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ANTISTAPHYLOCOCCAL METABOLITE FROM *AUREOBASIDIUM PULLULANS*: PRODUCTION AND CHARACTERIZATION

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***Aureobasidium pullulans* (NI.3) isolated from the leaves of *Dracaena reflexa variegata* produced intracellular antimicrobial metabolite the yield of which was 700-800 U from about 0.7-0.85 g of dry biomass. The antistaphylococcal metabolite showed strong activity against different *Staphylococcus* spp. The MICs ranged from 1.25 to 3.6 U/ml. The metabolite was only moderately sensitive to temperature. After storage at 40°C and 70°C for one hour, it lost only 20% and 60% of its activity respectively. However, it was completely inactivated upon exposure to 121°C for 20 min. The antistaphylococcal metabolite was insensitive to various protein-denaturing detergents and enzymes like trypsin, proteinase K, lipase and lysozyme. The activity was fairly stable over a wide range of pH (5.7 – 8). When *S. aureus* was grown in the medium in presence of antimicrobial metabolite (10 U/ml), the number of CFU progressively declined, with most of the cells loosing their viability after nine hours exposure. A slower killing of the *S. aureus* was noted when cells were kept in buffer containing antimicrobial metabolite (5 U/ml). Antimicrobial metabolite induced efflux of potassium ions from cells of Staphylococcus indicating that the mode of action is by formation of pores or channels in the cytoplasmic membrane.**

Key words: *Aureobasidium*, Antistaphylococcal activity, Potassium efflux

INTRODUCTION

Of the six thousand and more microbial metabolites described to date, four thousand are antibiotics, 1% of which has been used clinically (1). More than three thousand antibiotics have been isolated from *Actinomycetes* and a few from fungi (2). The large number of other microorganisms are yet unexplored for their potential to produce antibiotic. Screening of unusual fungi and other microorganisms not yet explored from the natural sources around the world might be productive in yielding new bioactive metabolites including antimicrobial metabolites.

Aureobasidium pullulans, a fungus, has not been adequately investigated for its antimicrobial potentials. Only few studies regarding its ability to produce antimicrobial compound are reported (3-4), however, detail investigation is necessary. In this paper, we report on production, characterization and mode of action of intracellular antimicrobial metabolite from indigenous strain of *A. pullulans* NI. 3.

MATERIALS AND METHOD

Organisms and their cultivation

Aureobasidium pullulans NI.3 culture was maintained on Saboraud dextrose agar, *Staphylococcus aureus* reference strain for assay, *Staphylococcus* MGHM 1 (Mahatma Gandhi Hospital, Mumbai), *Staphylococcus* MGHM 2, *Staphylococcus* MGHM 3, *Staphylococcus* MGHM 4, *S. aureus* NCIM 2079 (National Collection of Industrial Micro-organisms, National Chemical Laboratory, Pune, India.) and *S. epidermidis* MTCC 435 (Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) were maintained on nutrient agar slants and sub cultured every month and stored at 4-10°C until studied.

Isolation of A. pullulans from leaves of Dracaena reflexa variegata

A. pullulans NI.3 strain was isolated from *Dracaena reflexa variegata* leaves by using procedure described by Pollock *et al* (5), identified and characterized by procedure of Barnett *et al* and Takeo and Hoog (6-7).

Fermentation

Inoculum was prepared by transferring a loopful of cells from slant of *A. pullulans* in 10 ml of Saboraud Dextrose Broth (SDB), which was incubated for 24 hr at 28°C on shaker (120 rpm). One ml of inoculum was added to 100 ml of medium, containing (g/100 ml) glucose 2.0, $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.15, $MgSO_4$ 0.5, $CaCl_2$ 0.01, $FeCl_3$ and $ZnSO_4$ 5 µg/ml in 500 ml flask and incubated at 28°C on shaker. After two days of incubation, the medium was replenished with 20 ml of the same medium containing peptone 5%, and culture growth was continued for further four days.

The fermented broth was centrifuged at 5000 rpm for 20 minutes. Biomass was suspended in equal volume of ethanol and grounded in mortar and pestle with coarse silica for 15 – 20 minutes and centrifuged at 5000 rpm. The ethanol extract was evaporated to dryness and the residue was dried under the stream of nitrogen gas. During the fermentation, pH and biomass were measured.

Assay of antimicrobial activity

The dry residue obtained at the end of extraction was dissolved in 2 ml of 95% ethanol, and was loaded to paper disc as follows; 10 discs (Whatman paper No.1, disc diameter 5 mm) were soaked in 100 µL of ethanolic extract and kept at room temperature overnight. The discs were placed on nutrient agar which has been previously inoculated with target culture bacteria. Plates were incubated for 24-48 hours at 37°C and the zone diameters of inhibition were measured. Discs similarly prepared using 95% ethanol alone served as control. One unit of antibiotic was the amount of antibiotic which gave 5 mm zone diameter of inhibition against *S. aureus*.

Determination of MICs of antimicrobial metabolite from A. pullulans NI. 3

The minimum inhibitory concentration of target organisms was determined by the Kirby-Bauer double dilution method (8).

Measurement of potassium efflux

S. aureus cells were suspended in 25 ml of 2.5 mM Na-HEPES buffer, pH 7.2 containing 6.24 units/mL of antimicrobial metabolite from *A. pullulans* NI.3 and kept for two hours at room temperature. 5 ml samples were withdrawn at different time intervals, centrifuged at 12, 000 rpm for 10 minutes and the supernatants were collected and analyzed for K^+ using flame photometer. *S. aureus* cells suspended in 25 ml of Na-HEPES buffer alone were used as control. Potassium released after heat treatment of cultures at 100°C for 10 minutes was used as reference.

Characterization of antimicrobial metabolite from A. pullulans NI.3

The effects of temperature, pH, detergents and enzymes on antimicrobial metabolite were determined.

RESULTS

Production of antimicrobial metabolite by A. pullulans NI.3

The production of intracellular antimicrobial metabolite started after 24 hours and continued almost at a constant rate for 5 more days. During the same period, biomass as measured in terms of dry weight also kept increasing. The maximum amount of antimicrobial activity extracted from biomass was 700-800 U from about 0.7-0.85 g of dry biomass (Fig.1).

The antimicrobial metabolite of *A. pullulans* NI.3, determined by agar disk diffusion assay, is summarized in Table 1. The extract of *A. pullulans* NI.3 produced well-defined inhibition zone diameter of 14-23 mm against *Staphylococcus spp.* Absence of inhibition zones with control ruled out possibility of inhibition due to residual ethanol.

Effect of temperature and pH on antimicrobial activity of metabolite

The effects of temperature and pH on antimicrobial activity of metabolite against *Staphylococcus spp* was analyzed in term of residual activity (Fig. 2). When the metabolite was exposed to various temperatures, the loss of activity was gradual; at 50°C more than 70% of activity was still retained. After exposure at 70°C for one hour, there was only 60% of loss of activity as tested against *S. aureus*. However, the activity of the extract against *S. aureus* was lost completely after autoclaving. The antimicrobial metabolite was fairly stable for 34 days at room temperature showing loss of 46% of its activity as tested against *S. aureus*. It retained 84% of antimicrobial activity even after eight days of incubation at room temperature (Data not shown). The antimicrobial substance was very stable over a wide range of pH 5.7 – 8.0 (Fig. 2).

Effect of enzymes and protein-denaturing detergents on antimicrobial metabolite

Antimicrobial metabolite was treated with a variety of detergents and enzymes (Table 2). No loss of antimicrobial activity of the metabolite was observed upon treatment with any of the detergents except bile salt. In case of treatment with bile salt, zone diameter decreased from 24 to 19 mm. Upon treatment with proteinase K, trypsin, lysozyme and lipase, the activity of antimicrobial substance against *S. aureus* remained largely unaffected (Table 3).

Effect of antimicrobial metabolite from A. pullulans on growth and survival of S. aureus

Significant inhibitory effects were observed when antistaphylococcal metabolite (10 U/ml) was added to the growth medium (Fig. 3). After 3 hours of contact with the metabolite, more than 1.5 $\log_{10}$ CFU decrease was observed. A 6 hours incubation with antistaphylococcal metabolite led to killing of 8 $\log_{10}$ CFU decrease. In the controls, plain LB as well as LB containing 125 µL ethanol, the initial cell number increased by at least 1 $\log_{10}$ CFU. Similarly, when *S. aureus* cells was suspended in phosphate buffer containing antimicrobial metabolite (5U/ml), the viable cell count of *S aureus* decreased by 1.5 $\log_{10}$ CFU (Fig. 4).

Effect of reversal agents on activity of antimicrobial metabolite from A. pullulans NI.3

In order to determine whether antistaphylococcal metabolite from *A. pullulans* NI.3 inhibit any biosynthetic pathway, the metabolite was mixed with reversal agents and residual activity was determined. Inhibition of *S. aureus* was not reversed by any of the tested reversal agents (Table 4).

Determination of K^+ efflux from Staphylococcal cells in presence of antistaphylococcal metabolite from A. pullulans NI.3

In order to further understand the mechanism of action of antistaphylococcal metabolite from *A. pullulans* NI.3, we investigated the kinetics of potassium efflux from the cells (Fig. 5). Following incubation of cells with antistaphylococcal metabolite, the extracellular potassium concentration was significantly increased. The concentration of K^+ outside cells, treated with the metabolite for 10 minutes was 5 ppm and increased up to 6 ppm in 30 minutes. The largest amount of potassium released within 2 hours of incubation was 7 ppm. Extracellular levels of K^+ were negligible from *S aureus* suspended in buffer containing alcohol.

Table 1: Inhibition of *Staphylococcus spp* by antimicrobial metabolite from *A. pullulans* NI.3

Target organisms	Inhibition zone diameter † (mm)	MICs (U/ml)
Staphylococcus sp MGM 1	14	2.8
Staphylococcus sp MGM 2	18	3.6
Staphylococcus sp MGM 3	18	3.6
Staphylococcus sp MGM 4	15	3
S. aureus	24	1.25
S. aureus NCIM 2079	23	3
S. epidermidis MTCC 435	22	1.56
Control *	0	0

MIC values were determined by Kirby-Bauer double dilution method

* Disc containing 10 µl ethanol was kept as control; †each value represents the average of three measurements

Table 2: Effect of detergents on antistaphylococcal metabolite from *A. pullulans* NI.3

Detergents	Inhibition zone diameter † (mm)	
	No metabolite *	Metabolite
Tween 80	0.0	21
Tween 20	0.0	21
Triton X 100	0.0	21
SDS (0.01 g/ ml)	0.0	20
Cetrimide (0.01 g/ ml)	13	23
Bile salt (0.01 g/ ml)	0.0	19
Toluene	10	22
No detergents	0.0	24

Aliquots (200 µL) containing 96 units of antistaphylococcal metabolite were mixed with 20 µL of detergents and incubated for three hours at room temperature. The antistaphylococcal activity was then checked by using discs as described in materials and methods

*Control: 200 µL of antimicrobial metabolite mixed with 20 µL of ethanol.

*Detergent was mixed with distilled water (200 µL)

† Each value represents the average of three measurements.

Table 3: Effect of enzymes on antistaphylococcal metabolite from *A. pullulans* NI. 3

Test	Inhibition zone diameter † (mm)
Trypsin + NI. 3 extract	12
Proteinase K+ NI. 3 extract	11
Lipase + NI. 3 extract	13
Lysozyme + NI. 3 extract	13
* Diluted NI. 3 (Control)	11

100 µL of antistaphylococcal metabolite containing (44 units) was added to 100 µl of enzyme solution (1 mg / ml) incubated at room temperature for three hours, 100 µl of the mixture was added to 10 sterile discs. Using these discs the antistaphylococcal activity was determined

* Antimicrobial metabolite was diluted to match with dilution caused by addition of enzyme

† Each value represents the average of three measurements.

Table 4: Reversal of antistaphylococcal activity of metabolite from *A. pullulans* NI. 3

Reversal agent			*A. pullulan* extract	Inhibition
	µL/well	µg /well	µL	zone dia † (mm)
Casamino-acids*	B 40	4	40	26
	B 40	4	60	37
Riboflavin	A 20	0.2	20	27
	B 40	0.4	60	40
Nicotinic acid	A 20	0.2	20	27
	b 40	0.4	60	38
Thiamine	a 20	0.2	20	26
	b 40	0.4	60	39
Adenine	a 20	0.2	20	28
	b 40	0.4	60	40
Cytosine	a 20	0.2	20	26
	b 40	0.4	60	39
Guanine	A 20	0.2	20	26
	B 40	0.4	60	38
Uracil	A 20	0.2	20	26
	B 40	0.4	60	39
Thymine	A 20	0.2	20	27
	B 40	0.4	60	40
H_2O	A 20	--	20	28

100 µl of *S. aureus* (OD_{660} 0.7) each was spread inoculated on NA. The plate was incubated at 37°C. Concentration of stock solution for casamino- acids was 100µg/ ml and for all other reversal agents was 10 µg/ ml.

a - 10mm diameter well; b- 5 mm diameter well; †each value represents the average of three measurements.

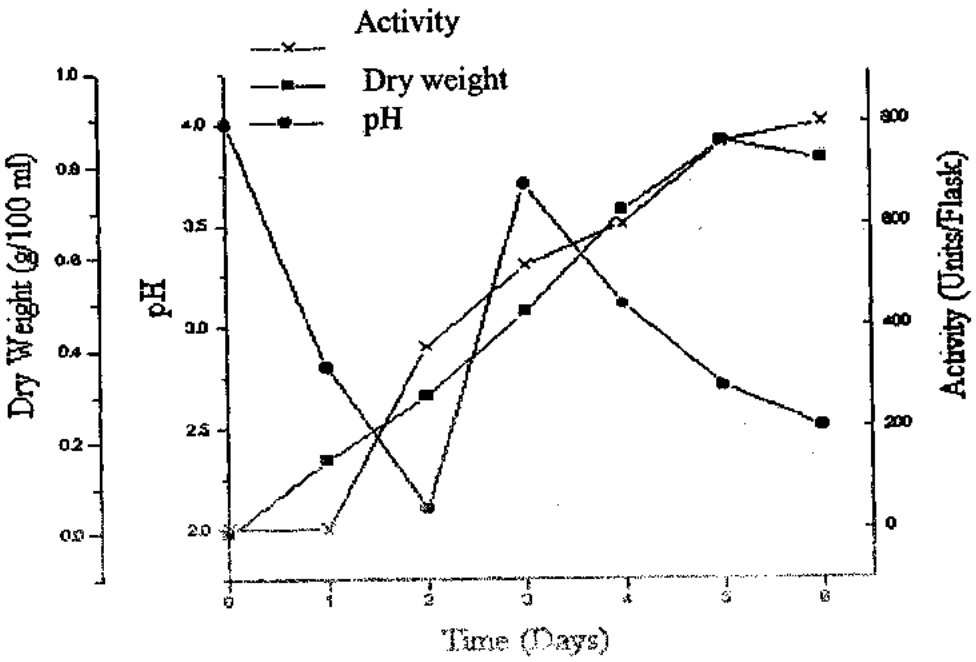

Fig. 1: Time course of production of antistaphylococcal metabolite *A. pullulans* NL3
Production of antistaphylococcal metabolite was studied in shake flasks, incubated at 120 rpm on a shaker, at 30°C for six days. Samples were removed periodically and growth, pH as well as metabolite production were determined.

