5 Daily am & pm t°	6 Functioning standby generator	7 Adequacy of freezer for vaccine storage	8 Normal freezer t°	9 Normal vaccine spacing in fridge/freezer	10 Vaccine within expiry date	Total score (%)
L1	YES	YES	NA	YES	YES	YES	YES	YES	YES	YES	100
L2	YES	YES	NA	YES	YES	YES	YES	YES	YES	YES	100
L3 (IKR)	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	100
L3 (KSF)	NO	NO	NO	YES	YES	YES	YES	YES	YES	YES	81
L3 (AJE)	YES	YES	NO	NO	NO	NO	YES	YES	YES	NO	69
L3 (ETI)	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	100
L3 (ALI)	NO	NO	NO	YES	YES	NO	YES	YES	YES	YES	75
Total (%)	71	71	40	86	86	71	100	100	100	92	

NA = Not Applicable

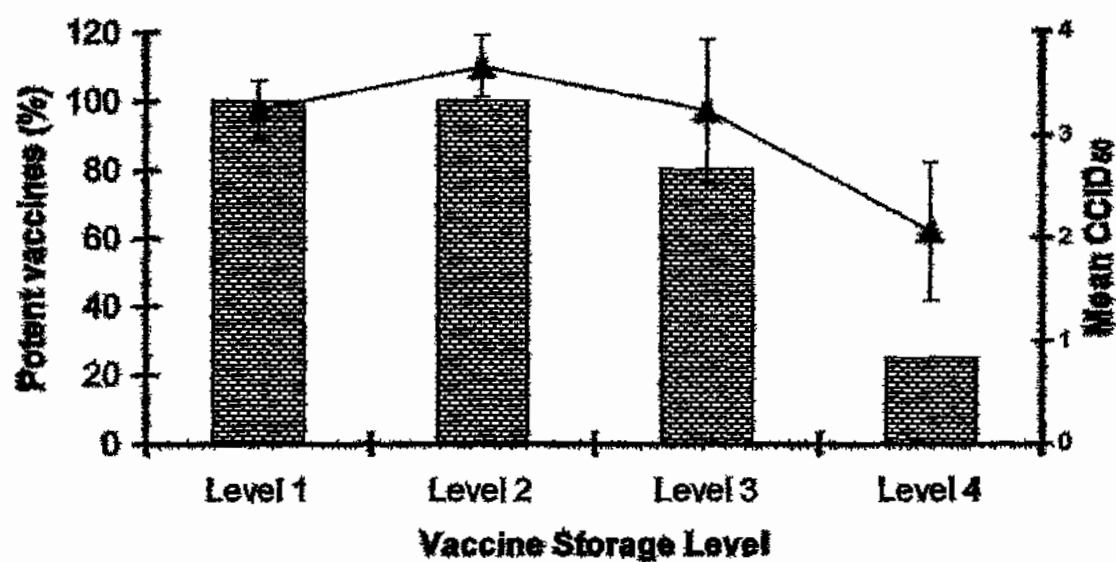


Fig. 1. (Oyefolu *et al.*)

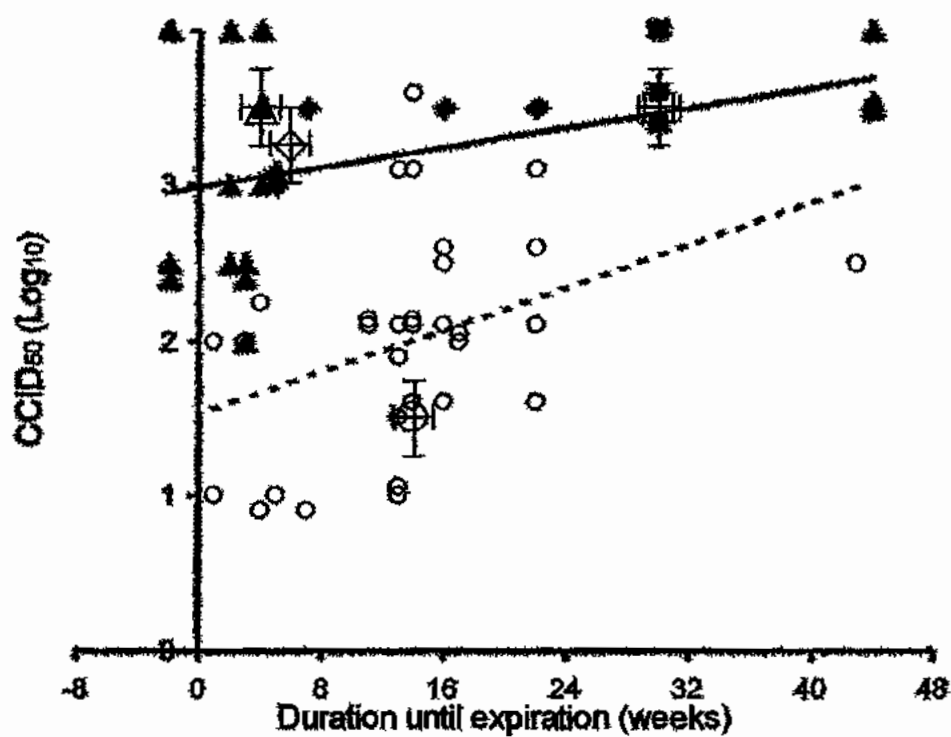


Fig. 2. (Oyefolu *et al.*)

DISCUSSION

When administrated under optimal conditions seroconversion rates to current measles vaccines (Edmonston-Zagreb strain) are >95% (13). With the exception of a small percentage of secondary vaccine failures, all of those who seroconvert are protected by long-lasting and robust immunity (13,14). However, the live-attenuated vaccine is highly sensitive to temperature, and a defective cold chain severely reduces vaccine efficacy (15). The WHO has a catalogue of recommendations as minimal requirements for maintaining a cold-chain at the different levels.

Both the L1 and L2 storage facilities were formally well equipped and maintained and this was reflected in the potency testing of the vaccines. However, many vaccines at L1 were within weeks from expiration. At L3 delayed forwarding was aggravated by considerable problems with refrigeration causing loss in potency both at L3 and L4. Expiration or time to expiration did not seem to be the main cause of loss of potency but rather delayed and inadequate handling.

It is therefore recommended here that vaccines are moved more rapidly through the system and that they are used at least 4 months before expiration. In fact, recommendations should be made for each level with respect to the allowed time to expiration for each batch, to avoid that batches close to expiration leave central stores. Batches that are close to their expiry date should have their potency recertified through laboratory testing before leaving the central stores as was suggested before (5,6).

Interruption of electricity supplied by the National Electric Power Authority (NEPA) was another general problem reported at all levels of vaccine storage and distribution, as has been reported before (5,6,8). Power failures are

notoriously paired with the lack of fuel for standby generators and their indiscriminate diversion at L3 for personal or other unrelated use by officials. While this must stop, we strongly recommend here to include to the WHO criteria, book-keeping of the exact periods of power failures, running time of generators and a complete inventory of fuel consumption.

Although, all L4 stores check-listed met all the EPI/NPI accreditation criteria, ¾ of the vaccines were sub-potent and this situation did not improve over the three year study period. In most vaccination centres (L4) this was due to inadequate handling of vaccines and attitudes among vaccinating staff. Personnel kept vaccines on thawed ice-packs at ambient temperatures and/or in their palms during conversations. Therefore there is an urgent need for continued training and education of vaccinating staff. Reprinting and redistribution of the summarised WHO-guidelines (14) for vaccinators could be a first step.

Although this study is relatively limited in scope and in some ways anecdotal rather than representative, it revealed considerable discrepancies between formal accreditation criteria and vaccine quality. More stringent recommendations are suggested with respect to moving of vaccines through the cold-chain and with respect to the closer monitoring of main and accessory power supplies and failures.

Acknowledgements

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Legends to Figures

Fig. 1. Vaccine potency at the different storage levels of the cold chain. At level L4 an aliquot of reconstituted vaccine taken just before injection was used for potency testing. Bars are percent of potent vaccines; mean CCID₅₀ (▲—▲).

Fig. 2. Vaccine potency (CCID₅₀) and time to expiration of vaccine sampled at the different storage levels L1 (◆); L2 (■); L3 (▲); L4 (●); potency trend line at L3 (—) and L4 (---). Large open symbols represent median potency ± S.D. of batches at the different storage levels L1 (◇); L2 (□); L3 (△); L4 (○). As in Fig. 1 potency testing at level L4 was performed on reconstituted vaccine just before injection.

BIOCHEMICAL BASIS OF HEAVY METAL INDUCED STRESS TOLERANCE IN THE N₂ FIXING CYANOBACTERIUM *ANABAENA DOLIOLUM*Phalisteen Sultan^{1*}, Shah, M.I.¹ Williams, P.² Arif Jan¹ and Naseer Ahmad³¹Deptt. of Microbiology and Mycology, RRL-Sanatnagar, Srinagar-190005.²Deptt. of Plant Protection and Plant Pathology, AAI-(DU), Allahabad, U.P-India³ Division of Biotechnology, Regional Research Laboratory, CSIR, Jammu-Tawi-180001Correspondence to : Phalisteen Sultan, Email: shakir_bio@yahoo.co.in**ABSTRACT**

The effect of heavy metals (Cd and Cu) on the nitrogen fixing cyanobacterium, *Anabaena doliolum* was observed in the present study. To explore the survival strategy of the test cyanobacterium, Chl/CAR content, protein content, antioxidative defense system (SOD, APX and GR) as well as biochemical fractionation (carbohydrates, lipids, protein, DNA and RNA) were studied. Increasing concentrations of metals inhibited the growth and survival significantly; chlorophyll and carotenoid content were found inhibited with increase in concentration of metals. Among the antioxidative enzymes, SOD and APX were increased with the increase in concentration of both the metals, whereas Catalase and Glutathione reductase were decreased at higher dose of Cd and Cu. APX played a major role for scavenging H₂O₂ rather than CAT. Results revealed decrease in all parameters with the duration of time. The role of metal induced PC in offering tolerance to UV-B was confirmed by measuring lipid peroxidation and antioxidant defense system of the cyanobacterium treated with Cd and UV-B as well as in the Cd pretreated cells of *A.doliolum* exposed to UV-B. Lipid peroxidation (measured in terms of MDA content) as well as SOD and APX were found to be less induced, thus showing less oxidative damage in case of interactive treatment when applied separately. However, CAT and GR which showed sensitivity at higher dose of both the stresses were found to be induced. Thus Cd appears to antagonize the effect of UV-B in test cyanobacterium. To know the actual reason for the antagonism, PC concentration was measured in the cells with and without BSO (a potent inhibitor of phytochelatin synthase) pretreatment. The results emphasized that the extent of antagonism was reverted in the BSO pretreated cells than the normal BSO non- treated cells. Nevertheless, the PC content was found to be more in case of Cd + UV-B than the individually treated cells, but the PC was more or less completely inhibited after BSO pretreatment in all the cases. The above finding was also visualized on the SDS-PAGE. Therefore this study showed that Cd induced PC has role in UV-B tolerance.

Introduction

Cyanobacteria are the major primary producers of the aquatic eco-system. They also account for the N₂ economy of the soil. These organisms also experience the various environmental stresses largely due to the increased heavy metal pollution. *Anabaena doliolum* is one of the potent nitrogen fixing cyanobacterium of rice fields, which faces the threat of heavy metals.

Certain heavy metals are essential microelements because they are required in very low concentrations by all living organisms. However, higher concentrations of these metals cause toxic effects on the organism. Due to anthropogenic activities (industrialization, use of chemical fertilizers, coal burning, mining etc) and natural sources (volcanic eruption, weathering of rocks, combustion etc), the heavy metal concentration is

continuously increasing in the soil and water bodies. The heavy metals Cd (non-essential) and Cu (essential) were chosen for the present work because they constitute a major percentage of the pollutant heavy metals and to see the adaptation strategies of the cyanobacterium to essential and non essential metals.

The major source of contamination to agricultural fields are traffic, metal processing industries, mining and by-products of mineral fertilizers e.g. DDT, Carbofuran, Machete, (18) and pesticides (6). The effects include inhibition of growth and photosynthesis, reduction in C^{14} uptake and inhibition of nitrogenase activity (21).

The metal toxicity has a great impact on the cyanobacteria population. To survive in the stressful conditions all organisms including bacteria will have to adopt to different strategies at ecological, physiological, biochemical and molecular level. The formation of various reactive oxygen species (ROS) by different environmental stresses viz. heavy metals, drought, pesticides, invasion by pathogens has been reported (12), (10). It has been generally accepted that active oxygen produced under stress is a detrimental factor, which cause lipid peroxidation, enzyme inactivation and oxidative damage to DNA and protein. To counteract the ROS- induced damage the antioxidant defense system is stimulated. This defense system consists of several enzymatic and non-enzymatic components. Among the antioxidative enzymes, the first enzyme is superoxide dismutase (SOD) which scavenges the O^{2-} and converts it into H_2O_2 that can be scavenged by either catalase (CAT) or ascorbate peroxidase (APX). Although H_2O_2 is less reactive than O^{2-} but in the presence of the reduced transition metal in a chelated form resulting in occurrence of OH (Fenton reaction) which is more detriuos. Further

more H_2O_2 also has the capacity of carrying out the chain reaction. Thus it becomes necessary to detoxify the ROS.

The most important protein induced during heavy metal stress is the metallothioneine (MT) which is low molecular weight protein and peptide. Algae, plant and some fungi also produce a class of metal binding peptides different from metallothioneines (23). The effect of heavy metal on test organisms, the cellular damage in terms of lipid peroxidation, enzymatic anti oxidant-SOD, APX, GR and CAT (total -SH and GSH) and change in the protein profile were studied. The main objectives of the present study were to observe the growth behavior, chlorophyll, carotenoid and protein content in the test cyanobacterium exposed to Cu and Cd and to study the biochemical fractionation of the cells to know the effect of Cu and Cd on DNA and RNA. and protein, lipid and carbohydrate content.

Materials and Methods

In order to study metal toxicity, the nitrogen fixing cyanobacterium *Anabaena doliolum* was selected and grown on Allen Arnon's media. The media was supplemented with Fe-EDTA as iron source and prepared according to (13), K_2HPO_4 and Fe-EDTA were autoclaved separately and added to cold sterilized culture media. The pH of the media was maintained at 7.5 for Allen Arnon's media and to avoid any alteration in pH, the medium was buffered with 2.0 mM Tris (Hydroxymethyl) amine -HCl. The test cyanobacterium, *A. doliolum* was grown exenically on modified Allen and Arnon's media (1) buffered with tris/HCl at $24 \pm 2^\circ C$ under 72 micro mol photon $m^{-2} S^{-1}$ PAR light intensity with a photoperiod of 14:10 hrs at a pH of 7.5. The cultures were shaken by hand 2-4 times daily. Stock solutions of $CuCl_2 \cdot 2H_2O$ ($100 \mu g ml^{-1}$) and $CdCl_2$ were prepared

in distilled water and sterilized by passing through a Millipore membrane 0.22mm.

Measurement of survival and growth

To measure the survival of *A. dololium* against Cu and Cd, cells were treated with different concentrations of Cu (0-25µm) and Cd (0-0.2µm). The LC₅₀ and lethal doses for both Cu and Cd were determined by the plate colony count method. Approx. 50 and 0 % survival of the test cyanobacterium was observed after 8.2µm of Cu1 and 20µm Cu2 respectively of Cu exposure. The LC₅₀ and lethal concentration for Cd used in this study were 0.02µm (Cd1) and 1.0 µm (Cd2). Both the treatments were given for 15 days, growth was estimated by measuring the culture density of the bacterium at 663nm in a Baush and Lomb spectronic-20 colorimeter every day upto 15th day using reference blank of basal culture medium.

Pigment Extraction and Estimation

For extraction of pigments a known volume of culture was suspended in a desired amount of acetone (80%). After overnight incubation at 4°C it was again centrifuged and the resulting supernatant was used for measuring chlorophyll and carotenoid content. The optical densities (OD) were recorded with the help of Baush and Lomb "spectronic-20" spectrophotometer at 663 and 480nm for chlorophyll and carotenoid content respectively. Chlorophyll was calculated as per Mackinney (14) and phycocyanin as per Broody. The total amount of carotenoid was calculated using the specific absorption coefficient described by Myers and Kratz (16).

Lipid peroxidation

Oxidative damage of lipid was measured in terms of the total content of thio barbituric acid (TBA). reactive substances and expressed as equivalent of Malondialdehyde (MDA) as per

Cakmark and Horst (5) with minor modification. Reactive substances from cell culture were extracted in 3ml of 1 % (w/v) Trichloro acetic acid (TCA) at 4°C and centrifuged at 13000xg for 2 minutes. An aliquot of 0.5ml from the supernatant was added to 1.5ml TBA (0.5% in TSA). Samples were incubated at 100xg for 5 minutes. Specific growth rate was calculated by using the equation, $M = \ln(N_2/N_1) / T_2 - T_1$ where M is specific growth, N₁ and N₂ are absorbance of culture suspension at given time interval and T₁ and T₂ stand for final and initial respectively. The results of final yield (expressed in terms of optical density) and specific growth rate were converted to percentage of control to permit comparison. After heating, the reaction was stopped using ice baths. The concentration of MDA was calculated for its extinction coefficient (155mM⁻¹ cm⁻¹).

Enzyme assay

Pellets collected from exponentially growing cultures of *A. dololium* were suspended in cell lysis buffer (pH 7.0) and sonicated in ice cold condition. The buffer contained 1mM EDTA and 1% poly vinyl pyrrolidone (PRP) with the addition of 1mM ASC in the case of APX assay. The sonicated sample was centrifuged at 15000xg for 30 minutes at 4°C. Total SOD activity was assayed by monitoring the inhibition of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries (11). A 3ml of reaction mixture containing 50mM potassium phosphate buffer (pH 8.0), 133mM methionine, 75µm NBT, 2µm riboflavin, 0.1 mM EDTA and 100µl of enzyme extract, the reaction mixture was illuminated for 20 minutes at a light intensity of 500m⁻²S⁻² PAR. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction. CAT activity was determined by measuring the consumption of

H_2O_2 , (extinction coefficient 39.4 mM cm^{-1}) at 240 nm for 3min. (1). The reaction mixture containing 50mM potassium phosphate buffer (pH 7.0), 10mM H_2O_2 and 200ul of enzyme extract, GR activity was determined by measuring the oxidation of H_2O_2 , (extinction co-efficient, $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$) for 3 min in 2ml assay mixture containing 50mM potassium phosphate buffer (pH 7.8) 2mM Na_2EDTA , 0.15mM NADPH, 0.5mM GSSG and 200ul of enzyme extract. The reaction was initiated by adding NADPH, correction were made for the background absorbance at 340nm without NADPH (20). APX activity was determined by measuring the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM cm^{-1}) for 1 min in 1ml reaction mixture containing 50mM potassium phosphate buffer (pH 7), 0.5mM, ASC, 0.1mM H_2O_2 and 200ul of enzyme extract the corrections were made for low, non enzymatic oxidation of H_2O_2 .

Biochemical fractionation

To a culture of 0.5 OD, 5ml per chloric acid was added and kept in ice for about 20 mins, centrifuged at 5000 rpm for 10 min. Pellet so obtained was dissolved in 3:1 (methanol : ethanol) and heated to 65°C . The supernatant was taken for lipid estimation whereas the pellet was dissolved in 5% TCA warmed for 20 mins and centrifuged at 5000 rpm. The supernatant obtained is used for DNA and RNA estimation and the pellet for protein estimation.

Phytochelation Estimation:-

Total thiol was estimated according to method of Ellman (1959). The total GSH content was estimated by the 5,5 dithiobis 2, nitrobenzoic acid (DNTB) glutathione reductase coupled assay as described by Anderson (3). Protein content was measured by using formula (total -thiol-GSH). All

the above parameters were measured in the control as well as BSO (2mM) pretreated cells.

Protein extraction and analysis

Cu and Cd treated cells of *A.dololium* were used for extraction of total cell protein. SDS – PAGE was carried out in 1.0 mm thick gels of 15% acrylamide . The gel loaded with 20ul sample as well as molecular weight marker (Sigma chemical Co.USA) were run at constant voltage of 220 V at 8°C and stained with coomassie blue as per the method of Sambrook and Russel (24).

Results were statically analyzed using a one way ANOVA, followed by Duncan's multiple range tests and correlationary coefficients (r). The number of independent replicates for each experiment were three.

Results

Inhibition of growth rate was consistent with the increase of metal concentration, Maximum inhibition of -298.8% was shown at 50um followed by 124,129 and 170% at 4.2 μm , 8.2 μm , and 15 μm Cu respectively. In case of Cd, maximum inhibition of 32.64% was observed at 0.1 μM concentration followed by 29.33, 28.92, 27.14 and 11.57% at 0.01 μm , 0.04 μm , 0.06 μm and 0.08 μm respectively. A simultaneous inhibition of pigment was noticed with increase of metal concentration. An inhibition of -41.74, -144.26,-125.24 and -80.78% chlorophyll and -156.02, -132.46, 129.6 and 92.73% carotenoid was observed at 50 μm , 152 μm , 8.2 μm and 4.2 μm Cu respectively where as in case of Cd, an inhibition of 64.80, 62.01, 157.26, 46.08 and 44.41% of chlorophyll and 65.09, 45.19, 53.16, 47.67 and 31.43% of carotenoid at 0.1, 0.04, 0.06, 0.08, and 0.01 μm Cd respectively as compared to control. For DNA estimation , 2ml of supernatant was mixed with 1ml of DPA reagent

(diphenylamine) and boiled for 10 min. The colour so developed was measured by recording OD at 595nm. For RNA estimation, 2ml of supernatant was mixed with 3ml of orcinol reagent and boiled for 10 minutes. The optical density of the mixture was recorded at 665nm. To estimate the protein concentration, A 10ul sample was mixed with 90ul cell lysis buffer and 1ml Bradford reagent, OD was measured at 595nm (4). To estimate the carbohydrate content, 1ml of algal sample was mixed with 1ml of phenol and kept for 15 minutes as such, 5ml of conc. H_2SO_4 was mixed and boiled followed by cooling. The colour so developed was measured by recording the OD at (8). For lipid estimation, the supernatant was used for estimation of lipid through TLC. Approximately 100ml culture was harvested, lipids were extracted from samples using chloroform: methanol (2:1 v/v) and dried under flash of nitrogen at 4°C. The total lipid obtained were estimated gravimetrically and stored at -10°C in deep freezer. The quantitative estimation of lipid were performed by TLC on 200mm plates impregnated with silica gel using chloroform: methanol: acetic acid and water (85:15:10:3) as solvent. Plates were activated for 20 mins at 120°C pooled with appropriate lipid extract and visualized by iodine vapours Table b. summarize the toxicity of Cu and table e, the toxicity of Cd on chlorophyll and carotenoid content of *A. dololium*. Simultaneous inhibition of pigment was noticed with increase of metal concentration. An inhibition of -41.74, -144.26, -125.24 and -80.78% chlorophyll and -156.02, -132.46, 129.6 and 92.73% carotenoid was observed at 50um, 152um, 8.2 um and 4.2um. Cu respectively, whereas in case of Cd, an inhibition of 64.80, 62.01, 57.26, 46.08 and 44.41% of chlorophyll and 65.04, 45.19, 53.16, 47.67 and 31.43% of carotenoid at 0.1, 0.06, 0.08 and 0.01um respectively as compared to control was observed. A marked reduction in the protein content of the

organism was observed with increasing concentration of Cu and Cd in the medium. A decline in the protein content was found to be 99.28, 72.04, 68.28 and 247.04, 56.56, 45.20, 37.07, 33.81 and 24.21% at 50, 15, 8.2, and 4.8um of Cu and 0.1, 0.08, 0.04 and 0.01um of Cd respectively as compared to control.

The impact of different doses of Cd and Cu on the carbohydrate and protein content of *A. dololium* has been incorporated in the tables (1-a) and (1-d). Carbohydrate and protein are found to be sensitive to metal and continuous decline was observed at different doses of these metals. An inhibition of 284.6, 638.4 and 762.7% of carbohydrate and 1208.6, 435.6, 1440.7 and 315.4% of protein at 8.2, 20 Cu, 0.02 and 0.1 Cd doses respectively as compared to control. However the table (1-b and (1-c) encompasses the data on the DNA and RNA in context of the organism. A simultaneous decrease in the DNA and RNA content was registered after the Cu and Cd treatment with the passage of time. From the first to fifteenth day, an inhibition of 1208.1, 435.6, 1440.7 and 315.4% of DNA and 1372.7, 331.9, 1424.0 and 257.3 % of RNA content was noticed at Cu 8.2, Cu 20, Cd 0.02 and Cd 0.1uM concentrations respectively over the control. On the other hand the lipid content was also found to be affected and showed a remarkable decline after the metal treatment.

Table (2-a, b, c and d) compiles data on the impact of different doses of Cu and Cd on the enzymatic antioxidant activities (SOD, CAT, APX and GR) of *A. dololium*. The SOD activity showed enhancement from 23.8, 61.9, 4.78 and 33.3% to 24.8, 90.5, 205.9 and 368.9 fold after the treatment of 8.2, 20um Cu, 0.02 and 1.0um Cd respectively as compared to control. Thus SOD activity was found to increase more in case of Cd 0.1um treatment. According to Pearson correlation

coefficient SOD activity was significantly correlated with APX activity and negatively with CAT. ($P<0.01$). However, CAT appeared to be metal sensitive and registered a decline in the activity after Cu and Cd treatment. In contrast to SOD activity, CAT showed decrease with the passage of time. activity was decreased by -42.2, -51.80 and 44.66 following the treatment with 20 μ M Cu, 0.02 and 0.1 μ M Cd on the fifteenth day as compared to control where as in case of Cu 8.2, CAT was found to be increased by 75.2% on the fifteenth day. CAT is negatively correlated with APX. ($P<0.01$) but highly significant with the GR in the early days of treatment. The APX activity registered an increase of 84, 141.8, 64.0 and 104.2 fold for Cu 8.2, Cu 20, Cd 0.02 and Cd 0.1 μ M respectively as compared to control. However the

magnitude of increase differed significantly between the two treatments of Cu and Cd (Duncan's multiple range test). During the initial days of metal stress, AP was non significantly related to GR but it showed a highly significant correlation in the late phase. Further the APX activity was significantly correlated with the SOD activity ($P<0.01$). A further enhancement was observed in case of GR activity after exposure of Cu and Cd. However, the enhancement was more at the lower concentration as compared to higher concentration of metal. A 6.2 and 8.7 fold enhancement in case of 8.2 μ M Cu and .02 μ M Cd was observed whereas 2.5 and 4.6 fold enhancement in case of Cu 20 and Cd1 as noticed, GR was highly correlated with APX.

Table I (a) Effect of different treatments on the carbohydrate content of *A. dololium* over a time course of 15 days. The values in the parenthesis denote the percent increase or decrease.

Treatments	1 st day	3 rd day	6 th day	9 th day	12 th day
Control	0.171 \pm 0.006	0.108 \pm 0.013	0.130 \pm 0.006	0.195 \pm 0.30	0.251 \pm 0.003
Cu (8.2 μ M)	0.143 \pm 0.004 (-15.9)	0.324 \pm 0.052 (362.2)	0.602 \pm 0.007 (362.2)	0.557 \pm 0.310 (185.6)	0.996 \pm 0.018 (284.6)
Cu(20 μ M)	0.100 \pm 0.004 (-41.2)	0.155 \pm 0.012 (42.8)	0.458 \pm 0.003 (251.7)	0.465 \pm 0.044 (138.9)	1.51 \pm 0.018 (501.50)
Cd(0.002 μ M)	0.127 \pm 0.022 (-25.5)	0.233 \pm 0.019 (113.9)	0.373 \pm 0.019 (168.0)	1.63 \pm 0.028 (738.6)	1.85 \pm 0.006 (638.41)
Cd(0.1 μ M)	0.061 \pm 0.004 (-64.0)	0.389 \pm 0.025 (257.2)	0.504 \pm 0.008 (290.6)	1.19 \pm 0.013 (510.51)	2.16 \pm 0.009 (762.7)

Table I (b) Effect of different treatments on the DNA content of *A. dololium* over a time course of 15 days. The values in the parenthesis denote the percent increase or decrease.

Treatments	1 st day	3 rd day	6 th day	9 th day	12 th day
Control	3.00±0.0020	3.72±0.005	9.07±0.002	8.87±0.003	8.05±0.003
Cu (8.2µM)	7.90±0.003 (102.71)	7.46±0.005 (100.5)	28.1±0.003 (210.4)	60.2±0.004 (579.4)	11.2±0.004 (120.86)
Cu(20µM)	6.55±0.003 (68.0)	4.22±0.004 (13.4)	23.3±0.001 (157.6)	56.1±0.016 (533.2)	45.5±0.00 (435.6)
Cd(0.002µM)	5.46±0.002	6.07±0.001 (63.2)	24.1±0.076 (165.9)	58.9±0.056 (565.0)	130.9±0.002 (1440.7)
Cd(0.1µM))	4.06±0.003 (4.1)	4.23±0.001 (13.9)	19.2±0.003 (11.5)	26.1±0.006 (195.1)	35.5±0.003 (315.4)

* All values are mean + SD of three replicates. Values in parenthesis show % increase over control and those with a negative sign indicate percent inhibition.

Table I(c) Effect of different treatments on the DNA content of *A. dololium* over a time course of 15 days. The values in the parenthesis denote the percent increase or decrease.

Treatments	1 st day	3 rd day	6 th day	9 th day	12 th day
Control	24.84±0.005	26.0±0.006	22.7±0.005	20.2±0.002	20.3±0.001
Cu (8.2µM)	49.8±0.001 (100.2)	67.1±0.002 (157.6)	108.0±0.002 (370/8)	146.8±0.003 (624.0)	299.4±0.003 (1372.7)
Cu(20µM)	35.2±0.005 (41.7)	53.5±0.003 (104.7)	58.2±0.002 (155.41)	45.1±0.001 (122.5)	87.8±0.003 (331.9)
Cd(0.002µM)	21.0±0.002 (-19.5)	54.3±0.005 (108.5)	72.0±0.060 (238.2)	173.5±0.014 (756.1)	309.9±0.003 (1424.0)
Cd(0.1µM))	12.1±0.003 (-51.4)	79.1±0.003 (203.5)	100.4±0.006 (340.7)	55.8±0.003 (175.5)	72.6±0.002 (259.3)

Table I (d): Effect of different treatments on the protein concentration of *A. dololium* over a time course of 15 days. The values in the parenthesis denote the percent increase or decrease.

Treatments	1 st day	3 rd day	6 th day	9 th day	12 th day
Control	7.91±0.007	9.20±0.003	9.58±0.008	10.2±0.008	10.6±0.009
Cu (8.2µM)	3.66±0.004 (-53.7)	2.97±0.006 (-67.7)	1.92±0.010 (-79.9)	1.37±0.004 (-86.7)	0.059±0.002 (-94.4)
Cu(20µM)	3.48±0.011 (-56.0)	2.99±0.006 (-67.5)	1.78±0.015 (-81.4)	1.36±0.005 (-86.6)	0.379±0.007 (-96.4)
Cd(0.002µM)	4.06±0.024 (-48.6)	3.20±0.025 (-65.2)	2.32±0.015 (-81.4)	1.17±0.008 (-88.5)	0.068±0.002 (-93.5)
Cd(0.1µM)	3.49±0.017 (55.8)	1.96±0.024 (-78.6)	1.701±0.021 (-82.2)	1.06±0.003 (89.6)	0.024±0.001 (-95.9)

* All values are mean + SD of three replicates. Values in parenthesis show % increase over control and those with a negative sign indicate percent inhibition.

Table2 (a): Effect of the different treatments on the SOD activity of *A. dololium* over a time course of 15 days.

Treatments	1 st day	3 rd day	6 th day	8 th day	12 th day	15 th day
Control	0.251± 0.002	0.190± 0.004	0.157± 0.003	0.188± 0.006	0.011± 0.004=5	0.152± 0.003
Cu(8.2um)	0.253± 0.004 (0.049)	0.433± 0.004 (127.8)	0.528± 0.003 (235.5)	0.829± 0.002 (391.2)	0.945± 0.003 (821.9)	2.51± 0.003 (1249.2)
Cu (20um)	0.250±0.006 (0.385)	0.423±0.003 (122.7)	0.461±0.002 (193.2)	0.496±0.004 (194.3)	0.105±0.002 (831.6)	2.183±0.005 (1249.2)
Cd (0.02uM)	0.255±0.006 (1.48)	0.412±0.003 (116.5)	0.479±0.005 (204.5)	0.696±0.004 (9312.4)	1.16±0.002 (1011.7)	2.183±0.005 (1335.7)
Cd(0.1uM)	0.267±0.003 (6.21)	0.454±0.004 (138.5)	0.741±0.004 (370.9)	0.934±0.005 (453.2)	1.24±0.006 (1089.8)	3.34±0.004 (2089.9)

Table2 (b): Effect of the different treatments on the CAT activity of *A. dololium* over a time course of 15 days.

Treatments	1 st day	3 rd day	6 th day	8 th day	12 th day	15 th day
Control	0.056±0.006	0.060±0.006	0.068±0.005	0.097±0.022	0.101±0.001	0.102±0.002
Cu(8.2um)	0.063±0.112 (1028.3)	0.597±0.051 (888.3)	0.522±0.006 (652.6)	0.311±0.007 (230.5)	0.203±0.010 (101.8)	0.185±0.002 (75.2)
Cu (20um)	0.979±0.017 (1681.1)	0.235±0.009 (295)	0.135±0.004 (97.5)	0.109±0.004 (15.7)	0.068±0.005 (-37.6)	0.066±0.001 (-42.2)
Cd (0.02uM)	0.486±0.027 (764.0)	0.320±0.009 (433.8)	0.161±0.008 (135.2)	0.163±0.007 (52.63)	0.122±0.003 (20.74)	0.49±0.001 (-51.8)
Cs(0.1uM)	0.440±0.102 (798.0)	0.363±0.027 (503.3)	0.275±0.018 (297.1)	0.178±0.006 (86.6)	0.151±0.004 (57.7)	0.059±0.001 (-44.7)

*All values mean±SD of three replicates Values in parenthesis show percent increase over control as those with a negative sign indicate percent inhibition.

Table2(c): Effect of the different treatments on the APX activity of *A. dololium* over a time course of 15 days.

Treatments	1 st day	3 rd day	6 th day	8 th day	12 th day	15 th day
Control	0.926±0.005	0.568±0.008	0.749±0.004	0.695±0.00	0.942±0.004	1.75±0.039
				I		
Cu(8.2um)	1.30±0.003 (12.1)	2.56±0.013 (255.0)	5.35±0.002 (272.3)	27.9±0.010 (3928.7)	47.5±0.014 (4970.4)	14.9±0.016 (8437.2)
Cu (20um)	1.22±0.013 (33.2)	2.59±0.005 (272.3)	3.81±0.002 (408.5)	50.59±0.02 (7147.6)	52.66±0.035 (5526.0)	254.3±0.019 (14184.0)
Cd (0.02uM)	2.02±0.007 (82.47)	15.7±0.047 (1303.1)	18.9±0.097 (2478.6)	27.2± (377.3)	38.4±0.016 (4007.6)	115.4±0.043 (6489.5)
Cs(0.1uM)	1.89±0.008 (105.2)	11.75±0.027 (1513.3)	23.04 ±0.007 (2963.0)	29.9±0.025 (4165.4)	54.4±0.125 (5627.0)	184.0±0.125 (10428.4)

Table2(d) Effect of the different treatments on the GR activity of *A. dololium* over a time course of 15 days.