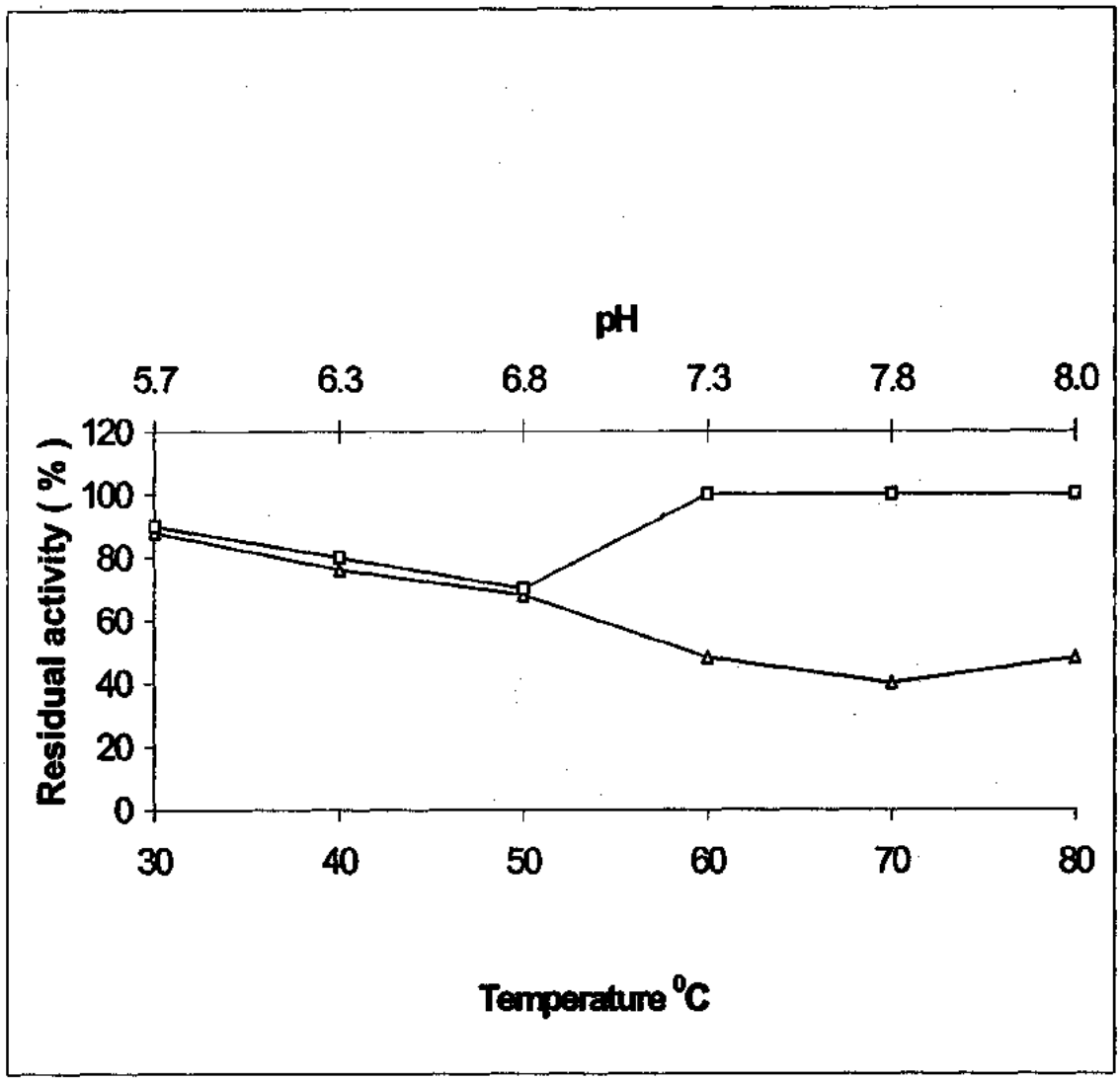

Fig. 2: Effect of temperature and pH on antistaphylococcal metabolite from *A. pullulans* NL3
Aliquots (200 µl) of metabolite containing (88 units) were kept in water bath at different temperatures for one hour and residual antimicrobial activity for each aliquot was measured. Similarly, 200 µl (88 U) of antistaphylococcal metabolite was mixed with 200 µl of phosphate buffer of different pH and residual antimicrobial activity for each aliquot was measured.
□ = pH, Δ = Temperature

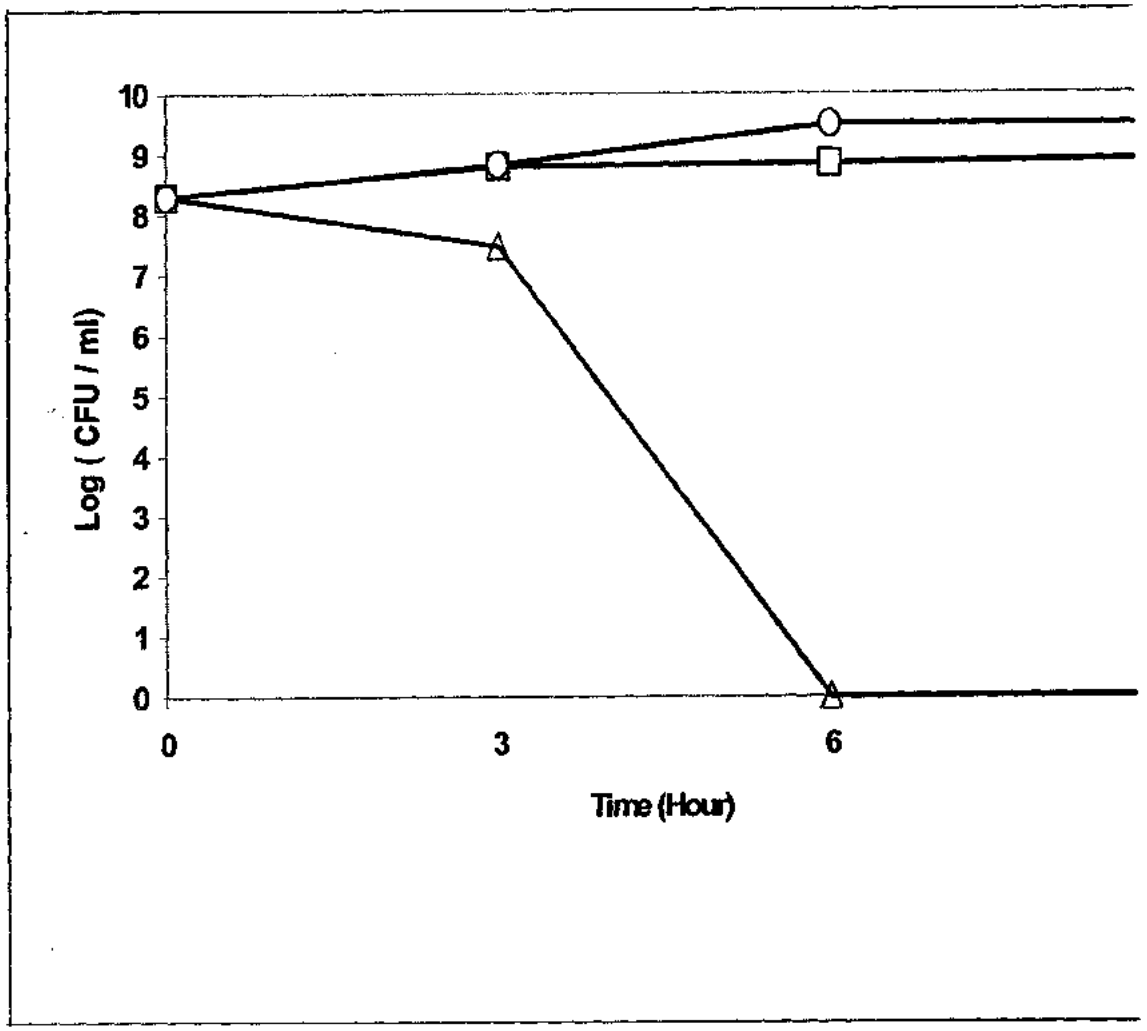

Fig. 3: Effect of antistaphylococcal metabolite from *A. pullulans* NI. 3 on growth of *S. aureus*
S. aureus cells were inoculated to 10 ml of LB (○), LB containing 125 µl ethanol (□) and to LB containing 10 U/ml of antimicrobial metabolite (Δ) and incubated for the indicated period at 37°C on shaker at 120 rpm. Growth was measured in terms of total viable counts (TVC).

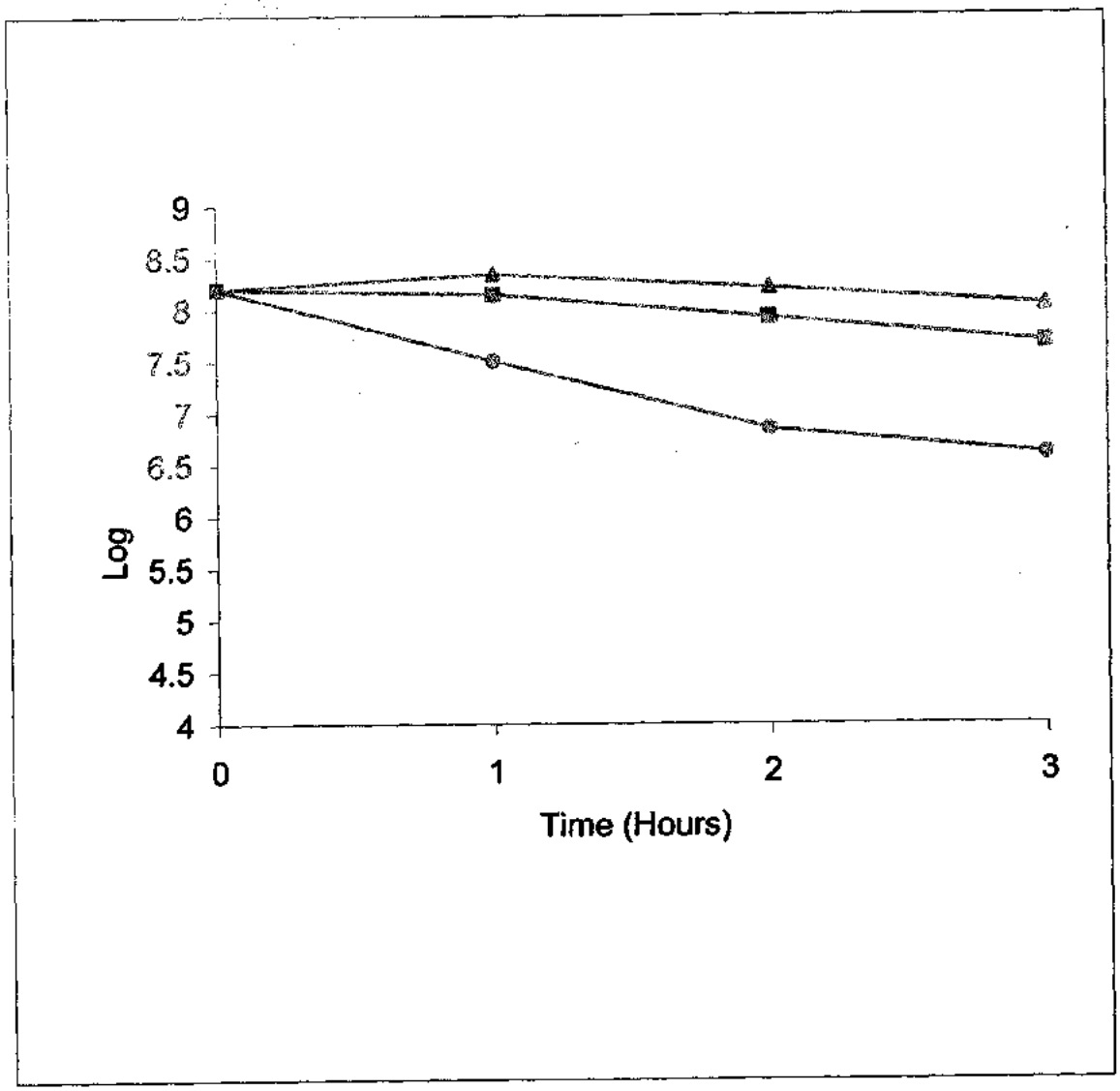

Fig. 4: Effect of antistaphylococcal metabolite from *A. pullulans* Nl. 3 on viability of *S. aureus*
S. aureus cells were added in 10 ml of 25 mM phosphate buffer (▲), phosphate buffer containing 100 µl of ethanol (■) and phosphate buffer containing 5 U/ml of antimicrobial metabolite (● 5U/ml) and incubated for the indicated period at room temperature and total viable counts (TVC) were determined.