Treatments	1 st day	3 rd day	6 th day	8 th day	12 th day	15 th day
Control	0.204±0.007	0.251±0.001	0.239±0.003	0.257±0.005	0.252±0.005	0.283±0.005
Cu(8.2 uM)	0.302±0.007 (53.8)	0.776±0.002 (202.3)	0.884±0.012 (240.8)	0.948±0.003 (264.8)	1.802±0.009 (621.0)	7.51±0.003 (1779.8)
Cu (20 uM)	0.187±0.002 (-26.6)	0.509±0.005 (108.1)	0.699±0.012 (177.4)	0.724±0.01 2 (195.4)	0.902±0.004 (259.8)	10.9±0.015 (13715.5)
Cd (0.02 uM)	0.172±0.001 (-13.5)	0.740±0.008 (191.8)	0.772±0.011 (215.4)	0.878± 0.002 (261.4)	2.465±0.019(8 76.1)	5.208±0.014 (1822.6)
Cd(0.1 uM)	0.149±0.002 (-3.3)	0.533±0.004 (131.2)	0.656 ±0.002 (163.4)	1.418±0.001 (364.6)	1.418±0.001 (465.2)	10.54±0.009 (3666.6)

*All values mean±SD of three replicates Values in parenthesis show percent increase over control as those with a negative sign indicate percent inhibition.

SOD activity of the cell was much elevated in case of Cu 20 (90 fold) as compared to Cd (36.8 fold). Similarly the increase of APX, was found to more in 20 uM Cu (141.8 fold). Than .1uM Cd (104.2 fold). Contradictory to this GR activity was enhanced much in .1uM Cd (36.6 fold) as compared to Cu (37.1 fold) whereas the activity of CAT was found to be inhibited much by Cd 1 (-44.2% then by Cu 20 (-42.2%). The logarithmic phase of cells of *A. dololium* were taken and exposed to 20uM Cd and for 20 (UV1) and 50 (UV2) min exposure of UV B. These treatments were found to increase the extent of oxidative damage measured in terms of lipid peroxidation by 71.7, 89.2 and 108.7% respectively with respect to control. However, the BSA pretreated cells on subjecting to the above stress condition exhibited increase in lipid concentration by 78.6, 80.4 and

89.17 % respectively over the control. Further, both Cd and UV-B were found to enhance the activity of SOD which the first line of defense against ROS, catalysis the desiccation of O₂⁻ to O₂ and H₂O₂. SOD activity is also considered to be an indirect measurement of O₂ production and hence the extent of oxidative damage. However, increase in SOD after Cd in the cell without BSO was 87.1% whereas UV1 and UV2 produced a rise. The results pertaining to CAT. Although similar trend was observed in BSO treated cells. UV1 and UV2 exhibited a much greater increase in SOD activity of 205 and 475% respectively with respect to control. The results pertaining to CAT in table 3 a, showed that Cd induced the CA activity by 62.7%. However, UV1 and UV2 inhibited its activity by 64.4 and 74.2% respectively as compared to control. This demonstrates that UV-B has an

inhibitory effect on CAT activity. This is also supported by Streb (1993) who reported Photo-inhibition of CAT activity. On the other hand BSO pretreatment inhibited CAT in all the cases, probably due to toxicity of CAT. Our results also revealed that these two stresses significantly increased the APX activity (Duncan's multiple range test) by 5.6, 2.4 and 4.9 fold in case of non BSO treated cells whereas 5.7, 4.5 and 8.5 fold in BSO pretreated cells after the exposure of Cd, UV1

and UV2 respectively as compared to control. GR was increased by 2.84 and 1.09 fold after Cd and UV1 treatment respectively but decreased by 17.7% in the case of UV1 as compared to control. On the other hand the GR activity in BSO pretreated cells was found to be enhanced by 3.17, 1.88 and 1.59 fold after Cd, UV1 and UV2 stress respectively. A low molecular weight protein PC was found to be induced after Cu and Cd treatment.

Table 3(a). Effect of different concentrations of Cd and UV-B on malondialdehyde content, activities of the antioxidant enzymes (SOD, CAT, APX and GR) and phytochelatin concentration in cells of *Anabaena dolium*. All values are mean \pm SD of three replicates. Values having different letters are significantly different ($P < 0.05$). Different analysis was done for each column (Duncan's new multiple range test). Values in parenthesis show %increase over control and those with negative sign indicate percent inhibition.

Treatment	MDA content (μM mol ng^{-1} protein)	SOD (μM SOD mg^{-1} protein $^{-1}$)	CAT (μM min^{-1}mg protein $^{-1}$)	APX (μM min^{-1}mg protein $^{-1}$)	GR ($\mu\text{M min}^{-1}$ mg protein^{-1})	PC concentration ($\mu\text{M mg}$ protein $^{-1}$)
Control	0.015 ± 0.011	0.0131 \pm 0.011	0.045 \pm 0.002	0.953 \pm 0.020	0.056 \pm 0.002	0.054 \pm 0.000
Cd	0.023 \pm 0.002 (53.7)	0.246 \pm 0.002 (87.1)	0.074 \pm 0.003 (62.7)	5.373 \pm 0.030 (463.7)	0.160 \pm 0.003 (185.7)	0.074 \pm 0.001 (34.6)
UV1	0.029 \pm 0.002 (89.2)	0.288 \pm 0.005 (119.1)	0.016 \pm 0.002 (-64.9)	2.256 \pm 0.035 (136.7)	0.061 \pm 0.002 (8.9)	0.056 \pm 0.000 (2.6)
UV2	0.032 \pm 0.001 (108.7)	0.311 \pm 0.005 (137.2)	0.013 \pm 0.002 (-72.2)	1.676 \pm 0.025 (390.6)	0.046 \pm 0.001 (-17.8)	0.056 \pm 0.002 (23.6)
Cd+UV1	0.022 \pm 0.002 (41.3)	0.365 \pm 0.004 (178.5)	0.080 \pm 0.003 (75.5)	3.456 \pm 0.034 (262.6)	0.124 \pm 0.003 (121.4)	0.067 \pm 0.002 (23.6)
Cd+UV2	0.016 \pm 0.001 (9.3)	0.256 \pm 0.002 (119.1)	0.062 \pm 0.004 (35.7)	1.543 \pm 0.032 (61.9)	0.072 \pm 0.003 (28.5)	0.078 \pm 0.002 (42.9)

Table 3b. Effect of different concentrations of Cd and UVB on malondialdehyde content, activities of the antioxidant enzymes (SOD, CAT, APX and GR) and phytochelation concentration in BSO pretreated cells of *Anabaena dololium*. All values are mean \pm SD of three replicates. Values having different letters are significantly different ($P < 0.05$). Different analysis was done for each column (Duncan's new multiple range test) Values in parenthesis show %increase over control and those with negative sign indicate percent inhibition.

Treatment	MDA content ($\mu\text{M mol ng}^{-1}$ protien)	SOD (μM mg l^{-1} protien $^{-1}$)	CAT (μM min^{-1}mg protein $^{-1}$)	APX (μM min^{-1}mg protien $^{-1}$)	GR ($\mu\text{M min}^{-1}$ mg protein^{-1})	PC concentration ($\mu\text{M mg prtien}^{-1}$)
BSO	0.018 \pm 0.000	0.260 \pm 0.005	0.019 \pm 0.001	1.730 \pm 0.051	0.056 \pm 0.002	0.054 \pm 0.000
Cd	0.027 \pm 0.002 (46.6)	0.355 \pm 0.001 (87.1)	0.026 \pm 0.004 (43.0)	5.480 \pm 0.020 (463.7)	0.160 \pm 0.003 (185.7)	0.074 \pm 0.001 (34.6)
UV1	0.026 \pm 0.001 (80.4)	0.795 \pm 0.008 (119.1)	0.029 \pm 0.003 (-36.9)	4.360 \pm 0.052 (136.7)	0.061 \pm 0.002 (8.9)	0.056 \pm 0.000 (2.6)
UV2	0.029 \pm 0.003 (89.1)	1.500 \pm 0.010 (137.2)	0.020 \pm 0.003 (-55.5)	8.123 \pm 0.015 (390.6)	0.046 \pm 0.001 (-17.8)	0.056 \pm 0.002 (23.6)
Cd+UV1	0.028 \pm 0.002 (82.6)	0.981 \pm 0.004 (178.5)	0.015 \pm 0.005 (-67.1)	5.663 \pm 0.047 (262.6)	0.124 \pm 0.003 (121.4)	0.067 \pm 0.002 (23.6)
Cd+UV2	0.023 \pm 0.002 (51.9)	1.791 \pm 0.004 (119.1)	0.027 \pm 0.002 (-40.8)	6.383 \pm 0.076 (61.9)	0.072 \pm 0.003 (28.5)	0.078 \pm 0.002 (42.9)

Discussions

Heavy metal toxicity to algae is one of the most debated environmental problem. Algae, cyanobacteria and other aquatic plants show sensitivity to metal toxicity displaying metabolic disturbances and growth inhibitions by heavy metal content only slightly higher than the normal level. Our study reveals a reduction in the survival and growth of *Anabaena dololium* with increasing concentration of Cu and Cd thereby confirming the toxicity of these metals. With the increasing concentration of Cu and Cd there was a remarkable reduction of 124 and 32.6% at 50 μM Cu and 0.1 μM Cd respectively by the 15th day. These results are in agreement with the findings of Fillips and Pallaghy (7). Rachin *et al* (19) who reported inhibition of growth and metabolism of algae and

cyanobacteria by Cu and Cd. The toxicity of these two heavy metals may be either due to the disruption of the permeability of the cell membrane or inhibition of photosynthetic pigment and enzymatic activities. These results offer support to the contention that growth reduction is reasonable determent of metal toxicity and that the degree of response of algae is deep dependent on the amount of metal that traverse for reduction of growth of cyanobacteria after exposure to heavy metal. This reduction could be due to the binding of the test metals to sulphhydryl group, which is responsible for regulation of cell division in plants including cyanobacteria. A concentration dependent decrease in content of all pigments was also observed in *Anabaena dololium*. The trend of pigment inhibition in this organism was Chl followed by

Caretenoid. Likewise protein content also depicted a gradual decrease in the metal supplemented cultures. The inhibition of Chl could be due to the bleaching of the pigment. Further the loss of CAR indicates that this pigment has little role in regulating metal toxicity.

Further decrease in the lipid content (data not given) with time could be due to the increased peroxidation of the membrane lipid by the heavy metals. All the studied parameters e.g. carbohydrate, lipid, DNA, RNA and protein. Except carbohydrate registered decrease in their content with the increase in the concentration of both the metals (Cu and Cd). The increase in carbohydrate content might be due to the thickening of the mucilaginous sheath. The reason for the reduction in DNA content may be the reduction of growth. As the growth rate is inhibited the number of cells will decrease and proportionally the DNA content will also decrease. This decrease could in turn cause a loss in the transcription rate hereby reducing the RNA content. Further, the reduction in RNA content could also bring about a reduction in protein content could also be due to the oxidative damage caused by the metals as observed in table 2.a, b, c, d.

The results presented in table 2.a,b,c and d clearly demonstrate oxidative damage caused by Cu and Cd as expressed in terms of enzymatic oxidant activity. These stresses were found to produce ROS, which are highly deleterious to the cell. To scavenge the cellular defense system is stimulated inducing either production of antioxidants and stress proteins. The activity of the enzymatic antioxidant SOD was stimulated by Cd as well as Cu but more so by Cu. The increase in SOD activity after metal treatment could be due to the production of O_2^- anions whose detoxification is necessary for the growth of organism, thus it

converted into peroxide by the activity of SOD. This could be due to the redox nature of Cu, which facilitates the production of superoxide anion. Cd being non redox metal stimulates the production of peroxide. The data obtained clearly show that the enzyme activity increased consistently with time. It is worth stating that SOD is the first enzyme of the superoxide of the antioxidant pathway responsible for the scavenging and conversion of the superoxide anion into H_2O_2 . H_2O_2 is known to generate OH radicals by interacting with reductants such as transition metal ions (Fe^{2+}) generated as a result of the reduction of (Fe^{3+}) by the superoxide radical. Fe^{2+} complex + H_2O_2 → $Fe^{3+}(\text{complex}) + OH^- + OH^\cdot$. Both these species (H_2O_2 and OH^\cdot) have the potential to oxidize membrane fatty acids, thereby initiating lipid peroxidation OH^\cdot is more reactive and is more reactive and damaging than H_2O_2 because no specific enzyme is available for its scavenging. Moreover, its reaction with cellular components proceeds at diffusion controlled rates (10^8 - $10^9 M^{-1} s^{-1}$). Thus even a trivial and transient expression of OH^\cdot can cause damages to the membrane lipid (Asada 1999). Under these circumstances, it is essential that H_2O_2 produced scavenged either by CAT or with the help of well known enzymes of the Halliwell Asada pathway (APX and GR). The activity of SOD and GR was remarkably enhanced. Contrary to this CAT activity was suppressed with the passage of time. Table 2-b reveals that by -51.8 and 44.7% Cu (8.2 μM) on the other hand inhibited its activity by -42.2% by the 15th day. The inactivation of CAT can be explained in the light of the fact that Cd and Cu can replace the metal present in CAT, which is responsible for its proper functioning (Hall,2002). Thus the insignificant effect of Cu and Cd on CAT leaves the problem of H_2O_2 scavenging unresolved. Under the circumstances other peroxide scavenging enzyme

have to take up the job. Our results show a 84, 14, 64 and 104 fold increase after the treatment of Cu1, Cu2, Cd1 and Cd2 respectively provide a testimony to the view that APX has greater affinity for H₂O₂ than CAT and is a major H₂O₂ scavenging enzyme under stress conditions (Streb *et al.* and Foyer *et al.*, 1994). Further, the GR was also found to be increased by 17, 37, 18 and 36 fold after the treatment of Cu (8.2uM and 20 uM). Our results are also supported by Nagalakshmi and Prasahad (17) and Mallick and Rai (15) who reported increase in APX and GR activities at increasing doses of Cu in *Scenedesmus bijugatus* and *Anabaena doliolum* respectively. Cd and Cu induced increase in GR activity can be explained on the basis of the transcriptional activation of gr1 and gr2 genes. Further APX activity was also induced due to increase in H₂O₂ concentration, which not acts as signal for apx operon but also as direct inducer of apx gene. Nevertheless, the decrease in the oxidative damage and suppression of antioxidant enzyme activity as well as lipid peroxidation in the Cd pretreated cells exposed to UV-B clearly supports the hypothesis that PC probably has a role in UV-B tolerance.

Conclusion

All the studied parameters viz. growth, Chl/CAR content, biochemical fractionation as well as antioxidative defense system clearly demonstrated that Cd and Cu induce the oxidative damage in the cells of *A. doliolum*. The SOD activity in control cultures did not increase with time, but the other enzymes were found to increase continuously. Further in the metal treated cells the activity of SOD does not increase but metal stress tremendously increased its activity. This observation can be of significant practical importance in that the SOD activity can be used as a bio-marker of metal pollution. Further it is also

found that under stress conditions the activities of the enzymes increased continuously with time until the 15th day. This shows that adaptation of the cell to metal stress is very much dependent on the antioxidative defense system.

An off shoot of this work was to study the involvement of PC in UV stress. The results revealed -very significant and first hand information that the induction PC after metal stress co-tolerance in the cyanobacterium to UV-B. However, the actual reason for this could not be established and this becomes an area of future research.

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THE ROLE OF GENITAL CHLAMYDIAL INFECTION IN ACUTE PELVIC INFLAMMATORY DISEASE,

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ABSTRACT

The polymicrobial nature of pelvic inflammatory disease (PID) underscores the need for a clearer understanding of the pathogenesis and etiology of PID especially among core groups most at risk. This study was designed to determine the role of specific microbial infections in leading to PID among women. Prevalence of genital chlamydial infection and other reproductive tract infections were determined in 100 women presenting at a health facility at Port Harcourt, Rivers State, Nigeria. The result showed that 11.1 per cent of women with acute PID were infected with *Chlamydia trachomatis* as compared to 4.3 per cent in the control group (odds ratio 2.75; 95% confidence interval (CI), 0.7-11.7). *Neisseria gonorrhoeae* was not detected in either of the two groups. Trichomoniasis (10% in PID cases and no case in control group) and bacterial vaginosis (17.5% and 4.3% in PID and control group respectively: Odds ratio 4.7, 95% CI, 1.0-21.1) were also significantly associated with the clinical picture suggestive of acute PID. It is recommended that where resources are limited, patients presenting with acute PID be treated empirically for *Chlamydia trachomatis*, trichomoniasis, bacterial vaginosis and gonorrhoea.

Key Words: Pelvic inflammatory disease, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Bacteria vaginosis, Trichomoniasis,

INTRODUCTION

Appreciating the etiologic relationship between sexually transmitted disease (STD), pelvic inflammatory disease and infertility is a complex exercise which involves looking at the correlation among varying conditions with varying definitions (1). The term pelvic inflammatory disease has come to represent clinically suspected endometritis and or salpingitis that have not been objectively confirmed pathologically. Thus, the diagnosis of PID is often made in sexually active women based on complaint of lower abdominal pain in addition to abnormal vaginal discharge, cervical uterine and adnexal tenderness.

Several investigations have emphasized the polymicrobial nature of pelvic inflammatory disease (2, 3, 4, 5, 6). In general, three major groups of microorganisms are recognized as

playing an etiologic role in PID which includes *N. gonorrhoeae*, *C. trachomatis* and a wide variety of anaerobes and aerobic bacteria. *C. trachomatis* and *N. gonorrhoeae* are associated with the initiation of tubal infection while the anaerobic and aerobic bacteria that constitute the normal vaginal flora are involved as secondary invaders in an infected female. The rates at which Chlamydia, *N. gonorrhoeae* and other organisms have been found in patients with symptomatic PID differ widely (5, 7). For example recovery rate of Chlamydia organisms varied from 25% to 50 % in

symptomatic PID patients in industrialized nations (7). However, there is paucity of data in developing countries. There is therefore an urgent need for prevalence studies of Chlamydia and other reproductive tract infections among groups most at risk of PID especially in resource poor settings. A clearer understanding of the etiology of PID and confirmation of the role of specific vaginal infections in leading to PID might reduce the need for invasive diagnostic procedures and permit a more rational basis for selecting antimicrobial therapy in the individual patient.

This study therefore gives preliminary information on microbiological indicators in patients with lower abdominal pain satisfying the diagnostic criteria for PID as outlined in the methodology compared to a control group of patients without symptoms suggestive of PID.

Materials and Method

As part of an on-going study on reproductive health of women in Rivers State which started in 1997, a total of 100 women referred to the clinic were recruited into the study after their consent was obtained. For the purpose of this study, a probable case of PID was defined as a patient presenting with lower abdominal pain and self reported copious foul smelling vaginal discharge only, (this was included to assess the validity of treating such patients syndromically for PID) and a case definition of PID was taken as a patient presenting with self reported abnormal vaginal discharge, lower abdominal pain and adnexal tenderness. A control case was defined as a woman referred for counseling for family planning purpose with no clinical evidence of lower abdominal pain or abnormal vaginal discharge. Questionnaires with basic demographic data and gynecological history

were compiled. Gynecological examination was done.

Laboratory investigations: High vaginal swabs were taken. Wet mount microscopy was done to identify *T. vaginalis*, clue cells and yeast cells. Bacterial vaginosis was further identified by presence of clue cells in Gram stained smear (8). Two endocervical swabs were taken from each patient. One was inoculated in Thayer Martins selective medium for isolation of *N. gonorrhoeae* and incubated under anaerobic conditions for 48 hours. Each batch of plate was quality controlled with *N. gonorrhoeae* standard strain ATCC 49226. The second cervical swab for Chlamydia antigen was processed within a week of collection using an Enzyme Linked Immunosorbant Assay (ELISA) (MASTAZYME; Mast laboratory, Bootle UK). All ELISA positive and borderline specimens were confirmed by direct immunofluorescence assay (MICROTRAT; SYVA, Berkshire UK). Briefly, test specimens were vortexed and centrifuged at 13000rpm for 30 minutes. Pellets obtained as residue were stained on immunofluorescence slide according to procedure outlined on the kit and then observed under immunofluorescence microscope. Slide with elementary bodies appearing as individual pinpoints of medium to bright apple green fluorescence was considered positive for Chlamydia. A positive control was included in each run.

Result

Of the 100 women initially followed up 40 patients were classified as probable cases of PID and 27 as PID cases. The data for the rest was considered incomplete and therefore not analyzed. The age range was 15-42 years with a mean age of 26 years ($SD \pm 5.8$). The prevalence rate of vaginal infection in the study population is as indicated in table 1 while table 2 and 3 highlights the prevalence of

reproductive tract infections in women with lower abdominal pain and those who met the criteria of PID as defined in the study respectively.

vaginosis as defined by gram-stained smear (presence of clue cells - curved gram variable cocco-bacilli surrounding epithelial cells, absence of pus cells) gave a prevalence rate of 13.8% in the overall study population and 25.9 per cent when considering only those with PID.

The prevalence rate of Chlamydia was 6.9% in the study population. The rate increased to 12.5% when considering women presenting with abnormal vaginal discharge and lower abdominal pain. While it was 11.1% in the PID study group. Prevalence rate of *T. vaginalis* was 4.6 per cent in the overall study population. The rate increased to 10.0% in patients presenting with lower abdominal pain and abnormal vaginal discharge and 14.8% when adnexal tenderness was added to the two cardinal symptoms. There was little difference in the prevalence rate of yeast infection, 51.7%, 47.8%, and 47.8% in the overall study population, PID cases and control cases respectively.

There was a case of mixed sexually transmitted infection with Chlamydia and *T. vaginalis*. Also *Neisseria gonorrhoeae* was not isolated during the study period.

Endocervical polymorph nuclear cell (PMN) counts were not predictive of acute PID or Chlamydia infections. 24.3% of patients with PID had PMN counts greater than 10 per oil immersion field as compared to 27% in the control cases. However, in TABLE 1: Overall distribution of reproductive tract infection in the study population

Organisms	Prevalence rates (%)
<i>Chlamydia trachomatis</i>	6.9
<i>Neisseria gonorrhoeae</i>	0
<i>Trichomonas vaginalis</i>	4.6
Yeast cells	51.7

Bacterial

the patients with acute PID, 66.7% of the chlamydial infection was described clinically as presenting with mucopurulent vaginal discharge.

TABLE 2: Prevalence of reproductive tract infections

among patients presenting with lower abdominal pain, and abnormal vaginal discharge

	*Probable PID cases (n=40)	Control cases (n=23)	
<i>Chlamydia trachomatis</i>	12.5	4.3	(Odds ratio 3.1, 95% CI, 0.9-10.8)
<i>Neisseria gonorrhoeae</i>	0	0	
<i>Trichomonas vaginalis</i>	10.0	0	
Yeast cells	55.0	47.8	
Bacterial vaginosis	17.5	4.3	(Odds ratio 4.7, 95% CI, 1.0-21.1)

TABLE 3: Prevalence of reproductive tract infections among patients presenting with all 3 criteria (lower abdominal pain, abnormal vaginal discharge and adnexal tenderness).

	*PID cases (n=27)	Control cases (n=23)
<i>Chlamydia trachomatis</i>	11.1	4.3 (Odds ratio 2.75, 95% CI, 0.7-11.1)
<i>Neisseria gonorrhoeae</i>	0	0
<i>Trichomonas vaginalis</i>	14.8	0
Yeast cells	51.9	47.8
Bacterial vaginosis	25.9	4.3 (Odds ratio 7.7, 95% CI, 2.3-24.3)

Discussion

This study alerts us to the fact that Chlamydia may be an important etiologic agent in the development of acute PID in Port Harcourt. The prevalence of

Trichomoniasis and gram stained smear appreciation of curved gram variable coccobacilli and clue cells typical of bacterial vaginosis have been strongly associated with acute PID compared with the control group. Few studies in Nigeria have been carried out with the aim of attempting an etiologic base for acute PID. No published article was available on prevalence of chlamydia in acute PID in Port Harcourt. Therefore, it is clear that a more comprehensive well designed study is needed to better appreciate the association highlighted in this preliminary study.

genital chlamydial infection in the overall study group was 6.9 %. The percentage increased to 11.1% in the PID case compared to 4.3% in the clients without PID, (odds ratio 2.75, 95%, CI 0.7-11.7).

Patients with *C. trachomatis* infection were significantly younger and were more often unmarried compared to patients without *C. trachomatis* infection. These findings are comparable to other studies (1, 2). The addition of adnexal tenderness to our definition did not significantly alter the prevalence of Chlamydia. This would be most interesting if confirmed by a more elaborate study. The entry point in the syndromic algorithm for PID management in the National Manual on Syndromic Management of STD is lower abdominal pain complemented by abnormal vaginal discharge. If Chlamydia

recovery is not enhanced by addition of the third criteria of adnexal tenderness as this study suggests, it would be useful information in evaluating management of PID in this setting.

T. vaginalis is not usually described as a pathogen of the cervix. However, studies are beginning to associate the organism to cervical infection (10) and recently with PID in women infected with HIV (11, 12). In most cases, PID is associated with *N. gonorrhoeae* or *Chlamydia trachomatis* (13). Our study showed a case of one mixed infection of *T. vaginalis* and *C. trachomatis*. *Trichomonas vaginalis* recovery is strongly associated with acute PID in this study, even more so when adnexal tenderness was added to the lower abdominal pain and abnormal vaginal discharge; 10.0 per cent and 14.5 per cent respectively compared to the control cases where the organism was not isolated. This result tends to suggest that some of the PID cases may in fact be trichomoniasis. This finding has also been observed in South Africa (14).

The absence of gonococcal infection may be explained by self-prescription of antibiotics amongst the study population. Self medication may not affect Chlamydia infection since the drug need to be taken for a longer period and compliance is not likely to be good enough to complete the dosage.

Bacterial vaginosis has been associated with acute PID, though in a rather undefined manner. This study shows a pronounced association between the gram appearance typical of bacterial vaginosis and acute PID compared to the control. The picture of the clue cells defined as curved gram variable coccobacilli distorting the epithelial cell lining and lack of gram positive bacilli is indicative of bacterial vaginosis. (15) Studies linking this picture with anaerobic mobiluncus culture confirm that while the "whiff test" would indeed be

complementary to the microbiologic picture, the gram stained smear is sensitive and specific enough to suggest bacterial vaginosis. It is argued that primary cervical pathogen such as Chlamydia may in effect alter the vagino-cervical micro-environment leading to an alteration of the flora and presenting as bacterial vaginosis (1, 17).

In conclusion genital Chlamydial infection is shown to be associated with the clinical picture typical of pelvic inflammatory disease. Trichomoniasis and bacterial vaginosis were also associated with this condition when compared to the control group.

In resource poor setting where one is not likely to have access to laparoscopy to make a diagnosis of acute salpingitis or the facility to culture Chlamydia, it is recommended that empirical treatment for acute PID should include treatment for chlamydial infection, bacterial vaginosis and trichomoniasis. Other studies have outlined the importance of also treating for *N. gonorrhoeae*. These preliminary findings have shown the importance of designing a comprehensive study to better appreciate the polymicrobial etiology of acute pelvic inflammatory disease.

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AN OUT-BREAK AND OBSERVATIONS ON TRYPANOSOMIASIS IN FRIESIAN CATTLE AT SABON-BIRNI, KADUNA STATE OF NIGERIA

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ABSTRACT

Bovine trypanosomiasis has clinical features characterized by anorexia, emaciation, anemia and the prognosis is usually guided culminating in death. An out-break of trypanosomiasis was reported and subsequently investigated in Batagarawa farms, Sabon-birni, Rigachukwu LGA Kaduna State of Nigeria. Clinical observations were made on parameters including appearance, temperature, pulse rate, respiratory rate, color of conjunctiva and lachrymal secretions. Record of pre and post treatment indices were also taken.

Trypanosoma congolense was isolated from five (5) Friesian cattle out of forty (40) Friesian cattle sampled representing 12.5%. Several workers had reported the occurrence of trypanosomiasis in cattle and goats. The present study deals with the observations on naturally occurring trypanosomiasis in Friesian cattle.

Haematological examinations were performed employing routine procedures. Biochemical activities and parameters were determined by standard calorimetric method using blood chemistry analyzer. Animals were treated with start doses of Beremil (3.5mg/kg body weight) intramuscularly.

Trypanosomiasis has been recognized as a disease of great economic importance as it generally causes heavy production losses by reduction in milk and other protein yields. There is emaciation in sub-acute and chronic conditions, low working capacity and high mortality in acute cases. This study confirmed that trypanosomiasis is a threat to introduction of exotic breeds of cattle into Nigeria.

INTRODUCTION

Trypanosomiasis is a serious disabling and debilitating tropical disease of man and domestic animal is caused by protozoan flagellate haemoparasite of the genus *trypanosoma*. It is transmitted by anthropoid tsetse flies (*Glossina spp.*) and is characterized by parasitemia, fever, anemia, loss of normal condition, reduced productivity and frequently high mortality (Ford, 1971; Iwuala *et al*, 1980., Robertson, 1976; and Seigmund *et al*, 1973).

Animal trypanosomiasis constitute a major threat to food security in Nigeria and other part of sub-sahara Africa (Onyiah, 1997, Swallow, 2000 and Abenga *et al*, 2002). Tsetse transmitted trypanosomiasis caused by single-celled parasites, trypanosome, has been recognized as a disease of great economic importance and major cause of livestock death in Nigeria and Africa each year leading to reduction in livestock numbers, reduce calving rates, milk yield, meat supply, work

efficiency of draft animals and mixed farming (Swallow, 2000). Control of the disease has explored the use of drugs, vector control and breeding of trypanotolerant livestock in order to enhance productivity. However, the difficulties associated with these control methods, include drug resistance, re-invasion of the controlled areas by tsetse flies and small population of the trypanotolerant cattle (Enwezor *et al*, 2003). Small ruminants may not often show the clinical signs of this disease. It is assumed that they are rarely affected under natural condition and that trypanosomiasis is not a serious problem (Kalejaiye *et al*, 1995). The occurrence of trypanosomiasis in local breed of cattle (Kumari *et al*, 2000, Rajguru *et al*, 2000), small ruminants (Kalejaiye *et al*, 1995, Mc, Guire *et al*, 1985, and White Law *et al*, 1985) Mcwuena, and Kanyari *et al*, 1986) and also in buffalos (Joshi, and Bhoopsingh, 2001) are well documented. But there is scanty report on the occurrence of trypanosomiasis in Exotic breed of cattle in Nigeria, and abroad. The purpose of this study was to investigate the clinical, haematological and biochemical features of naturally occurring trypanosomiasis in Friesian cattle.

MATERIALS AND METHODS

The outbreak occurred in dairy herd of 40 grade intensively managed Exotic Friesian breeds of cattle and 240 white fulani of both sexes with ages ranging between two (2) and five (5) years. This herd belonging to Batagarawa farms Sabon-Birni, Rigachikwun Local Government Area, Kaduna State of Nigeria. It covered 40 hectares. The report showed that animals were clinically healthy initially and there had been no recent introduction of cattle into herds, but occasionally neighbors' cattle would stray into the farm. They were housed in pens at night and released into a fenced paddock

to graze on improved grass and legume pasture during the day. The feed was supplemented with maize, citrus pulp, brewer's grain and salt lick. The animals were allowed free access to water from nearby natural free flowing stream (river) about 1000 meters to the fenced paddock. The river formed one boundary and cultivated land bordered the rest of the farm. Considerable thicket re-growth had occurred on parts of the farm.

All the animals, 40 Friesian cattle and 240 white Fulani (Bunaji) cattle weighing between 250-350kg body weight belonging to the same farm, but were located at different paddock, 1 km apart. The animals were initially prophylactically treated against trypanosomiasis and other haemoparasites on the 15th August 2004 with diminazine aceturate (Berenil® Hoechst, Hamburg, Germany) at 3.5mg/kg body weight intramuscularly. They were also treated against nematode parasite at 4 months intervals with Ivermectin (Ivomec® Merk Sharp and Dome Haavlem, Netherlands) at 0.02mg/kg body weight subcutaneously. Routine disease control measures consisted of once weekly hand spraying of all cattle with 0.3% of toxaphene emulsion to control ticks notably *Boophilus microplus* and *Rhipicephalus evertsi*

All the forty (40) Friesian cattle were sampled on 18th May 2005. About 5 mls of blood was collected from the 40 Friesian cattle via the jugular vein, into the ethylene tetra-acetic acid (EDTA) bottle for routine test before treatment. The samples were subjected to haematocrit centrifugation techniques (HCT) by Woo (1971) and Buffy Coat Method (BCM) for detection of trypanosomes (Murray *et al*, 1977). The Packed Cell Volume (PCV) was used to assess the anemia while the motility in wet film and morphological

appearance in Giemsa stained film were used to identify the trypanosome species.

All the forty (40) Friesian cattle were given a dose of Berenil (3.5mg/Kg body weight) intramuscularly. Blood was again collected 7-10 days after treatment to assess or ascertained effectiveness of the treatment.

Clinical observations including general appearance, posture, temperature, pulse rate, colour of the

conjunctiva and lachrymal secretions were recorded before and after treatment. Haematological examination were performed employing routine procedure (Schlam *et al*, 1975, Rajkwowa *et al*, 2003). Biochemical parameters were estimated by standard colorimetric method using blood chemistry analyzer (RA-50), Bayer diagnostics, Gujarat, Rajkhowa *et al*, 2003.

Table: Haemato-biochemical profiles (before and after treatment) of Exotic Friesian cattle suffering from typanosomosis (Mean \pm SE)

Parameters	Before treatment	After treatment	Normal values (n=30)
Hb (gm%)	9.30 \pm 0.30	12.80** \pm 0.00	13.51 \pm 0.15
PCV (%)	29.50 \pm 0.50	39.5* \pm 0.50	40.50 \pm 0.20
TEC $\times 10^6/\Phi l$	8.80 \pm 0.25	11.91** \pm 0.15	12.91 \pm 0.15
TLC $\times 10^3/cumm$	4.26 \pm 0.15	1.70** \pm 0.20	2.30 \pm 0.25
DLC			
Neutrophils (%)	52.80 \pm 0.80	48.05 \pm 0.05	47.02 \pm 0.65
Lymphocytes (%)	45.00 \pm 0.00	49.00 \pm 1.00	50.0 \pm 0.65
Monocytes (%)	2.50 \pm 0.25	2.00 \pm 0.00	1.95 \pm 0.35
Eosinophils (%)	2.50 \pm 1.00	2.00 \pm 0.25	2.05 \pm 0.45
Basophils (%)	0.0 \pm 0.0	0.0 \pm 0.0	0.50 \pm 0.05
Blood glucose (mg%)			
TSP (gm%)	30.00 \pm 1.00	36.00** \pm 3.00	36.83 \pm 0.17
SGOT (U/L)	6.40 \pm 0.15	6.30 \pm 0.05	6.50 \pm 0.05
SGPT (U/L)	102.50 \pm 0.15	161.50** \pm 1.50	162.15 \pm 3.35
	35.00 \pm 2.00	34.50** \pm 0.50	36.02 \pm 2.00

DLC = Direct Leucocyte Count. n=number of animals. *p<0.05 ** p<0.01

RESULTS AND DISCUSSION

During a social visit to the farm it was noted that many of the cattle had developed skin lesions and

that three (3) females pregnant Friesian cow out of forty (40) Friesian cattle aborted a seven (7) to eight (8) months fetus on the 14th May 2005, while

seven (7) other Friesian cattle were found clinically unhealthy, suspected for trypanosomosis and were examined physically before and after treatment for the presence or absence of Cachexia and other clinical signs suggestive of trypanosomosis (Budd, 1999). Ten (10) of the Friesian cattle had a very prominent ribs and three (3) Friesian cattle are disabling, while seven (7) Friesian were debilitating conditions. Six were found to have elevated rectal temperature, one as high as 41.1°C and were examined thoroughly by standard laboratory procedure. (Rajkhowa *et al* 2003).