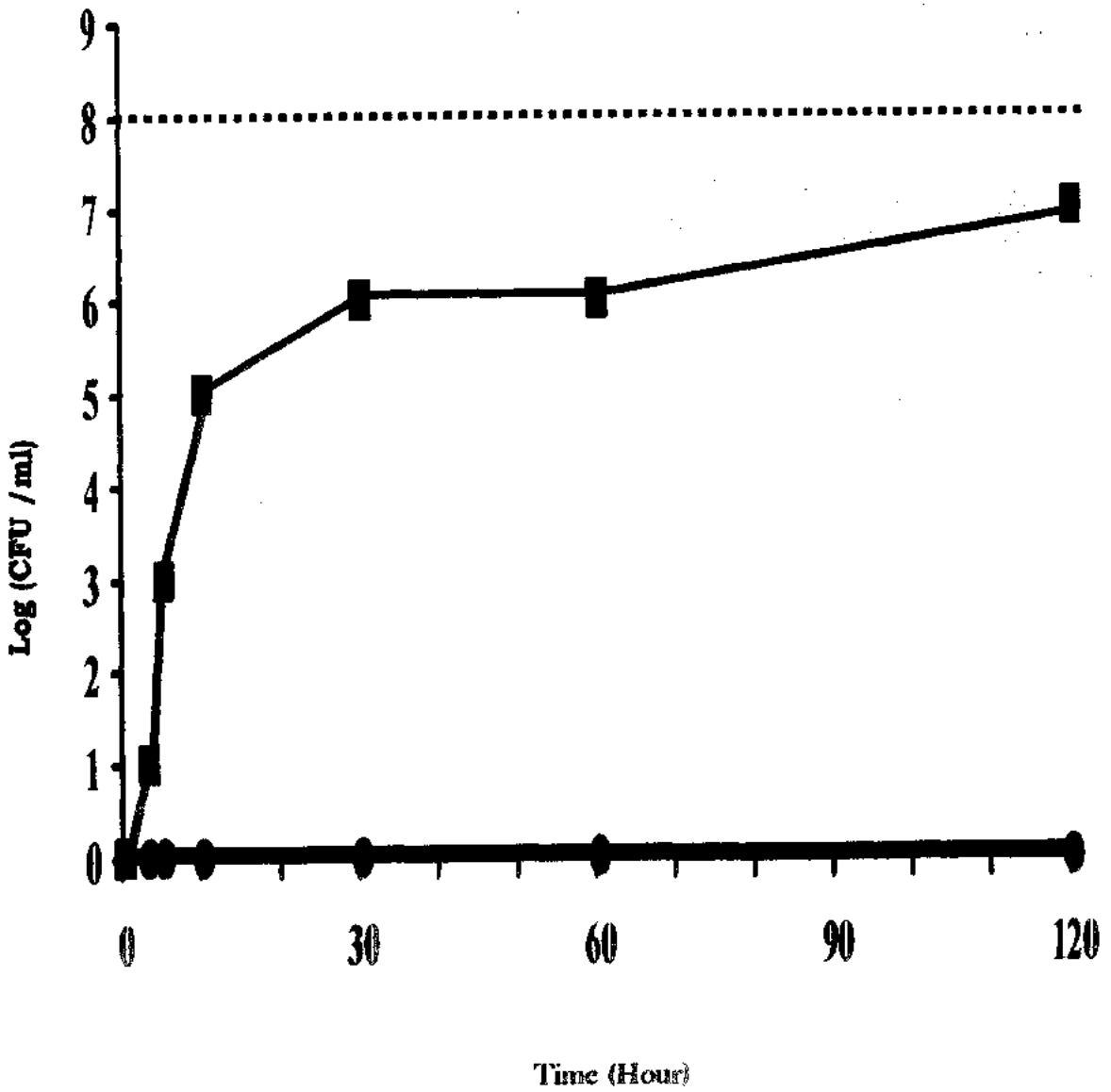

Fig. 5: Kinetics of potassium efflux from *S. aureus* in presence of antimicrobial metabolite
***S. aureus* cells were suspended in 25 ml of 2.5 mM Na- HEPES buffer (▲), Na-HEPES buffer containing antimicrobial metabolite (6.24 units/ml, ●), incubated for two hours at room temperature. Five ml of sample was withdrawn at 30 min intervals and centrifuged at 12000 rpm for 10 min. The potassium content of supernatant was determined by flame photometer. Maximum potassium ion released from cells heated at 100°C for 10 minutes is shown by dotted lines (- - -).**

DISCUSSION

In the past several years, the field of chemotherapy had witnessed both the emergence and rapid spread of resistance in microorganisms. A significant percentage of clinical isolates, including species of streptococci, staphylococci and enterococci, are resistant to commonly used antibiotics, such as the new β- lactams and glycopeptides (9-11). Factors such as these make the search for newer antistaphyloccocal metabolite(s) imperative and essential. Moreover, significantly large numbers of microorganisms are yet unexplored for their capacity to give mankind the potential antistaphylococcal compounds of desired properties. *A. pullulans* NI.3, which is a safe and cosmopolitan organism (12), produced intracellular antistaphylococcal metabolite that inhibits growth of *Staphylococcus spp.* The effect of antistaphylococcal metabolite from *A. pullulans* on Gram-positive bacteria has not been investigated extensively. Our results on production of antistaphylococcal metabolite are encouraging for elimination of human infections.

The results presented in this paper clearly suggest that *Aureobasidium pullulans* NI.3 strain accumulated intracellular alcohol extractable metabolite, capable of inhibiting different *Staphylococcus spp.* The intracellular antistaphylococcal metabolite of *A. pullulans* NI.3 was moderately heat sensitive and active over a wide rage of pH. Proteolytic treatment of metabolite did not abolish its antistaphylococcal activity against *S. aureus*. It is therefore tempting to propose the presence of non-proteinic compound, which has potent antistaphylococcal activity. However, failure of proteolytic enzymes to inactivate antifungal metabolite produced by *A. pullulans* is not unusual (13). Also, treatment of antistaphylococcal metabolite from *A. pullulans* NI.3 to various detergents provided full recovery of antistaphylococcal activity.

Our data show that addition of antistaphylococcal metabolite to *S. aureus* cells results in an immediate loss of cellular K^+. These results demonstrate that the cytoplasmic membrane is the primary target for antistaphylococcal metabolite from *A. pullulans* NI.3. Therefore, the staphylocidal effect of the metabolite is most likely due to the formation of pores in the cytoplasmic membrane. A potential use of *A. pullulans* and its metabolite need more studies including further purification, mass spectra, nuclear magnetic resonance (NMR) and evaluation of toxicity are needed and in progress for confirmation of this suggestion.

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SPECIES OF FUNGI ASSOCIATED WITH SKIN DISEASES OF DIFFERENT AGE GROUPS IN PLATEAU STATE, NIGERIA

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A survey was carried out on the species of fungi associated with skin diseases of thirty subjects of different age groups in Plateau State, Nigeria. The age groups included 1-10, 11-20, 21-30, 31-40 and 41-50 years, accounting for 27%, 33%, 23%, 8% and 4% of total number of individuals with fungal infections respectively. The skin diseases involved included ringworms, dermatitis, burns, impetigo and boils. The fungal isolates included *Microsporium canis*, *M. audouinii*, *M. ferrugineum*, *Trichophyton mentagrophytes*, *T. megninii* and *Aspergillus niger*, with frequencies of occurrence in the subjects being 80%, 60%, 40%, 60%, 60% and 20% respectively. The implications of the results are discussed.

Keywords: fungi, skin disease, age groups, Plateau State

INTRODUCTION

Cohen and Theodore (1) compare the skin as a biologic boundary between an organism and the physical environment to a psychologic mediator between a person and society, which for the emotionally stable person is an accurate register of feelings. Thus a person's attitude towards self and people's reaction to him may be greatly distorted as a result of a diseased skin. This malady is more accentuated in children and adolescents who depend largely on the love and security given them by parents.

The normal skin is known to harbour numerous non-pathogenic and potentially pathogenic bacteria and fungi, which remain harmless unless the skin is broken (2). These normal flora in different age groups, as reported by Somerville (3,) include Sarcina, Diphtheroids, Staphylococci and Micrococci, and Dermatophytes.

Various types of skin diseases affect school children but ringworm of the scalp is the most common (4). The causative organisms are able to feed on the keratin material of the hair, either from within the hair tuft or from outside and are accordingly termed endothrix and ectothrix hair infection. Somorin *et al* (5) reported 63 (1.8%) of 3,540 school children surveyed in Lagos in 1977 to be infected with dermatophytes of which 60.3% were males and 30.7% were females.

These organisms are present in the soil and are also known to cause ringworm in domestic animals and cattle. For example, *M. canis* has been reported to cause ringworms in dogs and cats while *T. mentagrophytes*, *T. verrucosum* and *T. equium* have been reported to cause ringworm in cattle and horses (6).

The present survey was designed to determine the species of fungi associated with skin diseases of different age groups in Plateau State and the factors that enhance their spread.

MATERIALS AND METHOD

For the survey, 30 subjects aged 1-50 years with various skin diseases were randomly selected. The skin diseases include dermatitis, ringworms, boils, burns and impetigo. Specimens from those with ringworms were collected by skin scraping from the active growth area of lesion with the use of sharp sterile blades, and placed in normal saline. From deep wound and burns, specimens were collected with sterile cotton swabs

and placed in a test tube containing one ml transport medium (Phosphate buffer saline, pH 7.2). All samples were transported to the laboratory immediately for processing.

The samples were examined first by wet film microscopy and then plated on plain Saboraud dextrose agar (SDA) and SDA containing chloramphenicol (0.05g/L) and cycloheximide 0.4 g/L (7). Sets of plates were incubated at 25°C and at 37°C for 7 to 28 days. Fungi colonies were subcultured to obtain pure cultures and identification was done according to Domsch *et al* (8), Samson *et al* (9) and Rippon (10).

RESULTS

The species of fungi isolated from different skin diseases examined included *M. canis, M. ferrugineum, M. audouinii, T. mentagrophytes, T. megninii* and *Aspergillus niger*, with frequency of occurrence in the subjects as 80%, 40%, 60%, 60%, 60% and 20% respectively (Table 1). The general colonial characteristics of the fungal isolates are shown in Table 2. Plates 1 (a-c) shows colonies of *M. canis, T megninii* and *M. ferrugineum*. The frequency of occurrence of fungal lesion in the different age groups is shown in Table 3. Age group 11-20 years had the highest frequency with 38% followed by 1-10 years 27%, 21-30 years 23%, 31-40 years 8% and 40-50 years 4%. Plate 3 shows the leg of a female student with ringworm; Plate 4 shows dermatitis of the face in a student and Plate 5 shows a housewife with ringworm of the leg.

Table 1: Species of fungi isolated from different skin lesions

Fungi isolates	Types of skin disease					Total	% occurrence
	B	Bn	R	D	IM		
M. canis (Bodin)	-	+	+	+	+	4	80
M. ferrugatum (Ota)	-	-	+	+	-	2	40
M. audouinii (Gruby)	-	-	+	+	+	3	60
T. mentagrophytes (Robin)	-	+	+	+	-	3	60
T. megninii (Blanchard)	-	-	+	+	+	3	60
A. niger (Van Tieghem)	-	-	+	-	-	1	20

R = Ringworm, B = Boil, Bn = Burns, D = Dermatitis, M = Impetigo, + = present, - = absent

Table 2: Colonial characteristics of the fungal isolates

Species	Colonial appearance	Microscopy of colony
M. canis	SDA: White, usually silk with radiating hyphae. Reverse: Deep yellow	Macroconidia: mature with one or more septa, immature appears aseptate Microconidia: Hyphae Other: Racquets seen
M. ferrugineum	SDA: Bright orange-yellow, heaped, may be folded. Reverse: No special feature	Macroconidia: Roundish, pear-shaped oval. Microconidia: Rough-walled, fusiform, multicellular. Other: Chlamydospores are present and hyphae appeared distorted
M. audouinii	SDA: Grey, buff or pale orange, dense and sometimes grooved. Reverse: Buff, pale orange or pink.	Macroconidia: Rough walled, fusiform, multicellular. Microconidia: Roundish, oval, pear-shaped, along the hyphae. Hyphae: ramified and septated usually quite straight
T. mentagrophytes	SDA: Pale to buff or pink, flat faltly or granular. Reverse: Buff or yellow-orange to brown	Macroconidia: Seen in some strains. Microconidia: Many Other: Often spiral hyphae and sometimes tangled hyphae.
T. megninii	SDA: Velvety surface, spoke-like grooves proceeding from a central button; upper side pink. Reverse: deep purple to dark violet, no diffusion of pigment into agar	Conidia: Borne in chains on the sterigma Conidiophores: Borne laterally on the hyphae, non-septate; numerous sterigma proceed from the apical cub-shaped swellings (head-shaped fructification organs) Hyphae: Septate

Table 3: Frequency of occurrence of fungi isolates in skin diseases of various age groups

Age groups (years)	No sampled	No with fungi isolated	% occurrence
1-10	9	7	27
11-20	11	10	38
21-30	6	6	23
31-40	3	2	8
41-50	1	1	4
	30	26	100

Plates 1 (a-c), 2, 3 and 4

DISCUSSION

The result obtained from the survey has shown that fungal species are associated with some skin diseases in Plateau State. Of the fungal isolates, *M. canis* occur most frequently in all the lesions, followed by *M. audouinii, T. mentagrophytes, T. megninii* and *Aspergillus niger* the least. This is in conformity with the report of Fakete (11) who found 2, 230 cases out of 8,013 patients in dermatological clinic at Kaduna and Zaria to have fungal diseases due to *M. canis, T. megninii* and *T. mentagrophytes*. Also in a survey by Egere and Gugnani (12) in Eastern Nigeria, 351 (34.1%) of 1030 specimens of scraping, hair and nail clippings were positive for dermatophytes with *T. mentagrophytes* being the most frequently isolated fungal species 30.4%, followed by *M. canis* 22.5%, *T. rubrum* 17.1%, *Epidermophyton flocossum* and *M audouinii* each with 6.5%.

When the percentage of occurrence of fungal isolates was compared with age, the highest frequencies of occurrence were in the 1-10 and 11-20 year age groups with 27% and 38% respectively. These age groups include school pupils and students and infection susceptibility in these groups could stem from prevailing nutritional level, low standard of hygiene and quality of medical care available (13). These factors act inter-relatedly and although individual can do little or nothing to modify the climate to suite his condition, nutrition-wise, a lot can be done.

Previous reports have shown that about 50% of the world's populations are undernourished (14) with about 75% of these being in the developing countries, Nigeria inclusive. Although, the nutritional standards of the average Nigerian can be said to be fairly good when compared to some other African countries, it is rather very poor when compared to the developed countries. There are still certain erroneous beliefs and practices with respect to food and nutrition in certain communities in Nigeria where children are given less meat in their food for fear that they might begin to steal if given sufficient meat to eat (15).

These children grow up lacking essential nutrients needed for strong body defense to infectious agents including fungi.

The sanitary condition of the average Nigerian is also poor. A close observation of Jos, Plateau State show most drainage systems to be frequently blocked thereby leading to floods after rains. Most of the pathogenic fungi are present in the soil and are carried in drainage water which overflows in such blocked drainage system. In a study by Ogbonna and Pugh (16) on soil sample in Jos, *M. gypseum, T. ajelloi* and *M. canis* were isolated. Since these fungi are common in the soil and with children usually playing with flood water, this could be one source of fungal infection. The subjects within the age group 21-30 years were found to have 23% frequency of fungal infection in the study. This group comprises students of high institutions of learning and some of them are known to use medicated soap and creams thereby subjecting their skin to damage and easy colonization or infection by pathogenic bacteria or fungi.

Many families are also known to share rooms with domestic animals and pets, some of which can be potential source of dermatophytes. Direct contact with infected animals can be another source of fungal skin infection (17). Since mice and guinea pigs and other rodents are reservoir of *T. mentagrophytes,* efforts at controlling fungal skin diseases should take into consideration the elimination of these reservoirs (18). Domestic animals and pets with such fungal reservoir or infection can be treated with antifungal agent such as Griseofulvin. A suspension of natamycin applied by sponging to 83 horses with ringworm infection caused by *T. equinum* successfully eliminated the fungi within four weeks (19). Continuous and meticulous cleaning of surroundings will minimize contamination of the environment by potentially pathogenic microbes.

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ORIGINAL ARTICLE

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EFFECT OF *TRYPANOSOMA CONGOLENSE* AND *TRYPANOSOMA BRUCEI* MIXED INFECTION ON THE PATTERN OF HAEMATOLOGICAL CHANGES IN MURINE TRYPANOSOMOSIS

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The effect of *Trypanosoma congolense* and *T. brucei* mixed infection on the pattern of haematological changes was demonstrated in a rat model. At the end of 21 days post infection (PI), anaemia which was characterised by drop in the packed cell volume (PCV), was found to be significantly ($P < 0.05$) severer in rats with mixed infection than those infected with *T. congolense* or *T. brucei*. Similar pattern of drop in the total white blood cell (WBC), differential WBC, and platelet counts was observed in the group with mixed infection. It was concluded that even though *T. congolense* and *T. brucei* may cause milder haematological changes in animals compared to *T. vivax*, mixed infection by these parasites may cause severer haematological changes in the natural hosts.

Key words: Mixed infections, pattern of haematological changes, rats, *Trypanosoma congolense*, *Trypanosoma brucei*

INTRODUCTION

African animal trypanosomosis still constitutes a major threat to food security in several parts of sub-Saharan Africa (1-3). Although several advances have been made on research into various aspects of the pathogenesis of African trypanosomosis, the exact factors involved in the disease process have not yet been fully known. *Trypanosoma vivax, T. congolense* and *T. brucei* are the major causes of disease in ruminants (4, 5). *T. congolense* and *T. brucei* also cause disease in dogs (5).

Anaemia constitutes a major pathological feature of trypanosomoses of man and domestic animals besides other haematological and serum biochemical changes (6, 7). Infecting trypanosome species are known to differ both in their host tissue of primary parasitization and disease pattern in animals (5).

The current understanding of the pathogenesis of African trypanosomes rests largely on the observation of results of experimental single trypanosome specie infections in animals (8-10) while the result of infection arising from mixed trypanosome species has not been properly investigated.

Several of the natural trypanosomoses in animals arise from mixed infections (11). There is therefore, the likelihood that the true impact of the disease on the animal has been underestimated. In West Africa, *T. congolense* and *T. brucei*, though pathogenic to animals, are less a threat to livestock when compared to *T. vivax* (4, 5). Not much has been known on the effect of mixed infection arising from these trypanosome species on disease course. In this study we attempt to investigate the outcome of *T. congolense* and *T. brucei* mixed infection on the course of haematological changes using rat as a model.

MATERIALS AND METHOD

A total of 80 albino rats were used for the investigation. All the rats were bred at our Institute in Kaduna and Vom. Commercial rat cubes and water were fed *ad libitum* through out the period of investigation. The rats were randomly divided into three groups; A, B and C of 20 rats each while 15 other rats served as control.

Trypanosome species used were *T. congolense* (NITR/BASSA) and *T. brucei* (NITR/LAFIA). Both parasites were isolated from cattle and cryopreserved in liquid nitrogen at the Institute from where they were sub-passaged once into rats before use. Rats in group A were inoculated with *T. congolense*, $1x10^3$ parasites through the intraperitoneal (IP)route while group B were inoculated with *T. brucei* using the same number of parasites as in group A IP. Group C rats were infected with a mixture of *T. congolense* and *T. brucei* made up of $0.5x10^3$ parasites each. Parasites for inoculation were estimated as described by Lumsden *et al* (12).

Daily parasitaemia was estimated from wet blood preparations made through tail prick while the packed cell volume (PCV) was determined as described by Kelly (13). At the end of 21 days post infection (PI), blood was collected from the heart of surviving rats using a 21 gauge hypodemic needle for the estimation of red blood cells (RBC), total white blood cell (WBC) and Platelet counts as described by Kelly (13). The Thin blood smears stained with Giemsa were used for WBC differentiation. The data collected were analysed using the student's t-test and analysis of variance (ANOVA).

RESULTS

Parasites were first detected in the tail blood of rats in groups B and C infected with *T. brucei* and mixed infection respectively, 3 to 4 days PI while that of *T. congolense* infected group A was not detected until 5 to 7 days PI. Thin smear made from mixed infection group on the first day of parasitaemia revealed *T. brucei* while *T. congolense* was detected in the smears 3 days later. Control rats showed 1.0% drop in PCV between day 0 and 21 PI while infected rats showed 9.1%, 6.7% and 17.6% drop in PCV in *T. congolense*, *T. brucei* and mixed infection groups respectively.

On the overall, the drop in mean PCV was significantly highest in group C with mixed infection ($P<0.05$) followed by group B infected with *T. brucei* and least in *T. congolense* infected group B (Fig 1). Drop in the mean RBC count at the end of 21 days followed a similar pattern of change in PCV (Table 1).

The total WBC counts also dropped most in the mixed infection group ($P<0.05$) but least in the *T. congolense* group. A similar pattern of decrease in the mean platelet counts was observed with the mixed infection group recording highest decrease in platelet counts at the end of 21 days PI (Table 1).

Absolute differential WBC counts of infected rats were as shown on Table 2. The decrease in the mean lymphocyte and neutrophil counts were also highest in the mixed infection group than in *T. congolense* and *T. brucei* infected groups. There was also higher increase in monocyte counts in the mixed infection group ($P<0.05$). Similarly eosinophilia occurred only in the mixed infection group. A total of 5 rats died within the last week of infection in the mixed infection group while no mortality was recorded in the other groups.

Table I: Changes in RBC, total WBC and Platelet Counts of control and trypanosome-infected rats at 21 days PI

	Control Rats n=20	Infected A n=20	Infected B N=20	Infected C N=15
RBC ($X10^6$/ul)	9.6 ± 1.0	*(9.8±1.7) 9.4±2.7	(8.7 ± 2.7) 6.4 ± 3.2	(6.8±2.5) 6.2±3.4)
WBC ($X10^3$/ul)	15.9 ± 4.5	(15.4±4.2) 12.1±4.7	(15.0±3.5) 11.0 ± 4.2)	(13.80±4.7) 5.9±0.1
Platelet Counts ($X10^3$/ul)	450.0± 0.1	(470.4±2.2) 385.1±0.7	(415.4±1.9) 268.1±4.2	(401.1±2.5) 133.3±29

*Pre infection values in brackets.

Table II: Summary of changes in absolute differential WBC (X10²/ul of blood) of control and trypanosome-infected rats at 21days PI

	Control Rats N=20	Infected A n=20	Infected B n=20	Infected C N=15
Neutrophils (x10³/□l)	3.3±1.5	*(3.1±1.6)2.1±2.5	(3.4±2.9)2.0±2.5	(2.8±2.0)1.5±2.1
Lymphocytes (x10³/□l)	12.2±2.1	(13.2±4.3)10.9±0.8	(11.9±4.6)6.8±1.3	(12.3±3.8)5.1±0.1
Monocytes (x10²/□l)	0.3±3.1	(0.2±3.5)0.2±2.5	(0.3±1.0)0.3±0.9	(0.2±3.6)0.4±2.4
Eosinophils (x10²/□l)	0.0±0.0	(0.0±0.0)0.0±0.0	(0.0±0.0)0.0±0.0	(0.0±0.0)0.1±0.5

* Pre-infection values in brackets.

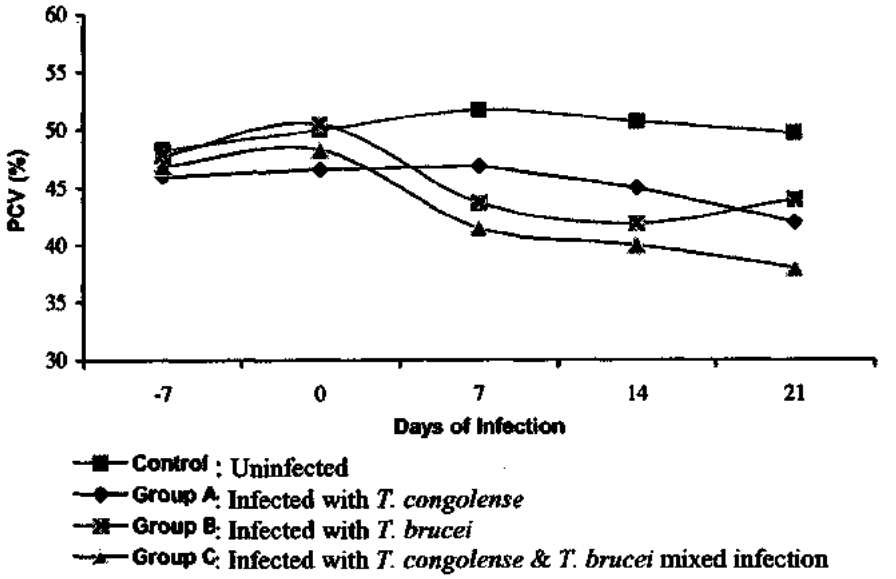

Fig 1: Mean Packed Cell Volume (%) of control and infected rats

DISCUSSION

Mixed infection did not appear to affect pre-patent period of either of the parasites in rats. Rather *T. congolense* and *T. brucei* mixed infection group exhibited more severe haematological changes characterised by marked significant drop in the PCV and RBC. The pattern of anaemia in *T. congolense* and *T. brucei* single infection groups did not differ from the pattern previously observed in *T. congolense* (14, 15) and *T. brucei* (14, 16) infected albino rats. By day 21 PI, anaemia in the *T. brucei* infected group seemed to recover. This has earlier been observed by Anosa (6) and arises from increase in erythropoietic activity in *T. brucei* infection. Similar observation was made in *T. gambiense* infected monkeys in which the PCV showed apparent recovery in the chronic phase (17).

Both *T. congolense* and *T. brucei* have been shown to differ in their sites of localization in the tissues of infected host. While *T. brucei* is extravascular and localise in solid tissues, *T. congolense* is largely intravascular (5, 18). Losos (5) classified trypanosome lesions into primary and secondary lesions; primary lesions being those changes caused by the direct effect of injurious mechanisms on the infected target organs while secondary lesions are those resulting from the subsequent malfunctioning of organs and tissues affected by the primary lesions. In *T. congolense*, primary lesions occur mainly in the blood, blood

vessels and lymphoid tissue, (5). In *T. brucei* infection, this occurs in the connective tissue of solid organs (2).

A combination of these different mechanisms of pathology may have been responsible for precipitation of an overall severer anaemia in the mixed infection group. Anaemia in trypanosomiasis arises from haemolysis, haemodilution, haemorrhage and dyshaematopoiesis (6, 19). The mechanisms involved in these factors probably become exaggerated in mixed infection. Although *T. brucei* group appeared to recover from anaemia, no such changes occurred in the mixed infection group. This may be due to severity of stem cell injury (19), and marked phagocytosis of erythroid cells (6, 20, 21).

Leucocytopaenia and thrombocytopenia was also a general feature in *T. congolense* and *T. brucei* infected rats, but were more marked in the group with mixed infection. This was also characterised by lymphopaenia, neutropaenia, eosinopaenia and monocytosis. Eosinophilia was observed in *T. evansi* infected buffalo calves (10, 22) and *T. brucei* infected deer mice (20). The pattern of fall in lymphocyte numbers suggests that *T. congolense* and *T. brucei* mixed infection also caused more marked antigenic stimulation leading to accelerated transformation of lymphocytes to plasma cells and transferred lymphocytes resulting to lymphopaenia (7, 23) in this group. Similarly marked depression of precusor cells and marked phagocytosis of neutrophil precursors in the bone marrow (7, 24) and spleen (6, 25) may have been responsible for the severe fall in neutrophil numbers in the mixed infection group.

Monocytosis may also be due to increased demand for removal of particulate matter (23) arising from severer pathology in rats with mixed infection, which was matched by proliferation of macrophages in several tissues (26). The aetiology of thrombocytopenia on the other hand is multifactorial and it include platelet phagocytosis by splenic and bone marrow macrophages (7, 21, 24), platelet agglutination (28, 29), ineffective thrombopoiesis (30) and splenic pooling (31). These factors were probably exaggerated leading to more severe fall in platelet counts in mixed infection rats.

It is concluded that *T. congolense* and *T. brucei* cause severer pathological changes under mixed infection and that the true impact of African Trypanosomes on animals has indeed been underestimated.

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SCREENING FOR *SCHISTOSOMA HAEMATOBIUM* INFECTION IN A RURAL COHORT OF PREGNANT WOMEN IN NIGERIA

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Studies were conducted to investigate the occurrence of *Schistosoma haematobium* infection among 37 pregnant Nigerian women in Ilie, Osun state, Nigeria and to determine the effects on haemoglobin concentration and clinical symptoms. Out of the 37 pregnant women seen over a period of nine months, 14 (37%) had urinary schistosomiasis, with a mean egg count of 82.5eggs/10 mls of urine. The mean haemoglobin values in women with schistosomiasis mothers were lower than in women negative for the parasite but the differences were not stastically significant (P > 0.05). Abdominal pain was the predominant complaint among the women seen in with 71% of the infected women while other complaints were dizziness, fever and headache. This study shows that schistosomiasis is prevalent among pregnant women in rural area and could contribute to anaemia and abdominal pain commonly seen in pregnant women in our environment.

Key words: Schistosomiasis, Pregnant women, Abdominal pain, Haemoglobin values, Nigeria

INTRODUCTION

Schistosomiasis is endemic in 74 countries and infects more than 200 million people world wide (1). As a result of this parasite, women of reproductive age may experience genitourinary tract infection with renal complications, and salpingitis and tubal obstruction that can lead to infertility and ectopic pregnancy (2). In sub Saharan Africa, up to 24 million women may be pregnant each year and this high rate is often associated with increase susceptibility to infections because pregnancy is accompanied by high hormone activity which may exert immuno-suppressive effects (3).

Schistosomiasis, along with other helminthic infections, is a common occurrence particularly in rural areas of Nigeria and some studies have reported the relationship between schistosomiasis during pregnancy and tubal obstruction, anaemia, low birth weight and acute appendicitis (4-7). For example the prevalence of hookworm infection among pregnant women has been estimated to be 32% (8). Given the high fertility rate, low nutritional status and poor hygienic conditions predominant in developing countries, schistosomiasis during pregnancy may contribute significantly to adverse pregnancy outcome.

The occurrence of schistosomiasis in pregnant women in Nigeria, their clinical manifestations and the disease association are largely unreported. This study serves as a preliminary investigation of prevalence and clinical effects of *Schistosoma haematobium* infection among pregnant women in a rural area of Nigeria.

MATERIALS AND METHOD

Subjects/Study area

The subjects were pregnant women visiting the antenatal clinic (ANC) of Ilie health post of Olorunda Local Government area of Osogbo, Osun State, Nigeria between May 2002 and January 2003. Ilie is a very small community with a population of about 3 000 people. The women are Nigerians of the Yoruba ethnic group, who have been residing in the community for at least 5 years. The people of the community depended largely on a very close by stream for almost all their water

related activity. The purpose of the study was explained to the women and informed consent was sought. Only 37 women who consented were recruited to participate in the study.

Sample collection/examination

Urine samples were collected from the women into a universal bottle. Ten mls of urine was centrifuged at 2,500 rpm and the sediment was examined for eggs of *S. haematobium* using the sedimentation technique (9). The frequency of macroscopic haematuria was recorded. The presence of microscopic haematuria was confirmed using the Medi Test strip (Combi 9, Mcherey-Nagel Dueren) and the concentration was recorded as 10, 50 or 250 Ery/µL of urine. The haemoglobin concentration was evaluated using iron determination technique (10). The major water source in the community was examined for presence of snail (molluscs) using the kitchen sieve net method (11). Snails found were taken to the laboratory for identification and individual snail was examined for infection by exposure to sunlight.