Trypanosoma congolense was isolated from five (5) Friesian cattle out of forty (40) Friesian cattle sampled representing 12.5%. Identification of trypanosomes were based on the following criteria: smallest of the size, active motility, the absence of free flagellum, the bluntness of free flagellum, the bluntness of posterior extremity and typical marginal position of the kinetoplast (Richardson and Kendall, 1964). Several workers had reported the incidence or occurrence of trypanosomosis in cattle and goats. (Ugochukwu; 1983, 1986). But considering the source of these Friesian cattle (Cape-town and Europe, (Netherlands)) there is not a single report available on the occurrence of trypanosomosis in this breed of cattle. The present study deals with the observation on naturally occurring trypanosomosis in Friesian cattle. Similar findings were also reported in some breed of cattle suffering from animal trypanosomosis (Kumari *et al* 2000). It is known that trypanosomiasis occurs in all domestic animals in tropical Africa, resulting in acute and chronic manifestation with regular fever, anemia, emaciation and sometimes photophobia. Authors like Kariuki and Jacobson, 1980; Richardson and Kendall, 1964; Smith, Jones and Hunt 1972, are of the opinion that pathogenesis of trypanosomiasis

in man and domestic animals is not thoroughly understood. There is a lot of literature on haematological and biochemical changes in trypanosomal infected animals.

The affected animals showed the symptoms of intermittent fever, dullness, emaciation, anemia palour and mucopurulent discharges from the eyes. All these symptoms disappeared after treatment (7th – 10th days of treatment). There were significant decrease in pulse rate, respiration rate and temperature after initiation of treatment and all these parameters returned to their normal levels by 3rd day post-treatment. Haemato-biochemical changes during trypanosomiasis are in the table I. Marked elevation of the body temperature was the first clinical manifestation following the appearance of the parasites in the peripheral circulation. The fever fluctuated during the course of trypanosomosis treatment irrespective of the level of parasitemia. Ikede *et al* (1977) made similar observation in rabbits infected with *T. brucei*/ *T. congolense*. Other clinical signs were dullness, emaciation and hyperopnoea – probably related to the fever, anorexia and anaemia respectively. Decreased haemoglobin (Hb), packed cell volume (PCV) and total erythrocytic count (TEC) were noticed in affected Friesian cattle before treatment, which is suggestive or indicative of anemia.

Similar findings were also reported in cattle suffering from trypanosomiasis (Kumari *et al* 2000). The decrease in Hb, PCV and TEC values in the present studies might be either due to inhibition of erythrocyte formation in the bone marrow or their lysis by endotoxin liberated by *trypanosomes*. The total leucocytic count (TLC) and differential leucocytic count (DLC) revealed leucocytosis associated with lymphopenia

neutrophilia and eosinophilia in affected Friesian cattle before treatment. No significant alteration in the percentages of monocytes and basophils were observed (seen or noticed). Present findings are in agreement with the findings Rajguru *et al* (2000) in cattle and naturally occurring trypanosomiasis in Mithus (*Bos frontalis*), Rajkhowa *et al* 2003.

Blood glucose showed significant decrease in affected Friesian cattle. The marked hypoglycaemia might be either due to rapid consumption of glucose by trypanosomes (Gill, 1977) or liver dysfunction (Blood and Radostits, 1989). Non-significant variation was observed in the value of total Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) level before treatment. These returned to their normal levels by 7th day after initiation of treatment with Berenil®. The increase in the levels of SGOT and SGPT might be due to liver dysfunction as reported by Blood and Radostits (*loc.cit.*). Increase levels of SGOT and SGPT were also reported in buffalo calves infected with trypanosomes *evansi* (Singh and Gaur, 1983).

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ENDOMETRIAL TUBERCULOSIS IN INFERTILITY: REPORT OF 2 CASES AND A REVIEW OF THE LITERATURE

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Tuberculosis is a chronic granulomatous disease affecting human and many other mammals. Most human diseases are caused by *M. tuberculosis* but some are due to *M. bovis*. (1).

Tuberculosis of the tubes and endometrium is intimately bound to the problem of sterility. Despite the decline in frequency of genital tuberculosis in industrialized world coupled with widespread use of antibiotics worldwide, it still remain a possible cause of female infertility especially in developing world. (2)

It was the commonest diagnosis among infertile population in India (3), Malaysia (4) and Saudi Arabia (5). Nigeria, U.S.A., and Pakistan have reported a low and infrequent findings (6,7,8).

Endometrial tuberculosis seems to occur when the ovaries and uterus are in a state of activity (9) and it derives its significance as a clinical entity because of its tendency in focusing on those in these reproductive age group.

Tuberculosis of the genital tract gives rise to few symptoms especially in its mild or moderate phase, its discovery is usually made unexpectedly but will render a large percentage of infected women sterile unless detected and treated adequately in its earliest phases. The cases presented below is to highlight its clinical presentation and to serve as a reminder that pelvic tuberculosis still exist and will not disappear unless tuberculosis is completely eradicated.

Case Reports

Case 1: Mrs. I.T is a 26years old Nigeian nulliparous woman who presented on 13th of September 2002 with 3years history of inability to get pregnant despite adequate unprotected sexual contact with her husband, on and off colicky lower abdominal pain, irregular menses with menorrhagia in the 2years preceding presentation.. She had her menarche at the age of 15.

On physical examination, significantly physical findings were suprapubic tenderness on deep palpation, bulky uterus of 8weeks size and positive cervical excitation tenderness. The husband's semen analysis shows a count of 20million spermatozoa per ml and 70% motility. Her PCV

was 33%, White blood cell (WBC) count of $4.8 \times 10^9/l$, differentials: neutrophils (57%), lymphocytes (37%), monocyte/eosinophil (06%) and ESR of 30mm/hour. High vaginal and endocervical swabs for microscopy culture and sensitivity shows no significant growth, the urinalysis and electrolyte, urea and creatinine were essentially normal. Ultrasound scan showed sizeable amount of fluid in the pouch of Douglas with internal echo giving an impression of Pelvic inflammatory disease. Hysterosalpingiography (HSG) shows a dilated right fallopian tube in its outer half in keeping with right hydrosalpinx. The left fallopian tube was not demonstrated. Chest X Ray was normal. Histopathology report of endometrial biopsy showed granulomas with

multinucleated giant cells, mononuclear cell infiltration and dilated tubular gland. Ziehl Neelsen stains confirm acid-fast bacilli. A diagnosis of primary infertility secondary to tuberculous endometritis was made. Patient was subsequently referred to medicine department for anti tuberculous therapy.

Case 2

Mrs LI, a 32years old para 1+0 housewife presented on the 23rd October 2003 with 2years history of inability to conceive despite regular unprotected intercourse, 6weeks history of abdominal pain and 2weeks of progressively increasing abdominal swelling and dysmenorrhea. The last childbirth was 7years earlier. There was no history of cough or contact with patient with chronic cough. No history of heat or cold intolerance and no weight loss. Patient had presented in a private hospital earlier where abdominal ultrasound and hysterosalpingography was done among other investigations. She is the second wife of the husband who had a child through the first wife 2years earlier.

On examination, there was a cystic, non-tender, non-mobile lower abdominal mass of 14weeks size. Uterus was deviated to the right but not enlarged.

The PCV was 30%, abdominal ultrasound scan shows a huge multicystic left ovarian mass probably undergoing torsion. Hysterosalpingography revealed bilateral tubal blockage. An assessment of secondary infertility with ovarian cyst and bilateral tubal blockage was made and patient was admitted for exploratory laparotomy.

Intraoperative findings include abdominal mass of 16weeks size, completely matted pelvic organs with inability to identify any organ (frozen pelvic), adhesion involving omentum and gut with purulent/caseous exudates obtained from cul-de sac of the mass and haemorrhage with minimal ascites .

Minimal adhesionolysis was done to permit exploration and samples of ascitic fluid and purulent caseous exudates were taken for cytology, Acid-fast Bacilli and tissue biopsy. Her postoperative condition was uneventful and patient was discharged home on the 8th day. Sample taken for histology showed extensive area of necrosis and numerous granulomas comprising of macrophage, plasma cells and lymphocytes. There were also multinucleated giant cells of the foreign body and langhans type. A diagnosis of tuberculous endometritis was made and patient was commenced on antituberculous therapy. However she was lost to follow up.

Tuberculosis still remains a major disease in the developing countries (1). The prevalence of endometrial tuberculosis varies between countries. It is rare in the U.S.A., higher in Europe and much higher in developing countries (1,5,7,).

Pelvic tuberculosis is always secondary to tuberculosis elsewhere in the body and the primary lesion may remain silent for many years (10,11,12). Female genital tuberculosis usually begins from an hematogenous spread to the endosalpinx from where it may spread to the endometrium(50%),ovaries(30%),cervix(10%), and vagina(1%)(13). Nogales-Ortiz et al (14) showed involvement of the tube in 100% of the patients, endometrium (79%), myometrium (20%), uterine cervix (24%) and ovaries (11%).

Our two cases presented at ages 26 and 32 years. Tuberculous endometritis is commoner in the reproductive age group especially in developing countries where they are commonest in the 26-35 years age group (7, 15,16,17,18). Though rare, tuberculous endometritis has also been reported in postmenopausal women (10,19). The rarity is possible because of the decreased vascularity of the tissue, the lack of regular endometrial shedding in such patients, however means there is no barrier to

the establishment of the infection and to its progression (20).

The discovery of endometrial tuberculosis are usually unexpectedly mostly by endometrial biopsy in the course of investigative studies to explain infertility. The two cases were discovered while been investigated for infertility. According to Ojo (21), Schaefer(22), Chattopadhyay(5) and Klein(17) ,between 0.7-9.3% of infertile women are affected with endometrial tuberculosis.

Both patients here presented with lower abdominal pain. An intermittent chronic ache in the lower abdomen is observed in 20-30% of cases, in nearly 20%, the pain is mistaken for sub acute peritonitis (23). One of the two cases was managed for concomitant acute pelvic inflammatory disease. Pain in such patients may be due to secondary organisms invading the tube (24). Menstrual function may remain normal, menstrual abnormalities seen include oligomenorrhoea (54%), Menorrhagia (19%), amenorrhea (14.3%), postmenopausal bleeding (1.6%) and dysmenorrhoea (rare) (23,24).

Our patients show no constitutional symptoms at presentation. Malaise, loss of weight, night sweats and fever are seen only during unusually active phase of the disease (24).

The most likely explanation of failure to diagnose tuberculous pelvic infection correctly is the difficulty in distinguishing it from various forms of pelvic inflammation (24). Symptoms of chronic pelvic inflammatory disease in a virgin or in infertile woman with no past history of post abortal or venereal infection should be assumed to be tuberculosis until proven otherwise (23,24). Also one may suspect tuberculosis if the pelvic inflammatory process does not respond to antibiotic therapy one would expect if the principal abnormalities were due to gonococcus or streptococcus (24).

X-ray diagnosis is useful but not conclusive and could not alone provide a basis for definitive therapy (10,17). In the early stages, no evidence of endometrial infection may be present and hysterosalpingogram may be normal. As the process advances, confluence of the affected areas with caseation and ulcer develops; it then demonstrates variation from the normal. In more advance cases, the X-ray will reveal distortion of the endometrial cavity. In rare instances, the uterine cavity may be entirely obliterated so that no dye will enter and hysterosalpingogram may show a portion of the cervical canal (25) This is further exemplified by our two cases.

Final diagnosis depends on pathologic and bacteriological study of tissues or secretions. The diagnosis and confirmation of o

ur cases was by histology. Accurate diagnosis with biopsy depends on a biopsy taken late in the cycle (20,24). The finding of epithelioid clusters with giant cell is highly suggestive but not conclusive, unless tuberculous bacilli can also be demonstrated in special stained preparations. Traditional Ziehl-Neelson staining with basic fuchsin is satisfactory. Modern laboratory processing use auramine-rhodamine staining and fluorescence microscopy. Part of endometrial tissues is sent for culture as well, otherwise endometrial tuberculosis may be missed in up to 50-75%cases (25). PCR can also be done on endometrial tissue. However, this may be positive even with dead bacilli and may not be reflective of an active disease. If endometrial sampling is not possible, the collection of first day menstrual discharge may reveal positive culture. Negative culture is not conclusive.

A chance of getting cured to restore fertility is uncommon (26). Some centers have reported pregnancy with IVF, which would appear to be the only treatment with any possibility of success (27).

Ectopic pregnancy following antituberculous drug therapy for pelvic tuberculosis is a recognized clinical syndrome (10,28,29).

With AIDS pandemic and increasing poverty in most developing countries, there may be a need to refocus on effective nationwide immunization against tuberculosis with health education campaign against AIDS. These are important factors in tuberculosis as pelvic tuberculosis will not disappear unless tuberculosis is completely eradicated.

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BELIEFS AND PERCEPTIONS ABOUT ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) OF A NIGERIAN RURAL COMMUNITY: IMPLICATION FOR PREVENTION AND POLICY INITIATIVES

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ABSTRACT

Acquired Immunodeficiency syndrome (AIDS) has assumed a disease of epidemic dimension both in Nigeria's rural and urban communities. Different people have varying knowledge and beliefs about this disease. This study was designed to assess the beliefs and perceptions of the people of Ihugh community in that regard.

A structured questionnaire was interviewer administered to assess their beliefs and perceptions about AIDS, results were analyzed by simple descriptive methods.

Of the 500 respondents, 65%(n=325) believed that AIDS is found only in cities while 69.7%(n=345) were of the opinion that AIDS can be cured by traditional means. Sixty five percent (n=326) believed that some people are destined for AIDS while 75.8%(n=482) were of the opinion that AIDS can be acquired through witches and wizards; 71.1%(n=488) believed AIDS can be acquired through curses. A large number of the respondents (63.0%) claimed they did not know where to go for routine HIV screening.

Conclusion: Health education program should be designed for the people of Ihugh community in the context of their peculiarities. This should include town cry, health talk at their worship centres and local gatherings. The electronic and print media are not the best based on their peculiarities.

KEY WORDS: Beliefs; Perceptions; Acquired Immunodeficiency Syndrome.

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INTRODUCTION

In 1981 when the first case of AIDS was reported in USA, little did the entire medical world anticipate that the entire world was marching towards yet another disaster in the near future (1,2). This was as a result of immense technological breakthroughs and discoveries available at the

moment for detection, prevention and treatment of various infectious diseases (3,4). The new disease demystified technological advancement of humanity. By the end of 2003, at least 40 million people worldwide were living with human immunodeficiency virus (HIV) the causative agent of AIDS.

As at July 2004, 20 million lives had been lost in sub Saharan Africa alone; at least 28 million

people on the continent are infected with the HIV virus. This constitutes about 70% of the total world's HIV infections. Not less than 12 million children are already orphaned from the disease, globally, 6,000 people are infected each day, one has led to the erosion of the social and economic progress of the past 20 years, among other nightmarish statistics, and there are 5,500 AIDS-related funerals every day in Africa alone(2,5-10).

The first case of AIDS in Nigeria was reported in 1986. In the late eighties, the prevalence of HIV in Nigeria was less than 0.1%. By 1991, it rose to about 1.8% and by end of 1999, 7% of the Nigerian population were living with this virus (11-14). This explains how fast the disease is spreading, and by the end of December 2003, HIV seroprevalence sentinel survey showed that Cross river state had the highest prevalence of 12%. This was followed by Benue state (9.3%), while Osun state had the lowest prevalence of 1.2% in the country (15).

Due to the rising scourge of this disease, the government(federal, state and local), non-governmental organizations(NGO), corporate This study was therefore designed to assess the knowledge, beliefs and perceptions of the people of this community about AIDS. This will in turn serve as a pointer as to what extent the current health education campaign has been successful in the locality. It is only then that a new health campaign strategy can be fashioned out to meet the peculiarities of the community so as to stem the wave of spread of this disease.

MATERIALS AND METHODS

Steady Area

The study was carried out at Ihugh, a village community in Vandeikya LGA of Benue state. It is located at 28 kilometres outside Gboko town, with a projected population of 6000 people based on 1991 population census and is entirely of Tiv

every 15 seconds. There are at least 3 million new infections each year leading to more than 2 million deaths annually. More than 85% of AIDS related deaths occur in people between 20 and 45 years of age.

This organizations and private individuals have taken the initiative to properly educate the general public about AIDS so as to reduce the alarming rate of its transmission and spread. This can be successful through correct character modification and positive behavioral changes.

Ihugh, a rural settlement in Benue state was quoted to have the highest prevalence of HIV(10.7%) in the state by the last sentinel survey (15). This was as compared to Makurdi(9.7%), the state capital and Otukpo(7.7%), an urban centre in the state by the same survey. Considering a prevalence of as low as 1.2% in Osun State and as high as 10.7% at Ihugh in Benue State calls for concern.

ethnicity. Over 95% of the people are peasant farmers.

Procedure

Five hundred subjects were recruited into the study within the age range of 15 to 70 years during the period of study (June-July 2004). Individual consent was obtained. Selection of subjects was done on market days in the locality using the EPI modified cluster sampling survey methodology. An interviewer administered questionnaire was used to collect data by five trained interviewers. Information on beliefs and views on AIDS among the respondents was obtained.

ETHICAL ISSUES

Ethical clearance was sought and obtained from the Benue state ministry of Health for this study.

DATA ANALYSIS

The data obtained was analyzed by simple descriptive method.

RESULTS

All the 500 respondent's questionnaires were properly filled by the interviewers, 299(60%) were males while 201(40%) were females. (Table I). The age range was 16 to 75 years and peak age range

was 26 to 35 years, mean age was 34 years with a bimodal age of 29 and 44 years. The median age was 33 years with a mean deviation of 1.34 and standard deviation of 1.52.

Figure I shows the educational level of the respondents. Two hundred and seven (41.4%) respondents had no formal education while 126(25.2%) of them attended primary school. One hundred and fifty (30.0%) attended secondary school and 17(3.4%) attended tertiary education.

Table I: Age and Sex distribution of the people of Ihugh community in Benue state.