Subject's bio-data

With the aid of a health worker, each participant was asked to complete a questionnaire, which contained information about demographic characteristics, duration of pregnancy, contact with stream and water usage, and clinical symptoms in pregnancy. Each woman had full examination normally carried out during routine ANC.

Statistical analysis

Differences in mean haemoglobin values between women positive for *S. haematobium* infection and women negative were tested for statistical significance using Chi square (X^2) analysis.

RESULT

Of the 37 pregnant women examined, 13 (37%) were infected with *S. haematobium* with an overall mean egg count of 82.5 eggs per 10 mls of urine. Microscopic haematuria was seen in 37% of all the urine samples while 29% presented with macroscopic haematuria. The age group > 30years had the highest prevalence (50%) and also the highest mean egg count of 140.5, compared to age group 21–25 years with 12% and mean egg count of 56. This difference is stastically significant ($P < 0.001$) (Table 1).

Table 2 shows the breakdown of infection per trimester of pregnancy and clinical symptoms. The most common complaint by the women is abdominal pain/discomfort seen in 71% of those with schistosomiasis compared to 17% in those without schistosomiasis. This difference is stastically significant ($P < 0.05$). Other clinical complaints include dizziness, headache and fever (data not shown). All the women with schistosomiasis in the first trimester complained of abdominal discomfort.

Table 2 also illustrates the effect of *S. haematobium* on the mean haemoglobin values. The haemoglobin concentration values of women with *S. haematobium* infection were lower than the values in women negative for *S. haematobium* but these differences were not stastically significant ($P > 0.05$).

Table 1: Prevalence, mean intensity and percentage microscopic haematuria of schistosomiasis among pregnant women by age

Age range (Years)	Number Examined	Number Positive	% Infected	Mean egg count/ 10mls	% with microscopic haematuria	% with macroscopic haematuria
15- 20	9	4	44	41.3	55	33
21 – 25	8	1	12	56.0	37	12
26 - 30	12	4	33	92.5	43	21
> 30	8	4	50	140.5	62	25
Total	37	13	35	82.5	37	29

Table 2: Prevalence, clinical complaints and mean haemoglobin concentration among pregnant women by trimester

Trimester	No. Exam	Positive women				Negative women			
		% SH +ve	% Abd Pain	% others	Mean HC g/dL	% SH - ve	% Abd Pain	%others	Mean HC g/dL
1ST	6	33	100	0	8.7 ± 1.6	66	20	16	9.2 ± 1.6
2ND	21	28	71	4	8.9 ± 1.5	66	21	38	9.9 ± 1.3
3RD	10	50	60	10	8.6 ± 1.3	50	0	30	10.3 ± 1.3
Total	37	35	71	5		62	17	32	

No. Exam = Number Examined
% SH +ve = Percentage *Schistosoma haematobium* positive
% SH –ve = Percentage *Schistosoma haematobium* negative
% Abd Pain = Percentage with Abdominal Pain
% others = Percentage with other Pregnancy related complaints
Mean HC= Mean haemoglobin concentration

DISCUSSION

This study has demonstrated that over 35% of pregnant women in rural areas endemic for schistosomiasis are infected with the parasite. The occurrence of this infection at high rate among the women is an indication of continuous pollution of water with schistosome eggs due to poor sanitation and improper sewage disposal. Studies in many parts of Nigeria have highlighted the hyper endemicity of schistosomiasis especially among school children in rural communities (12, 13, 14). Pregnant women are also at high risk of infection because of their close relationship with their children and the fact that they also engage in water related activities like washing in the stream, bathing, swimming and even fishing, which expose then to considerable contact with cercariae infested rivers.

The high incidence of abdominal pain among women with schistosomiasis may be due to congestion of pelvic vessels during pregnancy which facilitates the passage of eggs into the villi and intervillous spaces, with resultant inflammatory reactions and pain (5). Exacerbation of acute appendicitis during pregnancy by schistosomiasis has also been reported (4). Masses of schistosome eggs can lodge throughout the body and cause acute inflammation of the appendix, liver and spleen (5). Acute infection is often asymptomatic, but can present with a non specific influenza-like illness or in extreme cases as potentially fatal Katayama fever, with cough, abdominal pain, diarrhoea, hepatosplenomegaly and eosinophilia.

The low haemoglobin level among women with schistosomiasis is attributed to chronic blood loss and iron deficiency, caused by terminal haematuria from urinary infection (2, 15). The most important cause of chronic blood loss and iron deficiency anaemia in the tropics, are helminthic infections (6, 17), especially hookworm (16), *S. mansoni. S. japonicum,* and *S. haematobium* infections (15, 17), and malaria (18).

Our study demonstrates that urinary schistosomiasis is still being actively transmitted among pregnant women in Ilie community, Osun State, Nigeria and could contribute to the abdominal discomfort, haematuria and anaemia seen in these women. The implementation of a control programme based on chemotherapy with Praziquantel® will help in the reduction of the frequency. Because the treatment with Praziquantel® is relatively simple and considered safe at least in the second and third trimester of

pregnancy, case management during pregnancy can be considered and would likely have important benefits in endemic settings (19, 20).

Also there is need for government to improve the health care delivery to the rural communities in the country. Most of the pregnant women do not make use of the ANC facility partly because of low level of awareness and also because the clinics are not well equipped. Although some of the women still prefers patronizing homes of traditional birth attendants (TBA), this mentality can be corrected with adequate public enlightenment and equipping the existing clinics. Provision of potable water, improved personal hygiene and extermination of immediate hosts are recommended.

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BACTERAEMIA AND ACUTE PHASE PROTEINS IN NIGERIAN WOMEN WITH SPONTANEOUS RECURRENT ABORTION

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C-reactive protein, alpha – 2 macroglobulin, transferrin and bacteraemia were studied in women with recurrent abortion and compared with the pregnant women as well as non-pregnant women with *no history of* abortion (controls). The results showed a significantly reduced level of transferrin but significantly raised levels of alpha-2-macroglobulin and C-reactive protein in the pregnant women with recurrent abortion (P+R) compared with pregnant women without recurrent abortion (P-R) or the controls. Four genera of bacteria (*Staphylococcus aureus, Streptococcus agalactiae, Klebsiella species* and *Clostridium perfringes*) were isolated from the blood of women with recurrent abortion while there were 2 genera of bacteria (*S. aureus* and *Strept. agalactiae*) isolated from the blood of pregnant women without recurrent abortion. This study had shown that inflammation and bacterial infection contribute to spontaneous recurrent abortion.

INTRODUCTION

Recurrent abortion is defined as a history of three or more consecutive spontaneous abortions occurring prior to the 22nd week of gestation (1). Some of the previous data suggested that recurrent abortion might be caused by failure to activate natural suppressor cells (CD8+), presence of certain auto antibodies (2), blocking factor in the serum (2) and infections (3).

In humans, there is evidence that CD8+ cells recognize the trophoblast which when activated with IL-2 elicit lyses of the trophoblastic cells. The pregnancy associated protein TJ6 when expressed on CD56+CD16- NK cells and ß cells is seen to elicit recurrent abortion and thus this expression can be used as a diagnostic tool in predicting successful pregnancy (4). In women experiencing recurrent abortion, a circulating embrotoxin has been reported, whose action involves induction of MHC I and II antigens on its targeted tissues as well as activating the macrophage to produce TNF-alpha. This in effect would cause the recognition of the induced MHC expression and thus rejection of the foetal allograft by the maternal immune system would result.

In some cases of recurrent pregnancy loss, the mother carries antiphospholipid autoantibodies against negatively charged membrane phospholipids of the placenta (5). Other immunological factors that had been found to cause recurrent spontaneous abortion are: presence of anti TLX antibodies, HLA and rhesus incompatibilities (5).

The acute phase proteins were shown to provide information on the presence of inflammatory lesions, on the prognosis of the condition and on the response to treatment (6). Studies on the levels of acute phase proteins in normal pregnancy are scanty and none had assessed the levels of acute phase proteins in Nigerian women with spontaneous recurrent abortion. A study showed that C-reactive protein (CRP) level was found not to be useful as an early predictor of clinical chorio-amnionitis and a significant difference between

maternal and neonatal CRP was demonstrated, implying a lack of transplacental transfer during labour (7). Also, serum transferrin level was low in 2nd and 3rd trimesters of normal pregnancy (8) while significantly raised C3, reduced C4 accompanied by a significant increased C4d was reported in normal pregnancy (9). This previous study concluded that pregnancy induces activation of the classical complement and that C4d concentration cannot be used to monitor disease activity in patients with connective tissue diseases during pregnancy.

Infections with bacteria had been linked with recurrent abortion and the spectrum of bacteria involved was concluded to vary (10). Bacterial infection is a potent inducer of inflammation (10) but little attention has been giving to immunological indicators of inflammation in Nigerian women with spontaneous recurrent abortion. This study will elucidate the involvement of inflammatory immunological factors and the species of bacterial in women with spontaneous recurrent abortion attending Obstetrics and Gynaecology Clinic, University College Hospital, Ibadan, Nigeria.

SUBJECTS AND METHOD

Subjects

Informed consent was obtained from eighty-four women of childbearing age before sample collection. They were divided into three groups viz: twenty-four pregnant women with recurrent abortion (P+R), thirty-six pregnant women without recurrent abortion (P-R) and twenty-four non-pregnant women with no history of abortion as controls. All subjects were recruited from Obstetrics and Gynaecology Clinic of the University College Hospital, Ibadan. Ten milliliters of venous blood was collected into non-heparinized bottle and spun at 1500-x g for five minutes. This was allowed to retract and the serum separated for the measurement of acute phase proteins was stored at -20°C till needed for analysis.

Quantitation of acute phase reactants

Acute phase proteins were quantitated by the single radial immunodifussion method as modified by Salimonu *et al* (11). A volume of an optimally diluted nonspecific anti-serum was mixed with noble agar and poured on glass plate. Wells of equal diameter were cut in the antibody agar mixture. The wells were filled with test or standard serum. The plates placed in humid box were incubated at 4°C for 18 hours. After incubation the diameters of the precipitin rings were measured with micrometer eyepiece.

Determination of bacteraemia

The method described in a standard test (12) was adopted. Five (5) ml of blood was collected into 10-15 ml of broth. The content was thoroughly mixed and incubated at 37°C for 7 days. Each day the content was examined for turbidity. The turbid culture was inoculated into blood agar plate, chocolate agar and MacConkey plate and incubated at 37°C overnight. The characteristic appearance of the organism grown on each plate was noted and necessary biochemical tests were preformed.

Data Analysis

Data were presented as mean and standard deviation. Student t-test was used to test the significance of differences between mean values. The probability value (p) greater than 0.05 was considered insignificant

RESULTS

The mean age of the pregnant women without recurrent abortion (P-R) was 22.5 + 3.2yrs, the mean age for the pregnant women with recurrent abortion (P+R) was 21.0 + 5.3yrs while the mean age for the negative controls is

21.3 + 4.0yrs. There were no statistical significant differences between the ages of all subjects.

The result shows significant decrease in the mean level of transferrin in P-R or P+R compared with the controls. The Table also shows that the mean level of alpha-2-macroglobulin was significantly raised in P+R compared with the controls but was significantly reduced in P-R compared with the controls. The level of CRP was significantly higher in P+R subjects compared with the P-R or the controls.

Table 2 shows that there were four genera of bacteria (*S. aureus, Strept. agalactiae, Klebsiella spp.* and C. *perfringes*) in women with recurrent abortion while there were 2 genera of bacteria (*S. aureus* and *Strept. agalactiae)* in pregnant women without recurrent abortion. The prevalence of these bacteria was higher in P+R women compared with P-R women.

Table 1: Levels of acute phase proteins (mean ± 1sd) in pregnant women with or without recurrent abortion compared with controls

Subjects	n	Transferrin (mg/dL)	Alpha-2-Macroglobulin (mg/dL)	CRP (mg/dL)
C	24	110.3±9.0	705.5±34.9	4.3±0.9
P-R	36	100.7±25.7	609.1±27.2	7.4±0.3
P+R	24	66.8±17.1	1301.8±16.6	13.8±2.5
t, p-values[a]		2.04, <0.05	1.01,>0.2	2.65, <0.05
t, p-values[b]		6.10, <0.01	5.49,<0.01	9.00, <0.01
t, p-values[c]		22.8, <0.01	51.1,<0.01	22.9, <0.01

P-R = Pregnant subjects without recurrent abortion
P+R = Pregnant subjects with recurrent abortion
(a) = Controls compared with P-R
(b) = P-R compared with P+R
(c) = Controls compared with P +R

Table 2: Percentage distribution of different isolates of bacteria in the blood of women with recurrent abortion (P+R) and without recurrent abortion (P-R

Organisms isolated	P+R	P-R
S. aureus	8 (33)	3 (8.3)
Strept. agalactiae	3 (12.5)	1(2.8)
Klebsiella species	1(4.2)	-
C. perfringes	1(4.2)	-

Percentages are in parentheses.

DISCUSSION

CRP is a major acute phase protein, with its concentration increasing over 200 times from a low, virtually, negligible, normal level. Other recognized acute phase proteins are serum amyloid A (SAA), haptoglobin (HP), alpha 1-acid glycoprotein (AGP), fibrinogen (Fb), alpha 1-antiprotease (AP), caeruloplasmin (CP), alpha 2-macroglobulin and transferrin (13, 14). HP, AGP, Fb and AP are moderate acute phase proteins as serum concentration increases only 2-3 times during the response. Cp is a minor acute phase protein as it only increases by 60-70% in response to inflammatory lesions.

Investigators have related the presence of infection or inflammatory lesions with the acute phase protein response (13, 14, 15) and assess the diagnostic value of such biomarkers of disease but studies on the serum levels of acute phase proteins during normal pregnancy is scanty. Reduced level of alpha-2 macroglobulin was found in P-R subjects. Previous reports have associated high values of alpha- 2 macroglobulin with low birth weight (15, 16). Thus, the significantly low serum level of alpha-2 macroglobulin recorded in pregnant subjects without recurrent abortion may be of immense benefit to the outcome of the pregnancy.

Alpha-2 macroglobulin is a large plasma glycoprotein that binds many proteinases. Proteolytic enzymes released from damaged tissues as well as from phagocytic cells have their activity inhibited by being bound to alpha-2 macroglobulin (17). In addition, alpha–2 macroglobulin is also known to bind growth factors such as IL-8 (18), nerve growth factor, platelet derived growth factor-B and transforming growth factor-B (19) and transport them to their target cells where such cytokines affect cell growth and functions (20). Raised level of alpha-2 macroglobulin was observed in P+R. The possible increase in hepatic synthesis of alpha–2 macroglobulin to meet the requirement caused by tissue damage and as transport protein may account for the significantly high level of alpha–2 macroglobulin found in pregnant subjects with recurrent abortion in this study. This high level has been similarly documented in animal model (21).

Transferrin is the principal plasma protein for transport of iron. In states of iron deficiency, plasma transferrin level rises and returns to normal level upon successful treatment with iron (22.). Transferrin is a negative acute phase protein, and as such, its level is expected to reduce during inflammation, chronic liver disease, malnutrition or protein losing enteropathies (22). In view of the iron deficiency that is expected in pregnancy (23), antenatal routine iron supplement given to pregnant women affects transferrin level (22). High transferrin level observed in pregnant women without recurrent abortion could indicate a normal functioning of hepatic parenchymal cells that produces more transferrin to bind the circulation excess iron. In the present study, serum transferrin was significantly low in P+R compared to controls. During abortion, blood loose and RBC haemolysis is common (24). Overproduction of iron from periodic RBC lysis might have caused rapid consumption of transferin despite its adequate production.

CRP is a useful indicator of bacterial infection, capable of complement activation via classical pathways, platelet aggregation, inhibition of rosette formation by human T lymphocytes and inhibition of phyto-haemagglutinin induced by mitogenesis in vitro (7). The elevated levels of CRP observed in a number of our P+R subjects can only be attributed to bacterial infection that was shown to be more prevalent in P+R subjects. The elevated levels of CRP obtained in this study might also be related to state of inflammation caused by tissue damage that occurs in P+R subjects.

This study had shown that inflammation and bacterial infection contribute to spontaneous recurrent abortion in this environment.

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DETECTION OF CHLAMYDIAL ANTIGEN IN CERVICAL SPECIMENS FROM ANTENATAL CLINIC ATTENDEES IN BENIN CITY, NIGERIA

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Four hundred consenting antenatal clinic attendees were serologically screened for evidence of *Chlamydia trachomatis* infection. Infection with this organism is underreported in many countries including Nigeria. In the antenatal clinic setting in most developing countries, antigen detection has found widespread application in diagnosis due to lesser demands of cost, expertise, and time required to obtain results. In this study, Chlamydia antigens were serologically detected using an immunochromatographic method (Hexagon Chlamydia Rapid Test Kit manufactured and described by Human Gesellschaft fur Biochemica und Diagnostcal MbH-Germany). Overall, 40 (13.3%) of the 300 women screened had chlamydia antigens in their endocervical specimens while 100 women (control subjects) were negative for chlamydial antigens. There seems to be an association between chlamydial infection and vaginal discharge, abortion and infertility. We highly recommend the necessity to include chlamydia screening tests in antenatal health care in Nigeria to prevent unpleasant sequelae

Keywords: Chlamydial antigens, endocervical specimen, antenatal women, Benin City, Nigeria.

INTRODUCTION

Chlamydia trachomatis includes the agents of trachoma, lymphogranuloma venereum, and urogenital tract disease and inclusion conjunctivitis (1-4). Although *C. trachomatis* infection is underreported in most countries of the world, it is fast assuming a prominent role as the major aetiological agent of sexually transmitted non-gonococcal urethritis (5). Krul (6) has reported that 50 million new cases of *C. trachomatis* infection occur annually, worldwide.

Although most infections caused by *C. trachomatis* in women are asymptomatic, clinical manifestations include cervicitis, urethritis, endometritis, pelvic inflammatory disease (PID), or abscess of the Bartholin gland (7). Culture studies have shown that among women infected with *C. trachomatis*, 50 to 60% are infected at both the cervix and urethra, 30% have only cervical infections, and 5 to 30% have only urethral infections (8). The importance of cervical Chlamydial infection in the pathogenesis of pelvic infection is well recognized (9-12). Although the major impact of disease caused by *C. trachomatis is* on the female reproductive tract, the agent also causes infections in man and children.

The biggest challenge to the control of chlamydial disease is that as many as 70 to 80% of women and up to 50% of men who are infected do not experience any symptoms.(4,13-14). The study of Scholes *et al* (15) however provides evidence that, once women at high risk are identified and tested, the incidence of PID can be reduced.

In the less developed countries of the world, chlamydial infections in women are not routinely diagnosed in our hospitals. Until recently, cell culture of inocula from urogenital specimens was considered the "gold standard" for detection of *C. trachomatis* because it has a specificity that approaches 100% (16). However, antigen and nucleic acid detection technologies have found

widespread application in diagnosis due to lesser demands of cost, expertise, and time required to obtain results (17-20). Previous studies (21-22) have indicated prevalence rates of chlamydial infections in various Nigerian cities.

This paper reports on the prevalence of *C. trachomatis* infection among unsuspecting women attending an antenatal clinic in Benin City, Nigeria.

PATIENTS AND METHOD

Study population

The patient group consisted of three hundred consenting pregnant women (mean age 28 years, range 19 to 42 years) who were consulting the antenatal clinic of Central Hospital, Benin City, Nigeria. One hundred non-pregnant women within the same age range served as control. These were screened for the presence of *C. trachomatis* antigen in their endocervical specimens. Each subject gave her consent and responded to a questionnaire containing series of screening criteria such as: number of sexual partners, marital status, age, number of previous still births and abortions, etc. Only those who had not been on tetracycline or erythromycin therapy within the past 3 months before sampling were included in the study.

Methodology

The Hexagon chlamydia test kit (cat. No. 58012) used for this study employs an immuno chromatographic method for the direct detection of chlamydia antigen in extracts from patient's specimens.

Specimen collection and sample extraction

The clinician aseptically cleansed the vagina and cervix of traces of blood and mucus using a sterile cotton ball before inserting the Hexagon chlamydia collect swab (cat No. 58912) into the endocervical os. The swab was rotated for 15-30 seconds, carefully withdrawn and immersed into the extraction solution provided in the collection tube. This was left for 10-15 minutes at room temperature to allow for proper sample extraction. At the end of the extraction time, the liquid was removed from the swab by twisting it against the tube wall while removing the swab from the tube. The swab extract was used within 30 minutes.

Test procedure

The test device and the sample of the extract were brought to room temperature on a level workbench. 4 drops of sample extract were carefully added drop-wise onto the sample window on the device, and allowing each drop to be completely absorbed. This was allowed to incubate and read at 20 minutes. The test results as well as the controls incorporated within the test device were read and recorded.

RESULTS

Of the 300 subjects screened for the presence of chlamydia antigens, using the Hexagon chlamydia reagents, 40 (13.3%) yielded positive results while no chlamydia antigen was detected in specimens from the control subjects (Table 1).

Table 1: Prevalence of *C. trachomatis* antigens in antenatal clinic patients

No of patients	No positive for antigens (%)	No negative for antigens (%)
300	40 (13.3)	260 (86.7)
100 (Control)	0 (0)	100 (100)

$X^2 = 14.77$, $p < 0.005$

Of the 40 women demonstrating presence of chlamydial antigens in their cervical specimens, 10 had two previous abortions while only 1 had one previous abortion. All had vaginal discharge at the time of visiting the clinic. 30 out of the 40 positive cases were carrying nine months old pregnancies, while the remaining 10 women were in their first trimester. Also 30 out of the 40 positive women were childless.

DISCUSSION

The primary aim for undertaking this study was to use the rapid immunochromatographic test to detect chlamydial infection in unsuspecting women attending hospital clinics for their antenatal

check-up. Of the 300 women attending the antenatal clinic, 40 (13.3%) demonstrated presence of chlamydia antigens in their endocervical specimens; thus showing evidence of their having contacted *C. trachomatis* infection. Thirty of the 40 positive women in our study had advanced to their ninth month of pregnancy. Martin *et al* (23) have reported that pregnant women with *C. trachomatis* infection were 10-fold more likely to have poor outcomes such as still birth and neonatal death. Gestation periods were also significantly shorter in infected women. However, the present study, like the earlier works of Hardy *et al* (24) and Harrison *et al* (25) does not confirm this association, as the 30 pregnant women with chlamydial antigens had progressed to the ninth month of pregnancy.

Responses to our study questionnaires reveal that 11(27.5%) of the 40 women harboring chlamydial antigens have had previous abortions. Although we do not have evidence that these previous abortions were caused by infection with chlamydial organisms, two independent studies (26, 27) have however indicated an association between exposure to *C. trachomatis* and recurrent spontaneous abortions.

The 40 women positive for chlamydial antigens had vaginal discharges. This is one of the symptoms associated with chlamydial infections (16) and so, we suggest that routine screening of antenatal women, especially those with vaginal discharges, be carried out in order to prevent or reduce the incidence of PID and its adverse sequelae. A previous study (21) in Benin City has established a link between chlamydial infection and primary and secondary infertilities in women. It could be possible that chlamydial infection may have been responsible for the infertility observed in 30 of the 40 women carrying chlamydial antigens.

Analysis of the questionnaires dispensed during the present study, showed that 75% of women carrying antigens were found to be childless at the time of obtaining specimens from them.

A study by Ofor (unpublished observation) showed a high prevalence (23%) of *C. trachomatis* infection among pregnant women and 37% prevalence in non-pregnant group in Lagos. Also, Azenabor and Eghafona (22) had earlier demonstrated the presence of chlamydial infection in Benin City, this time, amongst infertile women where a prevalence of detectable chlamydial antibodies of 22% and 25% for primary and secondary infertility respectively was recorded. A prevalence rate of 13.3% was found in this study. Some workers (23, 28) have shown that the prevalence of *C. trachomatis* infection in pregnant women ranges from 2 to 35%.

Since it has been proven that pregnant women with chlamydial infections are at increased risk for adverse outcomes of pregnancy and post partum PID (23-25, 28), results from this study should stimulate our health planners and providers towards including chlamydial screening of antenatal women as effective preventive measures for our women folk. We disagree with the views of Obunye *et al* (29) that screening for chlamydial infection is not yet feasible, for reasons of high costs and the difficulty of reaching the target population. We believe that the proverbial journey of a thousand miles starts with a step!

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EFFECT OF STORAGE ON BACTERIOLOGICAL QUALITY OF BOREHOLE WATER

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The effect of storage on the bacteriological quality of water from a borehole was investigated. Water samples drawn from the borehole were stored in covered tap-fitted buckets of different colours at room temperature. Physicochemical parameters (pH and suspended solids contents) as well as bacteriological parameters (total bacterial and total coliform counts) were monitored over 12 days of storage. Generally, there was an increase in pH during storage. Their suspended solid content reduced by 75.0%, 92.3% and 40.0% during storage in the purple, blue and transparent buckets respectively. A total of eleven bacterial species were isolated at onset but only three of them: *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris* survived till the twelfth day of storage. There was also reduction in the total bacteria count by 82.8%, 83.35%, and 58.82% from an initial 17 x 10^4 CFU/ml during storage in the purple, blue and transparent buckets respectively. The total coliform count decreased by 99.18%, 82.35% and 91.36% in purple, blue and transparent buckets respectively from an initial 1100 MPN/100ml during the period of storage. The significance of storage as a means of enhancing water purification was discussed and suggestion provided on proper storage of water intended for drinking.

INTRODUCTION

Since the beginning of recorded history, water has been recognized as a potential carrier of disease [1]. It has been suggested that drinking water should be aesthetically acceptable; it should be free from apparent turbidity and odour, and from any objectionable taste [2]. The World Health Organization guidelines for bacteriological water quality recommends that all waters intended for drinking should contain no coliform organisms in any 100ml sample taken and should be free from all hazardous odour as well as being tasteless [3].

It is usually taken for granted that piped water supplies received in homes and institutions satisfy this requirement. The irregularity of piped water supply has however made it necessary to seek alternative water sources. This is found in rainwater and groundwater such as wells and boreholes. The contribution of groundwater to the total water supply is greatest in arid and semi arid region and in some places where geological conditions favour groundwater storage [4]. Although ground water especially from deep sources are considered free of bacterial contamination, some study [5] have found that water from some boreholes do not meet the WHO standard for drinking water. This gives credence to the assertion that no water in nature is 100% pure [6].

It is common practice for individuals and households to store water for domestic use especially where there is pressure on the water source. It was reported that a few days of storage of surface water would improve the physical and microbiological characteristics of the water [7]. In water treatment, storage is valuable as a preliminary step to other processes, because it reduces the bacterial content of the raw water and also reduces the amount of suspended matter in it. However, it cannot be relied on as sole measure of purification. Storage acts in three ways; sedimentation, equalization, devitalization [8].

Several individuals and households collect water from boreholes into various types of container especially plastics. The collected water is usually stored sometimes for as long as two weeks depending on the size/number of collecting containers and/or the size of consumers. The storage of water could serve in checking the outbreaks of diseases associated with drinking of contaminated water.

This study investigates the effect of storage on the bacteriological quality of borehole water over a period of 12 days. In assessing the effect of storage, the total bacterial count, and total coliform count were examined at interval as well as some physicochemical parameters (pH, temperature and total suspended solids).

MATERIALS AND METHOD

Plastic buckets with lids were fitted with plastic tap at 5cm from the base. They were rinsed with 70% alcohol and then with sterile distilled water. Water was drawn from the borehole between the academic area, students' village and bus stop in University of Ilorin (Permanent Site) following the procedure described by WHO [3]. The buckets were carefully opened and filled with water to about 3cm from the top. They were tightly covered and moved into the laboratory for storage at room temperature in a stationary position. Samples of the water in each bucket were collected through the tap following the procedure of WHO [3]. The pH and suspended solids content of the water samples were determined according to the American Society for Test and Measurement Standards [9], while the total heterotrophic bacterial counts were determinéd using the pour plate method [10]. The total coliform counts were determined as Most Probable Number (MPN) using the multiple tube fermentation tests [11]. Bacterial species isolated were characterized and identified as described in Bergey's manual of determinative bacteriology [12].

RESULTS

The initial pH of the water samples was 5.5 however it increased during storage. Variation in mean pH of the samples is shown in Figure 1. Variation in the mean suspended solids content is shown in Figure 2. The initial mean value of 16 x 10^{-2} was reduced by 75.0%, 92.3%, and 40.0% during storage in the purple, blue and transparent buckets respectively. Eleven bacterial species were isolated initially, but as storage progressed it was observed that some bacterial species were not encountered subsequently and only three of them were recovered at the end of 12 day of storage.

Table 1 shows the identity of the bacterial species and their succession during storage. Reduction was recorded in the total bacterial counts and total coliform counts. Figure 3 shows the variation in the total bacterial and total coliform counts. Reduction in the total bacteria count was 82.35%, 83.35% and 58.82% from an initial 17x10^4 CFU/ml for the purple, blue and transparent buckets respectively. The reduction in total coliform count was 99.18%, 82.35% and 91.36% in purple, blue and transparent buckets respectively from an initial 1100 MPN/100ml.