A ge Interval(Yrs)	M(%)	F(%)	TOTAL(%)
16-25	71(14.2)	33(6.6)	104(20.8)
26-35	87(17.4)	62(12.4)	149(29.8)
36-45	53(10.6)	45(9.0)	98(19.6)
46-55	41(8.2)	37(7.4)	78(15.6)
56-65	32(6.4)	11(2.2)	43(8.6)
66-75	15(3.0)	13(2.6)	28(5.6)
TOTAL	299(60)	201(40)	500(100)

Peak Age range = 26-35 Years

Mean Age = 34 Years

Modal Age = Bimodal = 29, 44 Years

Median Age = 33 Years

Mean Deviation = 1.34

Standard deviation = 1.52

Table II shows the scores of the various responses of the respondents to the statements on the questionnaire. Three hundred and twenty five (65%) of the respondents believed that AIDS is only found in the cities as compared to 175(35%) who denied it. A total of 345(69.7%) and 94(19%) of the respondents were of the opinion that AIDS can be cured by traditional medicine and orthodox medicine respectively. On the contrary, 150(30.3%) and 403(81%) respectively of the respondents denied the above two notions. Three hundred and fifty five (73%) of the respondents replied AIDS cannot be cured by any means while 128(27%) believed AIDS can be cured. A total 326(65.6%) of the respondents were of the opinion that some people are destined to have it while 171(34.4%) denied that notion.

Concerning the mode of transmission of AIDS, a large proportion of the respondents was of the opinion that AIDS can be acquired: from witches and wizards (75.8%); through dreams (67.0%) and a smaller proportion consented to the view that AIDS can be acquired through breathing air (12.5%).

Only 23 out of 500(4.6%) respondents had done HIV screening in the past, while 77 out of 493(15.4%) had listened to a talk about AIDS.

Table III gives the various reasons why the respondents could not avail themselves for routine HIV screening. A large number of the respondents did not know where to avail themselves for the test (63.0%), 298(77.2%) respondents could not do the test due to financial reasons and 337(76.0%) was for fear of stigmatization.

DISCUSSION

The study was designed to find out the knowledge and perceptions of the people of Ithugh community in Benue state about AIDS. This was against the backdrop of the recently quoted National sentinel report of a 10.7% HIV seroprevalence¹⁵ of the community, a figure exceedingly high.

Of the 500 subjects studied, 41.4 % (n=207) did not attend primary school while 25.2 % (n=126) attended only primary school. According to WHO classification of illiteracy (16), 66.6 % (n=333) of the population are illiterate. This is an unhealthy scenario, since illiteracy gives rise to ignorance and all its attendant ills such as poverty and inability to

seek for proper medical attention. The situation is the reverse in the developed parts of the world (17-19) where people are well informed about their health and have unrestricted access to information about their health as well as other environmentally related health issues. This accounts for the low prevalence of HIV in those parts of the world (19-21). It is easier to effect changes in beliefs of well-informed people than that of the uninformed.

A large proportion of the respondents believed that AIDS could be cured by traditional methods. This poses a serious public health problem since the people may disregard all the scientifically proven preventive measures with the belief that they can be cured if they eventually go down with the disease. This will eventually render the control measures put in place irrelevant. It further buttresses the fact that, the ongoing health education campaign by the various organizations (governmental and non-governmental) has not yet been internalized by the people of this community.

Seventy five point eight percent (n=365) of the respondents believed that AIDS can be acquired through witches and wizards, 71.1 % (n=347) through curses and 67.0 % (n=318) through dreams. This explains the role superstition plays in the life of these people. These beliefs also have some religious connotations since majority of the people practice both Christianity and traditional religion. Generally, beliefs that hinge on people's religion are usually difficult to eradicate easily

(22,23). This could account for these deep-rooted wrong beliefs about AIDS transmission. The public health implication here is that, the actual modes of spread of this virus are not given adequate attention hence promoting the spread of the virus in the community.

Only 4.6 % (n=23) of the respondents had HIV screening in the past. This is extremely low and 83.6 % (n=416) had not listened to any teaching about AIDS. This calls to question the reliability of the media used by government and other agencies to reach out to the people of this community. The television is not accessible by these people; over 95% of the people do not own a radio. Hence, the heavily utilized electronic media (radio and television) by government and other agencies to reach out to these people has not yielded the desired results. This situation may be similar to many other rural settlements spread across the country and other parts of tropical Africa (24,25). The modes of information dissemination in countries like Japan, Germany and Italy are quite impressive (26,27).

Seventy-seven percent (n=298) of the respondents attributed their inability to carry out routine HIV screening to lack of funds. In other words, the cost of HIV screening is beyond the reach of an average Nigerian. This problem can be overcome if government would supply HIV screening kits at highly subsidized rate and within comfortable reach to the majority of her teeming population. The 63.0 % (n=302) respondents who said they do not know where to avail themselves for routine HIV test further strengthens this view. With out this approach the success of the present control program put in place by government will only continue to be a mirage.

Fear of stigmatization was also found to be a major factor hindering people's submission to routine HIV test as 76.0 % (n=337) attested to this fact. This boils down to lack of proper health education in the community. In other parts of the world where people are well enlightened, they are ever willing to submit themselves for routine HIV screening (28).

CONCLUSION

The high prevalence of HIV infection in Ihugh community is due to high level of illiteracy, their superstition coupled with their religious belief. It is also due to failure of the heavily utilized electronic and print media by government and other agencies to reach the people of this community.

There is therefore the need for government and other concerned bodies to re-design a new prevention and control program bearing in mind

the esteemed cultural beliefs, values and traditions of these people. Town cry, health talks at village gatherings and worship centres in the locality can be effective alternatives. In addition, government needs to explore other equally effective alternative means of communication with this people that will overcome language barriers and at no cost to the target audience. It is only then that the government control policy and program will yield the desired result.

Finally, since both the electronic and print media are not the best of means to reach out to the people of this community, home grown resource persons should also be adequately equipped with the basic infrastructure to carry this doctrine of AIDS to their door step.

ISOLATION OF *AEROMANAS SPECIES* FROM CHILDREN WITH AND WITHOUT DIARRHOEA IN JOS, NIGERIA.

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ABSTRACT

An investigation on the prevalence and antibiogram of *Aeromonas* species among children in Jos was conducted. The samples analysed included a total of 104 (52 diarrhoeal and 52 non – diarrhoea) stool samples collected from Vom Christian and Plateau Specialists Hospital in Jos. *Aeromonas* isolates were identified using standard biochemical tests. Of the total number examined, 6 (5.7%) were positive for *Aeromonas* species, 2 (3.9%) from diarrhoeal and 4 (7.7%) from non diarrhoeal samples ($P>0.05$). All isolates were identified as *Aeromonas hydrophila*. The highest number of isolates 3 (10.7%) were recovered from the group 7-12 months. No isolates were recovered from exclusively breast fed children while the highest number 4 (9.8%) was found in children fed with breast milk and formula. The isolates were found to be very sensitive to ciprofloxacin, but resistant to penicillin.

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INTRODUCTION

Diarrhoeal diseases constitute major childhood mortality and morbidity world wide especially in developing countries (1). Estimates show that diarrhoeal diseases cause nearly 5 million deaths annually in children under 5 years old in developing countries. Traditional aetiologic agents of diarrhoea include *Entamoeba histolytica*, *Giardia lamblia*, *Salmonella* species, *Shigella* species and *Vibrio cholerae* (2). However, other agents as *Campylobacter*, *Yersinia*, *Aeromonas*, *Plesiomonas* and *Cryptosporidium* have also been implicated in gastrointestinal diseases and are often referred to as new agents of diarrhoea (3,4).

Of growing importance in recent times is *Aeromonas* which affects all age groups but is said to be most common in children under 5 years, the elderly and the immunocompromised (5).

Aeromonas species are gram-negative bacilli of the *Aeromonadaceae* family. These motile bacteria are

involved in both intestinal and extraintestinal human infections (6) with clinical manifestations ranging from skin and soft tissue infection, bacteremia, to gastroenteritis (7). However, acute watery diarrhoea with a short duration is the most common clinical feature (8).

The first reported association of *Aeromonas* with gastrointestinal disease was in 1958 in Jamaica(9), since then numerous reports have appeared from several countries including Italy, England, Australia and the United States regarding the isolation of *Aeromonas* from faeces of patients with diarrhoea (10,11).

In Nigeria Obi *et al* (12) identified *Aeromonas* species and *Plesiomonas shigelloides* as bacterial agents of diarrhoea in urban and rural areas. *Aeromonas* have also been found in cases of acute diarrhoea and asymptomatic infections in Nigerian school children (13).

Reported frequency of isolation from symptomatic (diarrhoeic) as compared with asymptomatic (non-diarrhoeic) cases varies considerably, with some studies showing no significant difference in isolation rates (14, 15).

This study was therefore undertaken to examine the prevalence of *Aeromonas* species among children with and without diarrhoea and to identify the antibiogram of recovered isolates.

MATERIALS AND METHODS

Samples

The samples analysed in this study included a total of one hundred and four (52 diarrhoeal and 52 non-diarrhoeal) stool specimens collected from Vom Christian and Plateau Specialist Hospital in Jos.

Stool samples were collected from patients in clean, transparent wide-mouthed bottles. Information was also obtained from each subject regarding age, sex, major symptoms (diarrhoea, vomiting and fever) duration of disease, source of water and feeding pattern.

Processing of Specimens

The specimens were processed according to guidelines provided by Cheesbrough(16) for the laboratory diagnosis of enteric pathogens. These include, macroscopy, microscopy, gram stain, motility testing, culture, biochemical testing and antimicrobial sensitivity testing.

Specimens were inoculated into the medium of Agger *et al* (5) for the isolation of *Aeromonas* species (5% sheep blood agar containing 30µg/ml ampicillin). The inoculated plates were then incubated aerobically at 37°C for 24 hours. Resultant colonies were identified using biochemical tests.

Biochemical testing

Isolates that were beta haemolytic on sheep blood agar and gram -negative bacilli were identified as *Aeromonas* species using the following standard tests; oxidase test, indole test, urease test, citrate utilization test and test to determine motility after distilled water and peptone water subcultures. All tests were done using the methods

described by Collee and Miles (17) and Porter and Duguid (18).

Characterization of Species

Isolates were characterized to the species level based on seven biochemical tests as described by Carnahan *et al* (19). These included aesculin hydrolysis, gas from glucose, acid from arabinose, indole production, acid from sucrose, Voges-Proskauer reaction and resistance to cephalothin (30µg).

Antimicrobial Susceptibility Testing

Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar plates using the disc diffusion method of Scott (20). From a pure culture of the isolate to be tested a uniform streak was made on the agar plate. The antibiotic (Antec Diagnostics, UK) discs were placed on the plates and incubated at 37°C overnight. Interpretation of results was done using the zone sizes. Zones of inhibition of ≥ 18 mm were considered

sensitive while 13-17mm were considered intermediate and <13 mm were considered resistant. All isolates were tested for sensitivity to the following antibiotics, ciprofloxacin (5mcg) cotrimoxazole (25mcg) streptomycin (10mcg), gentamycin (10mcg), erythromycin (5mcg), tetracycline (10mcg) penicillin (5mcg) peflacin (10mcg) and tarivid (10mcg).

Statistical Analysis

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

RESULTS

A total of 104 (54 diarrhoeal and 52 non-diarrhoeal) stool samples were examined. The age range of the patients was 0-72 months. Of the total number of specimen examined, 6 (5.7%) were positive for *Aeromonas* spp. 2 (3.9%) of *Aeromonas* spp were recovered from diarrhoeal stool specimens while 4(7.7%) from non-diarrhoeal samples (Table 1). The difference is not statistically significant (

$P>0.05$). All the isolates were found to be *Aeromonas hydrophila*.

The highest numbers of isolates 3(10.7%) were recovered from the age group 7-12 months. The age brackets 13-18 months, 19-24 months and 67-72 months had 1 isolate each. No isolates were recovered from age group 0-6 months and from 25-66 months (Table 2). The difference is not statistically significant ($P>0.05$).

Macroscopic examination of the specimens showed that 36 were watery was found in children fed with breast milk and formula 4 (9.8%) followed by formula and family diet 2(4.7%). No

13 mucoid, 3 blood stained, 40 soft-formed and 12 hard-formed. The soft-formed specimens yielded the highest number of isolates 3 (7.5%), watery samples 2(5.6%) and, hard-formed 1(8.3%). The blood stained and mucoid specimens yielded no isolates (Table 3). This difference is not statistically significant ($P>0.05$).

Table 4 shows the prevalence of *Aeromonas* spp in relation to the feeding pattern of the children. The highest number of isolates were recovered from exclusively breast fed children. This result is not statistically significant.

Table 1: Prevalence of *Aeromonas* species among symptomatic and asymptomatic patients.

Patients	No. of Examined	Specimens	No. (%) Positive
Symptomatic (with diarrhoea)	52		2(3.9)
A symptomatic (without diarrhoea)	52		4(7.7)
Total	104		6(5.8)

$X^2 = 1.2$

$df = 1 \quad P > 0.05$

Table 2: Prevalence of *Aeromonas* species isolated in relation to age and sex:

Age Group (Months)	No of Specimens Collected		No (%) Positive		
	Male	Female	Male	Female	Total
0-6	5	14	0(0.0)	0(0.0)	0(0.0)
7-12	17	11	2(7.1)	1(3.6)	3(10.7)
13-18	10	5	1(6.7)	0(0.0)	1(6.7)
19-24	8	5	1(7.7)	0(0.0)	1(7.7)
25-30	5	2	0(0.0)	0(0.0)	0(0.0)
31-36	3	1	0(0.0)	0(0.0)	0(0.0)
37-42	3	2	0(0.0)	0(0.0)	0(0.0)
43-48	2	1	0(0.0)	0(0.0)	0(0.0)
49-54	1	0	0(0.0)	0(0.0)	0(0.0)
55-60	2	1	0(0.0)	0(0.0)	0(0.0)
61-66	2	1	0(0.0)	0(0.0)	0(0.0)
67-72	1	2	0(0.0)	1(33.3)	1(33.3)
Total	59	45	4(3.9)	2(1.9)	6(5.8)

$\chi^2 = 21.35, \quad df = 11 \quad P > 0.05$

Table 3: Types of samples treated and the number (%) of *Aeromonas* species isolated.

Types of Stool	No. Examined	No. (%) Positive
Watery	36	2(5.6)
Mucoid	13	0(0.0)
Blood stained	3	0(0.0)
Soft formed	40	3(7.5)
Hard formed	12	1(8.3)
Total	104	6(5.8)

$$X^2 = 132, \text{ df} = 4 \quad P > 0.05$$

Table 4: Prevalence of *Aeromonas* species in Relation to the type of Feeding

Type of Feeding	No. of Patient Tested	No. (% positive)
Breast milk	20	0(0.0)
Breast milk & formular	41	4(9.8)
Formular & family diet	43	2(4.7)
Total	104	6(5.8)

$$X^2 = 60.17 \text{ df} = 2 \quad P > 0.05$$

Table 5 shows the in-vitro susceptibility pattern of the isolates. Six (100.0%) of the isolates were sensitive to ciprofloxacin, 5 (83.33%) to gentamycin, peflacin and tarivid, 4 (66.67%) to erythromycin and streptomycin, 3 the highest isolation rates were found in infants 7-12 months. This result correlates with the findings of Abraham *et al* (21) and Regua *et al.*, (22). They both observed that the highest incidence of gastroenteritis in children was found within the age range of 7-12

The protective role of breast milk against diarrhoeal bacterial aetiology is well documented (23, 24).

Those who had their breast feeding interrupted with mixed feeding or stopped completely.

Another probable reason for the increase in incidence of gastroenteritis around 7-12 months of age might be due to faulty weaning practices and poor hygiene in preparing food.

The low isolation rate in asymptomatic children older than age 12 months might be attributed to immunity

(50.0%) to tetracycline and cotrimoxazole. All isolates were resistant to penicillin.

DISCUSSION

months where weaning practices begin in many parts of the world (Nigeria inclusive). The finding indicates that breast milk confers considerable protection to children as positive cases were not reported in children below 7 months whose mothers practice exclusive breast feeding.

Disease developed by the older children who may have come in contact with the agent through exposure. *Aeromonas* spp was found to be higher in males (6.8%) than in females (4.4%). This finding may be related to the number of male and female children from whom samples were collected. I.e. more samples were collected from males than females. However, this result is not statistically significant and no sex preference has been reported.

Aeromonas spp. were isolated more frequently from loose and watery stools.

The result of in-vitro antibiotic sensitive test showed 100% sensitivity to ciprofloxacin and more than 80% sensitivity to peflaxine, tarvid and gentamicin. Ciprofloxacin therefore is the drug of choice, when treating *Aeromonas* infections from this study. This presents cause for concern since it is expensive. Conventional and cheaper drugs like (cotrimoxazole, tetracycline, streptomycin and erythromycin) showed marked reduced in vitro susceptibility. This may be due to indiscriminate usage or an antibiotic (drug abuse) which has resulted in multiple drug resistance of many microorganisms in Nigeria (25). In addition, other enteric Bacteria isolated in patients with diarrhea in Jos are resistant to common antibiotics (26, 27).

rate from children without diarrhoea (7.7%) compare to those with diarrhoea (3.9%). However this result is not statistically significant. This agrees with findings from some researchers (Pitaragis *et al.*, (14) and Figura *et al.*, (15). *Aeromonas* spp was isolated from infant below 6 months.

Other common enteric pathogens like *Salmonella*, *Shigella* and *Escherichia coli* were not sought for in this study therefore it can not be concluded that the *Aeromonas* spp isolated were the actual cause or the diarrhoea in this study.

A total of 104 stool samples were analysed in this study in which the prevalence rate for *Aeromonas* spp was 5.8%. This result is similar to the 5% prevalence rate documented by Obi *et al.*, (12) for urban population in Edo, Lagos and Cross River States of Nigeria. All isolates identified were found to be *Aeromonas hydrophila*. This *Aeromonas* spp has been associated with many cases of diarrhoea (5).