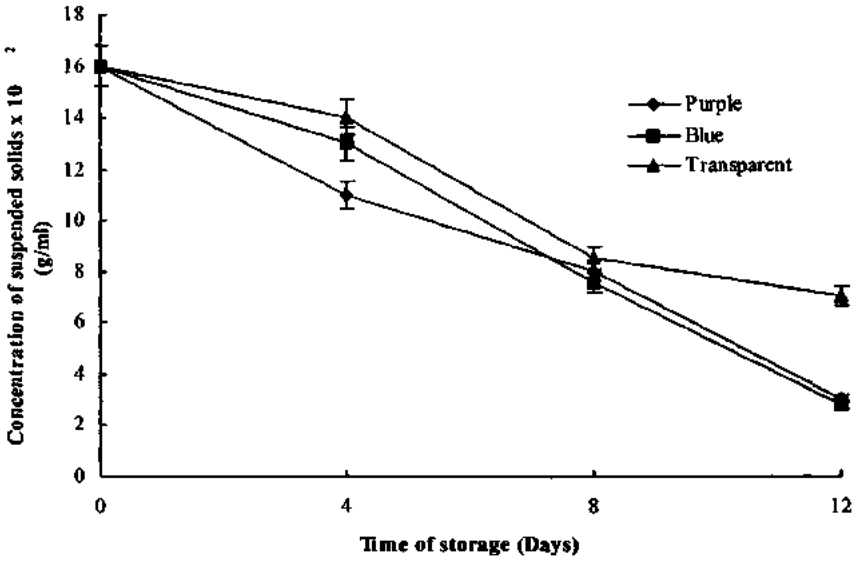

Figure 2. Change in mean suspended solid content of the water during storage.

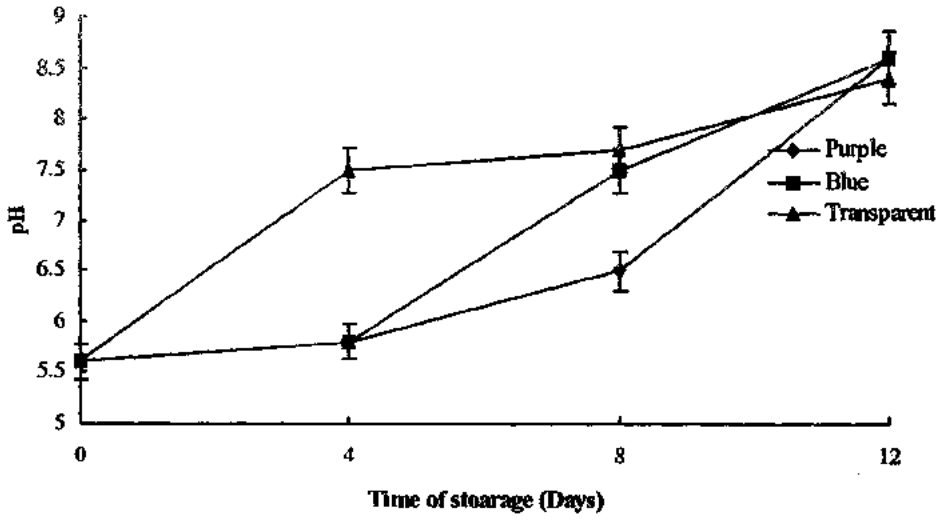

Figure 1. Change in mean pH of water during storage.

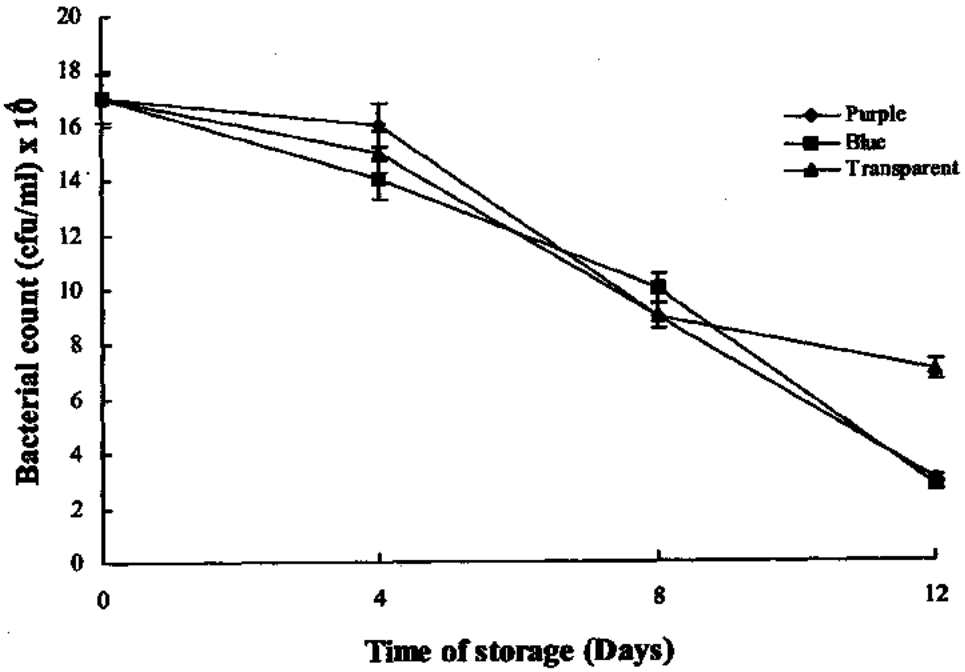

Figure 3. Variation in mean total bacterial count during storage

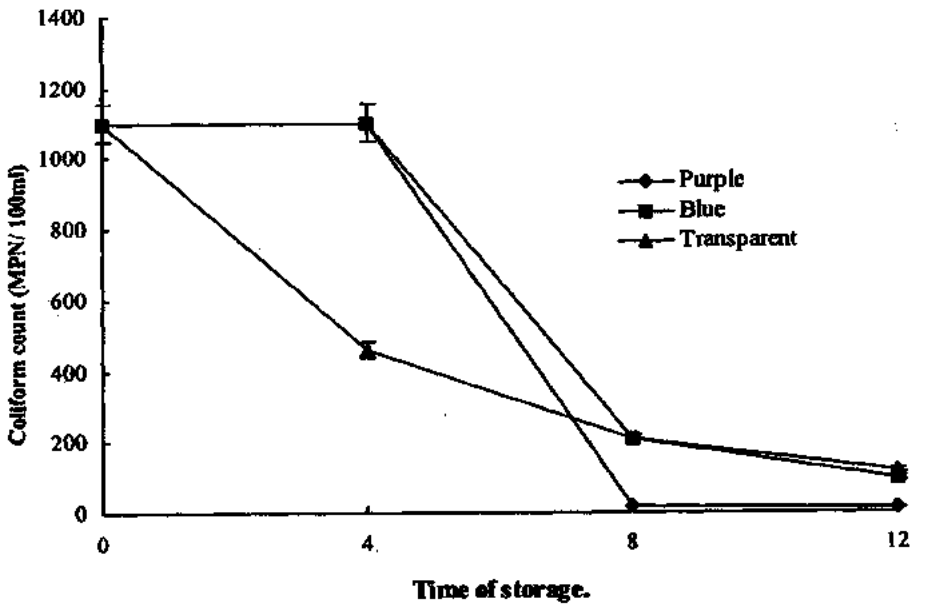

Figure 4. Variation in mean total coliform count during storage.

Table: Microbial succession in borehole water during storage

Isolate	Purple Bucket				Blue Bucket				Transparent Bucket			
Days	0	4	8	12	0	4	8	12	0	4	8	12
Acinectobacter spp	+	+	+	-	+	+	+	-	+	+	-	-
Micrococcus luteus	+	-	-	-	+	-	-	-	+	-	-	-
Serratia marcescens	+	+	-	-	+	+	-	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	-	+	+	+	-	-	+	-	-
Bacillus cereus	+	+	-	-	+	+	-	-	-	-	-	-
Enterobacter aerogenes	+	+	+	-	+	+	+	-	-	+	+	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	+	-	-	-	+	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	+
Proteus vulgaris	+	+	+	+	+	+	+	+	+	+	+	+
Shigella dysenteriae	+	+	+	-	+	+	+	-	+	+	-	-

DISCUSSION

The pH of the samples fell within the range of pH of water, which favours survival of bacteria. The importance of pH in the survival of microorganisms has been identified [13]. The observed increased in pH during storage could be due to activities of the resident flora and or their death, which result in the release of inorganic substance such as ammonia; this has been highlighted [5]. The observed progressive reduction in suspended solid content is consistent with the submissions of some researchers [14]. This is attributed to gravitational pull, which will cause the suspended materials to settle out with time.

The population of total bacteria and total coliform progressively dropped suggesting death of the resident bacteria during the storage period. Death of bacteria could occur due to depletion of nutrients. The decline in bacterial population could also result from sedimentation of suspended material in the water, which would include suspended bacterial cells. Sedimentation has earlier been identified as one of the ways by which storage act in reducing the bacterial content of water [8].

Eight of the eleven bacterial species isolated were not encountered after the storage period. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Shigella dysenteriae, Bacillus cereus, Enterobacter aerogenes,* and *Escherichia coli,* which were isolated, are well established as either pathogens or opportunistic pathogens. In addition, the presence of *Escherichia coli* is a well established index of faecal contamination. The presence of these organisms is a further confirmation of the report [5] that the water from the borehole fell short of the standard required of untreated drinking water as stipulated by the World Health Organization (WHO). Among the seven potential pathogens, only *Escherichia coli* and *Pseudomonas aeruginosa* survived to the twelfth day of storage.

Results obtained buttress the opinion that storage is valuable as a preliminary accessory stage of treatment but it cannot be relied on as a sole measure of purification [7]. A difference was observed in the rates of reduction of the suspended

solids, total bacterial and total coliform counts among the different coloured buckets. The reason for this is not yet known, however, the colour of the bucket is considered an important factor. To verify this, a collaborative study is being planned with colleagues in the Department of Physics of this institution to monitor the changes in the light or optical properties of the colours.

CONCLUSION

Generally, storage was found to be desirable as it brought about improvement in the quality of the water sample, reducing the types and number of bacteria in the water. It is therefore suggested that water drawn from the borehole should be stored for about a week if it is to be consumed.

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BACTERIOLOGICAL QUALITY OF WATER SAMPLES IN OSOGBO METROPOLIS

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The bacteriological qualities of samples of some sachet water, tap water and well water were examined. Some physicochemical parameters (pH and suspended solids) indicative of water quality as well as the total bacterial and total coliform counts were examined. The pH of the samples range between 6.5 and 7.2. Suspended solids content ranged between 3.3 and 18.5 x 10^{-2} g/ml. The total bacterial counts ranged between 7.0 to 12.0 x 10^{1} CFU/ml for sachet water, 0 to 20 CFU/ml for tap water and 2.0 to 20 x 10^{3} CFU/ml for well water. The coliform count (MPN) ranged between 0 to 1 coliform/100ml for sachet water, 0 to150 coliform/100 ml for tap water and 1200 to 1800 coliform/100ml for well water. A total of six bacterial species: *Escherichia coli*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus faecalis*, *Enterobacter aerogenes* and *Staphylococcus aureus* were isolated. Their distribution among the samples and the public health implication are discussed. The well water samples examined were found to fall short of the WHO recommendation for drinking water, while the tap water was adjudged fit for consumption.

INTRODUCTION

Man's initial assessment of the value of water is very low until he finds himself without it. Human water needs are usually met by water obtained from rainfall, streams, well, boreholes or tap depending on the locality and available technology. The availability of water in any given locality is however usually constant (1). Most organized society depends on piped treated water to meet their water requirement. It is often taken for granted that such water supplies are potable. However, it has been recommended by World Health Organization that the source of such water should be examined daily for coliform organisms, turbidity and pH at the point at which the water enters the distribution system. The piped water should contain no coliform organisms and no faecal coliform in 100 ml (2).

Ground water sources usually serve as alternative sources of water largely due to shortage of piped water or erratic supply. For this category of water, protection of the source by lining and covering, diversion of surface drainage, catchments protection to restrict human and animal access and paving of surroundings, have been recommended as means of preventing pollution of the water (2). The WHO guidelines also stipulate a coliform count of less than 10 per 100 ml (2). In contemporary time, the sale and consumption of bottled and sachet water in a bid to avoid consumption of contaminated water has increased. For bottled water, under which one can place sachet water, it is recommended that the water contains no coliform organism (2).

Water quality and supply affects health (3). To be safe for human consumption, water must be aesthetically acceptable and should be free from apparent turbidity and odour, and from any objectionable taste (4). The presence of faecal coliform or thermotolerant coliform organisms per 100 ml is an indication of some degree of faecal contamination (3). The presence of *E. coli* is particularly taken as

exclusively indicative of faecal pollution of water (5)

Sachet, well and tap water serve as regular sources of water for domestic requirement in Osogbo, Osun State in Nigeria. In this study, the bacteriological quality of sachet, well and tap water samples collected from popular regions were examined to ascertain whether or not they are fit for human consumption.

MATERIALS AND METHOD

Samples of sachet water, tap water and well water were collected from three areas of Osogbo: Oja Oba, Dada estate and Power line. Water samples were taken into bottles containing 3% sodium thiosulphate solution (6). The pH and suspended solid contents were determined as described by ASTM (7). Total bacterial count was determined using pour plate method. The plates were inoculated aerobically at 37°C for 24 hours. The total coliform count was determined as the most probable number (MPN) using multiple tube fermentation test (6). Colonies that developed were purified using the streaking method. The pure cultures were then characterized on the basis of colony morphology, cellular morphology, staining and biochemical reactions and subsequently identified (8).

RESULTS

The water samples were generally near neutral with pH ranging between 6.5 and 7.2. Well water samples were found to contain the highest amount of suspended solid while tap water had the lowest (Table 1). Table 2 shows the total bacterial and total coliform counts. The total bacterial counts ranged between 7.0 and 12.0 X 10^1 CFU/ml for the sachet water, 0 to 2 CFU/ml for tap water and 2.0 to 20 x 10^3 CFU/ml for well water. The coliform count (MPN) ranged between 0 to 1 coliform/100 ml for the sachet water, 0 to 150 coliform/100 ml for tap water and 1200 to 1800 coliform/100 ml for well water.

A total of six bacterial species: *Escherichia coli, Bacillus cereus, Proteus vulgaris, Streptococcus faecalis, Enterobacter aerogenes* and *Staphylococcus aureus* were isolated. Their distribution among the samples is shown on Table 3. *Escherichia coli* was found in tap water samples from Ojo Oba, and in the entire well and sachet water samples. The well water samples were found to contain all the organisms.