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Antibacterial activity of Methanolic extract of *Garcinia kola* (Heckel) Seeds and Standard antibiotics.

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ABSTRACT-The methanolic extract of *Garcinia kola* (Heckel) seeds and eight standard antibiotics were tested in-vitro for comparative activity against 10 isolates of each of six bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *streptococcus pneumoniae* and *Pseudomonas aeruginosa*, all from throat infections.

The methanolic extract exerted activity against all the bacteria tested almost in similar manner as gentamicin. Of the remaining seven standard antibiotics, chloramphenicol, erythromycin, and tetracycline showed activity against one organism or the other. Remarkably, augmentin[®], cloxacillin and cotrimoxazole had no activity against any of the bacterial isolates. Amoxycillin was able to affect only 2 of the 8 isolates of *Esch. coli*. This has lent credence to the ethnopharmaceutical claims of the curative affect of raw-chewed *Garcinia kola* seeds on throat infections as well as highlighting the bacterial resistance to standard antibiotics, particularly, the β -lactams.

INTRODUCTION

Garcinia kola (Heckel) is a plant in the family Guttiferae and tropical in its distribution⁽¹⁾. It has various local names in Nigeria but is commonly referred to as bitter/false/male kola. Both the pulp and seeds of the plant are edible⁽²⁾; the seeds are chewed raw and the roots serve as chewing stick locally. The seed of *Garcinia kola* has been associated with folklore claims on its curative effects. The powdered seeds as well as in the treatment of cough, threatened abortion, diabetes, palpitation, intestinal pains, jaundice, whooping cough, anaemia and angina as well as in the treatment of liver disorders, and as an antidote against poison⁽⁴⁾. When chopped up and steeped in water, beer or palm wine, the seed of *Garcinia kola* has a cleansing effect on stomach⁽⁵⁾ *Garcinia kola* seed extracts have been reported to be active on both Gram-positive and Gram-negative bacteria,

and fungi^(6,7) attributed principally to such phytochemicals as benzophenone, garcinol and xanthochymol^(8,9,10). Specific targeting of this antimicrobial activity against bacterial causative agents of throat infections and a comparative study of such activity with that of standard antibiotics are lacking in the literature. These have formed the basis for this study.

MATERIALS AND METHODS

BACTERIA

Sixty bacterial isolates consisting of 10 for every one of 6 bacterial spp were collected from different clinical sources of respiratory tract infections. Some conventional biochemical tests⁽¹¹⁾ were carried out to confirm the identities of the bacteria.

SOXHLET EXTRACTION OF *GARCINIA KOLA* SEEDS IN ORGANIC SOLVENTS

Sun dried *G. kola* seeds weighing 200g were turned into powdered form on a local hand grater

and blender successively. Extraction with a soxhlet apparatus using 20g of the powder in 200ml of each of methanol, chloroform and petroleum followed by filtration and then, storage at 4°C in a refrigerator until when needed.

CONCENTRATION OF THE METHANOLIC EXTRACT

The hot filtrate of methanolic extract was concentrated on an

SUSCEPTIBILITY STUDIES

The methanolic, chloroform and petroleum ether crude extracts of *Garcinia kola* seed powder, the methanolic extract concentrate and 8 different standard antibiotics (as disks) were used in separate experiments against the bacterial isolates. The preliminary results obtained on the more encouraging antimicrobial activity of the methanolic crude extract than any of the other two necessitated the preferential use of the methanolic extract in its concentrated form. The agar-cup diffusion method

ether, in turns. An exhaustive extraction in each case lasting for about 6 hr was

electrothermal water bath to a constant weight of 3.96g. The concentrate was then preserved in a refrigerator at 4°C for further use.

of susceptibility testing was employed on the crude extracts and the concentrate, while antibiotic disk diffusion method was used on the standard antibiotics against the bacteria as previously described⁽¹²⁾. The minimum inhibitory concentrations (MIC) of the methanolic extract concentrate against the bacterial isolates were also determined, using double-fold serial dilutions of the concentrate.

RESULTS

The methanolic extract of *Garcinia kola* seeds had the most

pronounced effect on all the bacteria tested particularly *Esch. coli* and *Staph. aureus*, relative to the effects of the chloroform and petroleum ether extracts. None of the solvents had antibacterial activity on its own. The Duncan's Multiple Range test employed in statistical analysis established a significant difference (at $P<0.05$) between the methanolic extract and either of the chloroform and petroleum ether extracts. Zone of bacterial growth inhibition (mm) was the parameter used in assessing the antibacterial activity. Among the 8 standard antibiotics used (in disk form), only gentamicin compared

favourably with the methanolic extract of *Garcinia kola* seeds (Table 1). Augmentin^R, amoxycillin, cloxacillin, erythromycin and co-trimoxazole showed no activity against not less than 8 isolates among each of the bacterial spp. Tetracycline and co-trimoxazole showed no activity against all the isolates of *Strep pyogenes*, *Strep. pneumoniae* and *Pseud aeruginosa*. Chloramphenicol could not affect any one of the isolates of *Pseud aeruginosa* but varied in its activity against other bacteria.

The methanolic extract concentrate gave varying MICs among the microorganisms tested (Table 2).

Table 1

Sample results of susceptibility testing on 10 isolates of each of 3 bacterial spp.

Isolate:	STANDARD ANTIBIOTICS								MET. EXTRACTS		
	GEN	AUG	AMX	ERY	TET	CXC	COT	CHL	O		
PA											
1084	30*	**	-	-	-	-	-	-	13.0	-	
1380	33	-	-	-	-	-	-	-	13.5	-	
2366	28	-	-	-	-	-	-	-	13.0	-	
2819	33	-	-	-	-	-	-	-	11.5	-	
0714	32	-	-	-	-	-	-	-	12.0	-	
3011	29	-	-	-	-	-	-	-	13.5	-	
3681	21	-	-	-	-	-	-	-	13.0	-	
3800	28	-	-	-	-	-	-	-	12.0	-	
4010	33	-	-	-	-	-	-	-	12.5	-	
4499	29	-	-	-	-	-	-	-	13.0	-	
SA											
1122	25	-	-	-	21	-	-	-	13.5	-	
1633	19	-	-	-	10	-	-	-	13.0	-	
2464	27	-	-	-	18	-	-	-	14.5	-	
2994	20	-	-	-	12	-	-	-	16.5	-	
0899	23	-	-	-	11	-	-	-	16.5	-	
3002	25	-	-	-	28	-	-	-	17.0	-	
3533	28	-	-	-	32	-	-	-	16.0	-	
3801	21	-	-	-	19	-	-	-	14.0	-	
4113	22	-	-	-	19	-	-	-	16.0	-	
4430	26	-	-	-	-	-	-	25	17.0	-	
SN											
1164	20	-	-	-	-	-	-	-	8.5	-	

1463	22	-	-	-	-	-	-	-	11.5	-	
2114	20	-	-	-	-	-	-	-	9.5	-	
2845	24	-	-	-	-	-	-	-	10.5	-	
0874	19	-	-	-	-	-	-	-	9.5	-	
3321	22	-	-	-	-	-	-	-	11.0	-	
3644	23	-	-	-	-	-	-	21	10.0	-	
3778	20	-	-	-	-	-	-	26	9.0	-	
4220	10	-	-	-	-	-	-	-	10.5	-	
4400	23	-	-	-	-	-	-	26	10		

NB:

- * = Zone of growth inhibition (mm) (sensitive)
 ** = No growth (No activity)
 PA = Pseudomonas aeruginosa
 SA = Staph. aureus
 SN = Strep. Pneumoniae
 GE = Gentamicin (10µg); AU = Augmentin^R (30µg)
 AM = Amoxycillin (25µg); ER = Erythromycin (5µg)
 TE = Tetracycline (10µg); CX = Cloxacillin (5µg)
 CO = Cotrimoxazole (25µg); CH = Chloramphenicol (30µg)
 ME = EXTRACT = Methanol extract
 C = Control (Methanol)

Table 2

Sample result of MIC determination for Methanolic extract concentrate

Isolate: MITC (mg/ml) Isolate MIC (mg/ml)

PA

1084	75	2819	49.50
1388	49.50	714	49.50

2366 49.50

SA

1122 24.75 2994 49.50

2464 24.75 1633 24.75

0899 49.50

SN

1164 99.00 2845

99.50

12463 49.50 0874 99.50

2114 99.00

NB:

PA = *Pseudomonas aeruginosa*

SA = *Staph. aureus*

SN = *Strep. pneumoniae*

DISCUSSION

The pronounced antibacterial effect of the methanolic extract of *Garcinia kola* seeds on all the bacterial isolates tested, including Gram - positive and Gram negative bacteria, has significantly lent credence to the ethnopharmaceutical claims of the curative effect of the raw chewed

Garcinia kola seeds on respiratory tract infections₍₃₎. The observation in this study that only gentamicin of all the eight standard antibiotics tested, could compare favorably with the methanolic extract emphasizes the earlier observed₍₁₃₎ worrisome dimension in bacterial antibiotic resistance, particularly to the β -

lactams. Most importantly, *Staph aureus*, *Strep. pyogenes*, *Strep. pneumoniae* and *Pseud. aeruginosa* have deservedly, due to their clinical importance, enjoyed attraction for their resistance to antibiotics. This has been substantiated in this study with respect to virtually all the β -lactams (augmentin^R, amoxycillin and cloxacillin) tested. It is interesting to note that the methanolic extract exhibited pronounced activity against *Esch. coli*, the hospital isolates of which were found to be resistant to amoxycillin - clavulanate₍₁₄₎ the same as Augmentin^R. There is need to exploit fully the therapeutic value of *Garcinia kola* seeds through definitive isolation of the active principle and its formulation into suitable dosage forms for the treatment of respiratory tract infections.

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HUMAN INTESTINAL PARASITISM IN A RURAL SETTLEMENT OF NORTHERN NIGERIA, A SURVEY.

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ABSTRACT

Intestinal parasites are still a common feature among our communities. This study was set out to ascertain this. One hundred and fifty respondents were recruited into the study. A pretested questionnaire was administered to the respondents with specific hygienic components such as: sources of drinking water, methods of sewage disposal and water purification among others. Stool samples were collected and analysed microscopically and findings analysed.

The overall prevalence of intestinal parasites in Mbangough community was 62%(96 out of 150). The commonest parasite encountered was *Ascaris lumbricoides* (44%), followed by *Enterobius vermicularis* (14%) and *Entamoeba histolytica* (11.3%). Other parasites were *Entamoeba coli* 3.3%, Hookworm 6.0%, *Schistosoma mansoni* 1.3%, *Taenia* species 7.3%, while the least common parasite encountered was *Strongyloides stercoralis* 0.6%. None of the respondents had access to pipe borne water or bore hole.

The prevalence of intestinal parasites in Mbangough community is still quite high. Government should invigorate in her pursuit towards the provision of primary health care facilities in our communities. Also health education should be made a compulsory course in all primary schools as well as all adult and literacy classes.

INTRODUCTION

Infections are regarded by World Health Organisation (WHO) as the foremost cause of morbidity and mortality among humans (1). Parasitic infections and infestations constitute quite an integral part of this estimate (2). Intestinal parasites continue to ravage both the tropical and subtropical regions of the world (3) despite various efforts employed by governments and organizations worldwide to curtail this menace (4). Among Africans and Nigerians, it is common to find in stool, parasites such as *Enterobius vermicularis*, Hookworm (*Ancylostoma duodenale* and *Necator Americanus*), *Ascaris lumbricoides*, *Entamoeba* and *Taenia* species (5).

These parasitic infections could lead to diseases such as anaemia, retarded growth and intestinal obstruction, but to mention a few (6). Their spread is heavily dependent on various socio-cultural practices such as: sources of drinking water (7), modes of preparation/ processing of food and methods of sewage disposal (8) among others.

This study was designed to find out the prevalence of intestinal parasites in Mbangough community and the level of availability of

Primary health care facilities. The findings from this study will be a good pointer as concerns the degree of observance of basic hygienic tenets of the people of this community. In like manner, these findings will be used to assess the level of success achieved by government in the implementation of ongoing National Primary Health Care policy in this locality in particular and that of Nigeria in general.

MATERIALS AND METHODS

Study Area: The study was carried out at Mbangough community, a settlement in South Eastern Ushongu Local government area of Benue State. It has a land surface area of about 28.2 KM² and circumference of about 18.8 KM. Her population is estimated at 3,800 based on 1991 population census. It is estimated to have about 340 households (household = a man, his wives, children and other dependents), and each household averagely has 9 persons (range 2 to 32). All the inhabitants are of Tiv ethnic extraction and over 96% of them are peasant farmers.

Study Design: The study was carried out in Mbangough community. Households were selected by a predetermined random sampling procedure where one after another household

was selected in each direction faced. Individuals were recruited using simple random sampling method with the aid of blind folded paper and statistical table of random numbers. A pre-tested structured questionnaire was interviewer administered to 150 respondents. Appropriate information such as age, sex, source of water supply, methods of sewage disposal and water purification methods were obtained. Informants Analysis of Results. The results were analysed using Epi Info statistical software version 2002 where applicable. Chi square (X^2) was used to compare association among proportions, while P values < 0.05 were considered significant.

Ethical Considerations: Ethical approval for the study was dully sought and approval obtained. Similarly, consent was obtained from each respondent

RESULTS

Out of 150 respondents recruited into the study, 64(42.7%) were males while 86(57.3%) were females. The overall prevalence of intestinal parasites in Mbangough community was 62%(93 out of 150). The prevalence among males was 25.4%(38 out of 150) and that among females was 36%(54 out of 150). The male female difference was not statistically significant ($X^2 = 0.33$, $P > 0.05$). The highest prevalence of 16.6%

of first degree relations were used in case of children.

Procedure: Samples were collected into special bottles and transported immediately to the health centre for processing. Normal saline and then iodine preparations were carried out on each stool sample without concentration procedure. Microscopy was carried out using X5, X10 and occasionally X40 objectives.

(25 out of 150) was recorded among the 10-19 age range. The combined prevalence of intestinal parasites among the age range 1 to 19 was significantly higher than the older ages of 50 years and above ($X^2 = 6.68$, $P = 0.009$), Table I.

Table II shows the prevalence of intestinal parasites identified among the residents of Mbangough community. The commonest intestinal parasite identified in the community was *Ascaris lumbricoides* which infested 66 subjects (44%). This was followed by *Enterobius vermicularis* 14.0%(21 out of 150) and then *Entamoeba histolytica* 11.3% (17 out of 150), while the least common parasite encountered was *Strongyloides stercoralis* 0.6% (1 out 150). There were several instances of multiple parasitic infestation.

Table III shows the pattern of multiple parasitic infestation. Only a single parasite was found in

76 subjects (81.7%), two parasites in 16 subjects 17.2% and three parasites in 1 subject (1.1%).

Table IV analyses the methods of sewage disposal and sources of water supply in the community. Eighty nine respondents (59.3%) use pit latrines while 61(40.6%) use open air

defaecation. None of the inhabitants of the community uses pipe borne water or bore hole since all of them derive their water from wells and streams. None of them boil their water before drinking.

Table I: Age and sex distribution of intestinal parasites among the people of Mbangough community of Benue state.

Age(Years)	MALE		FEMALE		TOTAL
	Negative	Positive	Negative	Positive	
1-9	3(2.0)	9(6.0)	3(2.0)	6(4.0)	21(14.0)
10-19	5(3.3)	14(9.3)	5(3.3)	11(7.3)	35(23.3)
20-29	7(4.6)	4(2.7)	6(4.0)	17(11.4)	34(22.7)
30-39	3(2.0)	3(2.0)	1(0.6)	4(2.7)	11(7.3)
40-49	3(2.0)	6(4.0)	4(2.6)	8(5.3)	21(14.0)
50-59	4(2.7)	1(0.6)	3(2.0)	8(5.3)	16(10.7)
60& Above	1(0.7)	1(0.6)	10(6.6)	0(0)	12(8.0)
TOTAL	26(17.3)	38(25.4)	32(21.3)	54(36)	150(100)

Parenthesis = Percent.

$\chi^2 = 0.33$

P > 0.05

Table II: Prevalence of intestinal parasites among inhabitants of Mbangough community of Benue state. N =150

Parasite	Number	% Infection
	r	
	Positive	
<i>Entamoeba histolytica</i>	17	11.3
<i>Entamoeba coli</i>	8	5.3
<i>Enterobius vermicularis</i>	21	14.0
<i>Ascaris lumbricoides</i>	66	44.0
Hookworm	9	6.0
<i>Schistosoma mansoni</i>	3	1.3
<i>Taenia species</i>	11	7.3
<i>Strongyloides stercoralis</i>	1	0.6
<i>Trichuris trichura</i>	0	0
TOTAL	93	62

Table III: Distribution of intestinal parasites among residents of Mbangough community by the number of species of parasites per stool sample.

Number of species per stool sample

No.	Total	Percent
Species/Sample	Number	(%)
	r	
One Specie	76	81.7
Two Species	16	17.2
Three Species	1	1.1
Four Species	0	0
TOTAL	93	100

Table IV: Assessment of the sources of water supply and methods of sewage disposal among residents of Mbangough community.

Sewage Disposal	Methods
Water Systems/ Latrines	Pit Open Air Defaecation
Number	Number
%	%
89	61
59.3	40.6

DISCUSSION

The prevalence of intestinal parasitism in Sources Of Water Supply Mbangough community was found to be 62%. This finding is quite high but compares favourably with the findings of Salako (9) in his study among primary school children in Lagos.

This further buttresses the fact that parasitic infection and infestation is still an issue of serious public health importance in Nigeria.

The commonest parasite encountered in this study was *Ascaris lumbricoides* (44%) which was followed by *Enterobius vermicularis* (14%). This is similar to earlier studies in Lagos (9), Brazilian Amazon (10), and Kenyan coast (11). Findings from other centres such as Oman emirate (12), hookworm infection was the commonest organism while findings from Santo Brazil (13) showed *Strongyloides stercoralis* as the commonest organism among alcoholics.

Nutritional status in concert with other external factors such as standard of hygiene could play a significant role in this high prevalence of intestinal parasitism.

Findings from Bangladesh (14) showed a strong relationship between vitamin A, Iron status and helminthiasis. Findings from other parts of the world (15-17) concur with this view. The prevalence of intestinal parasitism was less in the older age group possibly due to higher level of awareness for the need to maintain good hygienic practices.

Twenty-three persons had two parasites in stool while (7) persons had three parasites in stool. Salako (9) in Lagos, Ashford (11) in Kenya, Ashford (18) in Kenya and Higgins (19) in Indonesia also demonstrated this polyparasitism as a common feature in tropical and subtropical

parts of the world. The influence of nutrition could be an important factor in multiple parasitic infestations as demonstrated by Tanner (20) among rural children in Tanzania.

There is lack of portable drinking water in the community and about 40% of the inhabitants result to open air defaecation. These are well known unhygienic practices that promote the spread of infections in our localities. This is coupled with the fact that less than 5% of the community population boils their water before drinking. Salako (9) who recorded a 70.6% prevalence of intestinal parasitism in a population that lacked these facilities also emphasized the contributions of these factors towards the spread of intestinal parasites. Okpala (4) in an earlier study in Lagos recorded a much higher figure of 85.1% prevalence of intestinal parasites among (44) schools in Lagos with varying degrees of sanitary conditions.

The Alma-Ata international conference on primary health care held on 12th September 1948 in the Soviet Union was meant to provide primary health care for all communities of the earth. The target year of accomplishment was 2000 AD. However, findings from this and other contemporary studies is a far cry from near achievement even at 5 years behind the target

schedule. Some of the resolutions of Alma-Ata declarations include the following among others: standard sewage disposal facilities for all and unrestricted availability and accessibility to basic health facilities.

The success of that Russian conference is now put to question going by the poor rating of primary health care indices in Nigerian communities, other parts of Africa and beyond.

CONCLUSION

This study has found out that primary health care in our communities is still at very low ebb compared to the developed parts of the world. 78 has failed, posterity has given the "New millennium development goal" (MDG) with a target year of 2015 AD to reduce the world's poverty by half. We therefore call on various

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Provision of portable water for all communities irrespective of location, provision of The teaching of health education in our primary schools in the urban and most especially in the rural communities should be reemphasized. The curriculum should be reviewed where it exists to meet the contemporary needs of basic hygiene in our society. As a matter of necessity, this should be extended to all adult vocational and literacy classes. This will no doubt elevate the people's knowledge and awareness about the basic tenets of good hygiene both at personal and community levels. Although Alma-Ata tiers of government, international and local organizations to this great challenge to achieve this 7 years ahead of time.

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SOIL TRANSMITTED HELMINTHIASIS AMONG APPARENTLY
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ABSTRACT

The prevalence of soil transmitted helminth infections in apparently healthy children of mean age 12.2 years drawn randomly from one school in each of the four local government areas of Kano, northern Nigeria were evaluated. Stool sample from 570 children were analyzed using formol ether concentration technique. 130(22.8%) of the subject were infected by soil transmitted helminthes (STH). The overall prevalence by species were *Ascaris Lumbricoides* (7.9%), Hookworm (5.3%) and *Trichuris trichiura* (3.5%) respectively. 35(6.1%) of the subjects were infected with two or more soil transmitted helminthes. The prevalence in males (24.2%) was generally higher than that of females (22%), Hookworm infection was high(45.5%) in the 15-20 years old age group, while *Ascaris Lumbricoides* infection was high (100%) in the 6-10 years old age group. The mean number of eggs per gramme (epg) of faeces was moderate. The study shows that subjects had high intensity of infections for *Ascaris Lumbricoides*, hook worm, *Trichuris trichiura* as epg of faeces counted were high in them compared to what was obtained for mixed infections. It also reveals a moderately high prevalence across board for all soil transmitted helminth (STH) in Ungo local government.

(Keywords: Soil transmitted helminthes, Children, prevalence.)

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INTRODUCTION

It was estimated that more than one billion people in the world are infected with soil transmitted helminth (STH), mainly *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (Crompton, 1999).

The problem of STH is more in children of school age (although it may affect other age groups), and is often

associated with impaired cognitive function and learning ability, reduced physical activity and poor growth (Strepenson et al., 1998; Nokes et al., 1996). Mobility particularly acute in children who are most at

risk with heavy infection. Heavy worm load result in nutritional deficiency due to poor diet, and these are more

pronounced during child development and growth. STH causes major human illness (Hall et al., 1982; Udensi, 1983; Elkiwa, 1984). *Ascaris lumbricoides* has been associated with the obstruction of the large biliary and pancreatic ducts. Hookworm infection is linked with iron deficiency anaemia (Layrissee et al., 1983). There are also documented cases of persistent auto-infection with *Steniloides stercoralis* induced chronic intestinal dermatological symptoms. Immuno-suppresses patients are especially prone potentially fatal symptoms of hyper-infection with parasite (Cook, 1986).

By improving the living condition and sanitation, STH infections may be controlled. The high degree of poverty and very poor economic status has however affected its successful implementation.

This study was undertaken to determine the prevalence of STH infections among apparently healthy children in Kano, and to help proffer suggestions to policy makers depending on the findings.

MATERIALS AND METHODS

The Study Area and Subjects

The study population was from four(4) Local Government Areas of Kano municipality. Inhabitants of the area are mainly indigenous farmers, students and public servants who reside among the majority indigenous population.

The subjects were 570 apparently healthy primary and secondary school children aged 6-20 years, randomly selected, with lottery method (Ben et al., 1991) from a school in each of the four Local Government Areas. The different Local Government and the number of

subjects analyzed from them include Tarauni (105), Kumbotso (110), Fagge (175) and Ungogo (180). Verbal consent was obtained from the Headmasters and Principals of the schools before stool containers were distributed to subjects. Stool samples were voided, collected and analyzed the day using formol ether concentration technique as described by Monica Cheesbrough for the stool concentration and microscopical examination of helminth ova, and stool's counting method (technique) (Cheesbrough, 1999) for counting helminth epg.

The examination of the stool sample was carried out at the Parasitology Laboratory of the Department of Medical Microbiology and Parasitology, Aminu Kano Teaching Hospital (AKTH), Kano. To ensure consistency of the readings, second readings were performed in 20% of the slides randomly selected as described by Andrade et al. (2001).

RESULTS

The results obtained from the study were as shown in tables 1,2,3, and 4.

Table 1 shows the total prevalence of soil transmitted helminth (STH) infections by age and sex of the 570 stool samples examined, 130 (22.8%) were infected with soil transmitted helminth (STH). The prevalence in the males was (24.2%) while in the females, the prevalence was (22%). In the three age categories, STH infections were highest in 15-20 years group in both males and females.

Table 2 shows the general prevalence of STH by species among apparently healthy children in the

study area. *Ascaris lumbricoides* had the highest overall infection rate of 7.9%. Prevalence of *Ascaris lumbricoides* was also the highest in schools in three out of the four local government areas studied. Overall prevalence of other STH were Hookworm(5.3%) and *trichuris trichiura* (13.9% and 8.3% respectively) was observed in the school from Ungogo local government. In addition 35(6.1%) of the subjects were infected by two or more STH. Of these, 28 had double infection and 7 had infection. Infection rates were highest among children from schools located in Ungogo local government and Kumbotso local government (13.9% and 4.6% respectively).

Table 3 shows the distribution of helminth in different age groups. The highest prevalence of *Ascaris Lumbricoides* infection (100%) was recorded in the 6-10 years old age group, while its lowest prevalence (9.1%) was observed in the 15-20 years old age group.

The highest prevalence of hookworm (45.5%) was observed in the 15-20 years old age group, while the lowest was observed in the 6-10 years old age group. The highest prevalence of *trichuris trichiura* (21.4%) was observed in 11-14 years old age group.

Table 4 shows the egg count of each helminth per infected subject. The highest and lowest number of eggs per subjects (15,000epg and 800eog respectively) was seen in children from school in Ungogo local government. Also egg per gramme of faeces (epg) obtained from the four *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm as compared with what was obtained for the mixed infection.

Table 1. Total Prevalence of Soil Transmitted Helminth (STH) Infections by Age and Sex

Age (years)	Male			Female			Overall		
	No Exam	No Infected	% Prevalence	No Exam	No Infected	% Prevalence	No Exam	No Infected	% Prevalence
6-11	40	5	12.5	55	0	0	95	5	5.3
11-14	200	35	17.5	150	35	23.3	350	70	20
15-20	7-	35	50	55	20	36.4	125	55	44
Total	310	75	24.2	260	55	22.0	570	130	22.8

Table 2: General Prevalence of Soil Transmitted Helminth (STH) Among Apparent Healthy Children in the Study Area

School Local govt	K* No of Children Examined	A. lumbricoides		Hookworm		T. trichiura		Mixed infection	
		No Infected	% Prevalence	No Infected	% Prevalence	No Infected	% Prevalence	No Infected	% Prevalence
TRN	105	5	4.8	5	4.8	0	0	0	0
KBT	110	10	9.1	0	0	0	0	0	0
TGE	175	20	11.4	0	0	5	2.9	5	2.9
UNG	180	10	5.6	25	13.9	15	8.3	25	13.9
Total	570	45	7.9	30	5.3	20	3.5	35	6.1

* Number of apparently healthy children in each local government

TRN = Tarauni

KBT = Kumbotso

FGE = Fagge

UNG = Ungogo

Table 3. The Distribution of each Helminth and their Prevalence as seen in each Age Group

	6-7 years	11-14 years	15-20 years	Total Positive Examined
Ascaris lumbricoides	5(100%)	35(50%)	5(9.1%)	45
Trichuris trichiura	0	15(21.4%)	5(9.1%)	20
Hookworm	0	5(7.1%)	25(45.5%)	30
Mixed infection	0	15(21.4%)	20(36.4%)	35
Total Positive Infection	5	70	55	130

Table 4. Egg Count of each Helminth Per Infection Subject

Helminth	Tarauni LG	Kumbotso LG	Fagge LG	Ungogo LG
<i>Ascaris lumbricoides</i>	8000epg	i. 7000epg ii. 940epg	i. 12000epg ii. 10000epg iii. 8000epg	9000epg
<i>Trichuris trichiura</i>	-	-	4000epg	i. 6000epg ii. 7000epg iii. 5000epg
Hookworm	5000epg	-	-	i. 5000epg ii. 5000epg iii. 7500epg iv. 15000epg v. 5000epg
Mixed infection	-	2000epg	3000epg	i. 1000epg ii. 1000epg iii. 1000epg iv. 1200epg v. 800epg

DISCUSSION AND CONCLUSION

Data obtained from this study are similar to those observed in other epidemiological studies conducted in other parts of Nigeria. The overall studies (Obiamiwe, 1977; Nwosu, 1981; Obiamwe and Nmorsi, 1991; Udonsi, 1984; Ukpai and Ugwu, 2003), while the prevalence of helminth infections is similar to that observed in other epidemiological studies (Okpala, 1956; Ejezie, 1981; Odewolo, 1988), the common triad being *Ascaris lumbricoides* (7.9%), *Hook* (5.3%) and *Trichuris trichiura* (3.5%).

The high prevalence of *Ascaris lumbricoides* (100%) within 6-10 years old age group could be linked to the route of infection being faecaloral, since children within the age group are easily susceptible due to poor level of hygiene.

Similarly, the high prevalence of Hookworm (45.5%) in the 15-20 years old age group could also be due to the route of infection being skin penetration.

Children of this group are more vulnerable to infection while playing on moist or swimming in stagnant muddy water. This study also shows that helminthic infection decreases with age.

Generally, there is an appreciably high helminth count in the four Local Government Areas. However, the zero egg count observed for *Trichuris trichiura* in both Tarauni and Kumbotso Local Governments, as well as the one observed for Hookworm in both Kumbotso and Fagge Local Governments may not reflect the actual situation on ground because majority of the children may have been infected predominantly with male worms, hence reduced egg count. The reason for this high prevalence rate of infection may be attributed to shortage of portable water, indiscriminate defaecation, poor environment and lack of personal hygiene.

In conclusion, this shows a relatively moderate prevalence of soil transmitted helminth (STH) among the apparently healthy children population. In view of the morbidity and medical complication of soil transmitted helminthiasis (e.g. toxæmia, anaemia, tissue perforation and occlusion of the gut), routine medical check-up should be encouraged in school children. The use of chemotherapy (periodic deworming), health

education and improved socio-economic conditions should be advocated as a infection may be shortage water, indiscriminate defecation, poor environment and lack of personal hygiene.

In conclusion, this study shows a relatively moderate prevalence of soil transmitted helminth (STH) among the apparently healthy children population. In view of the morbidity and medical complication of soil transmitted

helminthiasis (e.g. toxemia, anaemia, tissue perforation and occlusion of the gut), routine medical check-up should be encouraged in school children. The use of chemotherapy (periodic deworming), health education and improved socio-economic conditions should be advocated as a means of effecting a proper control of STH, and reduction of morbidity caused by it.

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Comparative Antibiotic Sensitivity of *Staphylococcus aureus* isolates from two clinical sources

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Abstract

Fifty isolates of *Staphylococcus aureus* from pyoderma and wound infections were screened for their respective sensitivities to six β -lactam antibiotics by the microtitre plate broth-dilution method. The results consistently showed higher percentage sensitivity of the isolates from wounds. It is suggested that the site of isolation of a specific bacterium may influence the choice of antibiotic against an infection.

Key words: *Staphylococcus aureus* Antibiotic sensitivity, Pyoderma and Wounds.

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Introduction

Staphylococcus aureus is known to form suppurative diseases (1,2), which may or may not be easily treated with common antibiotics due to the recognized potential of the organism to offer antibiotic resistance. The resistance is normally attributed to plasmid genes which mediate the production of two antibiotic inactivating enzymes penicillinase and cephalosporinase (β - lactamases) (3,4).

Thus, both the β -lactamase sensitive and stable β - lactams have been regularly implicated in the resistance episode (5,6,7).

It has been established that many factors may influence antimicrobial susceptibilities (8,9) but the possibility of a correlation between the site of isolation of a specific bacterium and its antimicrobial susceptibilities (8) remains a novel suggestion. In a preliminary

study, such correlation was drawn between antimicrobial susceptibilities and site of infection with *Staph. aureus* and *Staph. intermedius* from dog and cat, as an hypothetical suggestion (9).

This study is an attempt to determine the possibilities of human isolate of *Staph. aureus* varying in their antibiotic susceptibilities with their sites of isolation.

Materials and Methods

The 50 isolates of *Staph. aureus* used in this study were from different clinical specimens collected at the Routine section, Department of Medical Microbiology and Parasitology, University college Hospital, Ibadan, Nigeria. Of the 50 isolates, 25 were from each of pyoderma and wounds. All the isolates were identified as Gram - positive cocci producing β -haemolysis on blood agar and wound were isolates for pyoderma and wound were then ranked on percentage basis. In all the tests carried out, *Staph. aureus* NCTC 6571 served as the control. then ranked on percentage basis. In all the tests carried out, *Staph. aureus* NCTC 6571 served as the control.

Results

and acid from D-mannitol aerobically and anaerobically (10,11, 12,13). Most importantly, they were identified with free coagulase in a tube- test, as a clumping factor in human plasma (10).

The antibiotic susceptibility test was carried out on the 50 isolates by the microtitre plate broth-dilution similar to the Checkerboard MIC determinations (14,15) against penicillin G (Pn), ampicillin (Ap), amoxycillin (Am), Cloxacillin (Cl), Cefuroxime (Cf) and Cefotaxime (Ct). Bacterial growth as an indication of resistance was shown by a pinkish colour of formazan due to the growth indicator (2,3,5 - Triphenyl 2H - tetrazolium chloride monohydrate). The minimum inhibitory concentrations (MICs) obtained were used to describe the isolates as either sensitive or resistant. The numbers of sensitive and resistant isolates for pyoderma

For the sensitive strains of *Staph. Aureus*, the MICs occurred within the range of 0.02 μ g to 0.49 μ g/ml for the parent penicillins (penicillin G, ampicillin and amoxycillin), 0.12 μ g/ml to 0.97 μ g/ml for cloxacillin, and 0.03 μ g/ml to 0.97 μ g/ml for cefuroxime and cefotaxime. In respect of the resistant strains, the MICs varied between 1.95 μ g/ml and

beyond 250µg/ml for all the six antibiotics. The percentage antibiotic sensitivity of *Staph. aureus* isolates from pyoderma ranged between 24 and 64% against 4 and 44% of the isolates from wounds (Table 1). For either of the two

clinical sources, lower percentage sensitivities were obtained for penicillin, ampicillin and amoxycillin while higher levels occurred for cloxacillin, cefuroxime and cefotaxime, which are known to be β -lactamase inhibitors.

Table 1

Percentage antibiotic susceptibilities of *Staph aureus* isolates from pyoderma and wound infections.

Antibiotic	Pyoderma isolates (%)	Wound isolates (%)
Pn	24	4
Ap	24	8
Am	24	8
Cl	48	36
Cf	60	44
Ct	64	44

NOTE:

Pn	=	Penicillin G
Ap	=	Ampicillin
Am	=	Amoxycillin
Cl	=	Cloxacillin
Cf	=	Cefuroxime
Ct	=	Cefotaxime

DISCUSSION

The percentage susceptibility results showed that the *Staph. aureus* isolates from pyoderma were consistently more sensitive to the six antibiotics used than those isolates from wounds. This lends credence to the suggestion that antimicrobial susceptibilities of a specific

bacterium may vary with its site of infection (8). The results also agreed with the hypothesis stated in a previous report (9) on *Staph. aureus* and *Staph intermedius* isolates from canine and feline origin of dog and cat. Further studies involving more bacterial species and clinical sources should facilitate a more reliable

rational choice of empirical therapy. In this study however, it is noteworthy that the site of infection may be an important factor in choosing the proper antibiotic treatment for human-borne pathogens in some environments.

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