Table 1: Physicochemical characteristics of water samples from different locations

Samples	Physiochemical characteristics	
	pH	Suspended solid content (g/ml X 10^{-4})
S1	6.6	3.6
S2	6.8	3.3
S3	7.0	3.3
T1	6.9	4.5
T2	7.2	4.0
T3	7.1	3.7
W1	6.5	17.4
W2	6.6	18.5
W3	6.5	14.6

S1= Satchet water samples from Oja Oba
S2= Satchet water samples from Dada Estate
S3= Satchet water samples from Power Line
T1= Tap water samples from Oja Oba
T2= Tap water samples from Dada Estate
T3= Tap water samples from Power Line
W1= Well water samples from Oja Oba
W2= Well water samples from Dada Estate
W3= Well water samples from Power Line

Table 2: Mean population of bacteria in water samples

Samples	Population of bacteria	
	Total bacterial count (CFU/ml)	Total coliform count (MPN/100 ml)
S1	1.2 x 10^2	1
S2	1.6 x 10^1	0
S3	7.0 x 10^1	0
T1	2.0 x 10^1	150
T2	0	0
T3	0	0
W1	2.0 x 10^4	≥ 1800
W2	2.0 x 10^3	≥ 1200
W3	1.2 x 10^4	≥ 1800

Table 3: Distribution of bacterial species in water samples

Bacterial species	Sample								
	S_1	S_2	S_3	T_1	T_2	T_3	W_1	W_2	W_3
Bacillus subtilis	+	-	-	+	-	-	+	+	+
Escherichia coli	-	-	-	+	-	-	+	+	+
Staphylococcus aureus	+	-	-	-	-	-	+	+	+
Proteus vulgaris	-	-	-	-	-	-	-	+	-
Streptococcus faecalis	-	-	-	-	-	-	-	-	+
Enterobacter aerogenes	+	-	-	+	-	-	+	+	+

S1= Satchet water samples from Oja Oba, S2= Satchet water samples from Dada Estate
S3= Satchet water samples from Power Line, T1= Tap water samples from Oja Oba, T2= Tap water samples from Dada Estate, T3= Tap water samples from Power Line, W1= Well water samples from Oja Oba, W2= Well water samples from Dada Estate, W3= Well water samples from Power Line
+ = Present; - = Absent.

DISCUSSION

The pH of the water samples was generally within the limit considered suitable for human consumption. The well water was observed to have high suspended solid content and high bacterial count. The high concentration of suspend solids in the well water could be due to the way the water is fetched, which may stir the bottom stratum of the well. This will make materials that had settled at the bottom to be re-suspended in the water and may account for the presence of the bacterial species in the water samples. Another factor that could have accounted for the high bacterial count is the fact that the majority of those who fetch water from the wells are children, most of who are in poor state of hygiene.

The presence of *E. coli* in the sachet water portends serious danger as it is the most commonly consumed forms of water. The tap water was however found acceptable, as the samples were largely free of bacteria. *Bacillus subtilis* and *Staphylococcus aureus* were found in only one of the samples. This is indicative of proper treatment and distribution of the treated water. The presence of *E coli* in one of the samples, which also had a high coliform count (MPN of 150 coliform/100ml), suggests that the distribution network in the area could have suffered leakage at some point. Such leakages have been identified as being responsible for the presence of bacteria in treated piped water (5)

The presence of *E. coli* suggests contamination of the water sample with faecal materials. For the well water, this is most likely to result from cross contamination through the buckets used for fetching water, which are often placed on the ground. The presence of *Staphylococcus aureus* suggests human contamination as the organism is a commensal on the skin and nostril of humans (9). The organism may be associated with food poisoning which involves the elaboration of heat labile toxins (10). The presence of *Bacillus subtilis* in the well water further buttresses the fact that cross contamination of the well water occurred. This organism is a common saprophyte encountered in the soil (10) and could have been carried along with soil that sticks to the containers used for fetching water.

CONCLUSION

The well water samples were particularly observed to fall below the WHO recommendation which states that drinking water should contain no microorganism known to be pathogenic, and should also be free of bacteria indicative of pollution with excreta. The sachet and tap water were found to satisfy this requirement. It can thus be concluded that the tap water supply to Osogbo metropolis is bacteriologically safe for human consumption. However, the conditions under which sachet water are produced and packaged will need to be improved The National Agency for Food and Drug Administration and Control (NAFDAC) will need to properly monitor the production

and certification of these packaged water for domestic consumption. On the other hand, well water should not serve as drinking water without treatment.

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EVALUATION OF THE 48 HOUR, 72 HOUR AND 96 HOUR READINGS OF TUBERCULIN TEST FOR THE SCREENING OF TUBERCULOSIS IN CATTLE

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In this study, a cattle farm with a history of tuberculosis was examined over a period of three years to determine the usefulness of reading tuberculin tests (single intradermal cervical tuberculin test (SICTT) and single intradermal comparative cervical tuberculin test (SICCTT)) at 48 hrs, 72 hrs and 96 hrs intervals in the diagnosis of tuberculosis. On the onset, SICTT was conducted on a total of 145 cattle, 52 (35.9%) of these were positive at 48 hours, 56 (38.6%) at 72 hours and 68 (44.8%) at 96 hours (X^2 = 1.54, p = 0.46). After one year, 171 cattle were screened using SICCTT, 10 (5.8%) animals were positive at 48 hours, 12 (7.0%) at 72 hours and 14 (8.2%) at 96 hours (X^2 = 0.67, p = 0.72). During the third test conducted almost one year after the second test, 136 cattle were screened using SICCTT, 13 (9.6%) were positive at 48 hours, 17 (12.5%) at 72 hours and 17 (12.5%) at 96 hours (X^2 = 0.68, p = 0.71). With the pattern of this result, there may be need to review the policy which gave the 72 hr reading a preference over the 96 hr reading of tuberculin test.

INTRODUCTION

According to Francis *et al.*, (1) various forms of the tuberculin test provided the essential means for the diagnosis and control of tuberculosis but these had been modified over time. The tuberculin test in cattle has proven to be the most widely accepted *in vivo* diagnostic test when compared to other sero-diagnostic tests that have been employed in the diagnosis of bovine tuberculosis.

Serological tests for screening tuberculosis are ELISA technique, complement fixation, fluorescent antibody, direct bacterial agglutination, precipitin and haemagglutination tests (2); but these have little potential value for the routine diagnosis of tuberculosis. However, a diagnostic technique exploring the *in vitro* assay of cell mediated reactivity known as the interferon-gamma assay has been developed (2).

The more recent adaptation of the IFN-gamma assay is ELISPOT assay (enzyme linked immunospot) using synthetic peptides (ESAT-6 and CFP-10), which have been reported by Vordermeier *et al* (3) to be promising in the detection of *M. bovis* in cattle. Despite these elegant detection procedures for tuberculosis, the single intradermal test using the purified protein derivative of *Mycobacterium tuberculosis* (PPD) is still the routine screening tool for detecting carriers of tuberculosis (3).

PPD tuberculin is universally used in medical practice and has been the official tuberculin for testing cattle in Britain, Europe, South Africa and some of the developing countries including Nigeria (4, 5, 6). Reactivity to tuberculin made from either human or bovine bacilli (the mammalian tuberculins) is similar and usually greatest in sensitized hosts.

However, sensitization to *M. avium, M. paratuberculosis* and many tuberculoid bacilli may produce a state of greater sensitivity to tuberculin made from *M avium* (avian tuberculin) than to the mammalian tuberculins. This difference according to Francis *et al.*, (1) provided the basis for the comparative tuberculin test, which has been used universally.

The accuracy of this tuberculin test lies greatly on the time when readings are done post-inoculation of the PPD. Several authors, (1, 5, 6, 7) supported reading on the 72hours post-inoculation; while others (4), suggested taking the average of readings at 72hours and 96hours post inoculation. Radostits *et al*, (2) however supported readings between 48 hours and 96hours after injection with a preference for 48-72hours for maximum sensitivity and at 96hours for maximum specificity.

This study evaluated the 48hour, 72hour and 96hour readings of tuberculin test in making accurate diagnosis of tuberculosis. The results of tuberculin tests were also compared with other diagnostic tools of tuberculosis.

MATERIALS AND METHOD

This work was conducted in a resident cattle herd in Southwestern Nigeria over a period of three years. Before this study was carried out, postmortem findings revealed five cases of tuberculosis. All the cattle were screened using either the single intradermal tuberculin test (SICTT) or the single intradermal comparative cervical tuberculin test (SICCTT) with bovine purified protein derivative (B-PPD) and avian purified protein derivative (A-PPD) obtained from the Central Veterinary Laboratory Weybridge, UK. Measurements of skin thickness were taken before and after the inoculation as described by OIE (6).

Further confirmation of the disease was based on clinical symptoms, gross pathology lesions, histopathology, Ziehl Neelsen staining technique (acid fast microscopy), culture and microscopy using the Lowenstein-Jensen medium (5, 6).

1st year: This involved the screening of 145 cattle using SICTT followed by carrying out postmortem on two cattle that were positive following this test.

2nd year: This test was conducted nine months after test 1 was completed and 171cattle were screened using the SICCT. This was followed by postmortem examination of 11 of the positive animals. 3rd year: This was carried out one year after test 2. In all, 136 cattle remaining in the herd were screened using SICCTT.

RESULTS

Figure 1 shows the results of this study as explained below;

1st year: Altogether, 85 (58.6%) of the cattle were classified as reactors when individual hours are taken into consideration without counting the same animal twice between 48 to 96 hours. Fifty-two (35.9%) animals were positive at 48hours, 56(38.6%) at 72hours and 65(44.8%) at 96hours (X^2 =1.54, p=0.46). Of the two animals culled, postmortem findings in one of them showed no visible lesion in all the organs and lymph nodes, while the other animal revealed generalized tuberculosis, which affected all the lymph nodes, major organs and the reproductive tract.

For 2nd year, 18 (10.5%) animals were considered to be positive between 48 to 96hours following the criterion used in 1st year above. On individual hours, 10 (5.8%) animals were positive at 48hours, 12 (7.0%) at 72hours and 14 (8.2%) at 96hours using the total of 171 animals as the common denominator (X^2=0.67, p=0.72). Eleven animals were culled for postmortem examination with the revelation of varying degrees of tuberculosis lesions. The organs affected included the lungs, kidney, spleen, heart, liver, lymph nodes (i.e. retropharyngeal, mandibular, bronchial, mesenteric, mediastinal, and hepatic).

Histopathology revealed varying

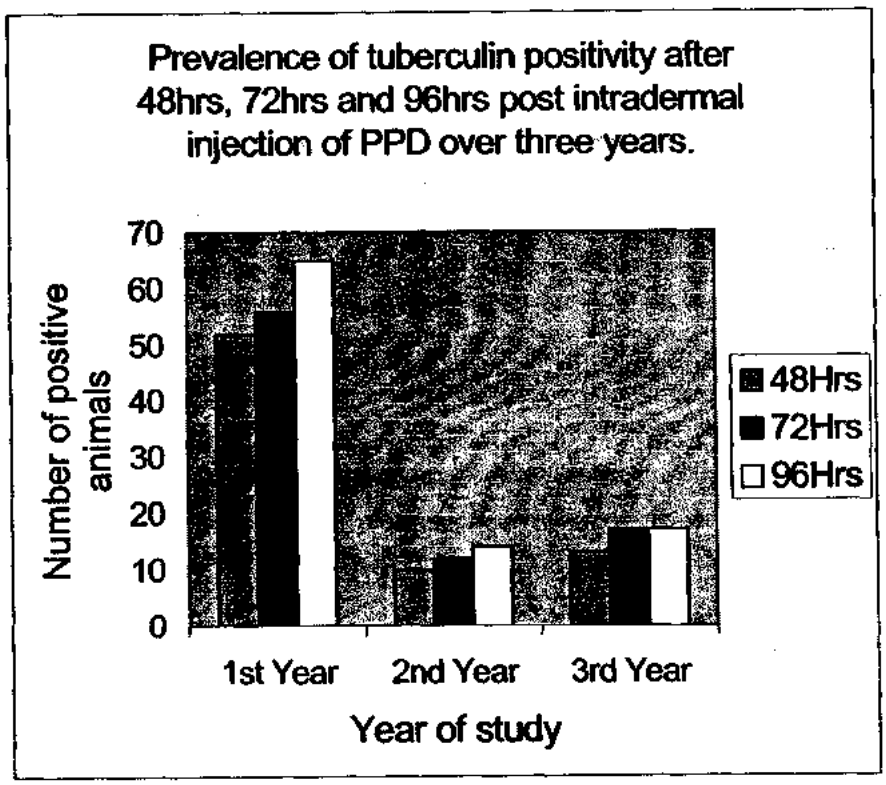
degrees of caseating granuloma, extensive areas of necrosis with parenchyma fibrosis and some areas of calcification. All the samples were acid fast positive. Only one of the 20 pairs of L-J media had growth on both L-J with glycerol and L-J with pyruvate indicating that there was a mixed infection in one of the animals. The other 19 pairs of LJ had growth only on the LJ with pyruvate. The confirmation of these positive growths was revealed by the numerous pink staining acid-fast bacilli on staining each culture smear with Ziehl Neelsen stain.

3rd year: Out of the 136 animals that were screened, 20 (14.7%) animals tested positive between 48 hours to 96hours following the criterion used in 1st year. 13 (9.6%) animals were positive at 48hours, 17 (12.5%) by 72hours and 17 (12.5%) at 96 hours ($X^2 = 0.68$, p=0.71). All the five animals examined for gross-pathology had gross lesions of TB with a very young calf of about six months also showing gross pathological lesions on the liver.

DISCUSSION

It was pointed out that paratuberculosis, an avian-tuberculosis, and nocardiosis gives positive

Figure 1:



reaction to SICTT (2, 8), therefore SICCTT, which is a more confirmatory test in confirming bovine-TB is recommended. This assertion is corroborated by Ayanwale (8). This explains why both SICTT and SICCTT were used in this study. Our finding shows that more animals were positive during the 96-hour readings than at the 48hours or 72 hours readings. This could be explained from the immunological point of view that at 96-hour, all the cells responsible for cell mediated immunity would have

been fully activated; hence, more reactors at this hour than at the 48 or 72-hour when fewer cells would be immunologically active. This is in line with the findings of Radostits *et al.*, (2) who reported that at 96 hour, maximum specificity is achieved for tuberculin test; while at 48-72 hour maximum sensitivity is obtained.

Histological study of this work also revealed that positive reactors were more in proportion in 72 hours and 96 hours post inoculation. This assertion is partially supported by Ayanwale (4) when he suggested the use of average reading at 72 hours and the 96 hours post inoculation for the diagnosis of TB.

CONCLUSION

The results obtained over the three years were consistent with the fact that the 96hour readings produced more positive reactors than the 48 and 72-hour readings; suggesting that more tuberculous animals would have been identified and destroyed in order to reduce the spread of TB within the herd.

This investigation strongly supports readings tuberculin test at 96-hour post inoculation, and therefore calls for the review of preference for 72 hour readings of tuberculin tests.

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INFANT IMMUNIZATION COVERAGE IN DIFFICULT-TO-REACH AREA OF LAGOS METROPOLIS

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A retrospective survey of infant immunization coverage was conducted among 210 children aged 12 to 23 months in difficult-to-reach coastal suburb of Lagos, Nigeria. This was to assess immunization coverage for BCG, DPT, OPV and measles vaccination and to investigate reasons for failure to be immunized and evaluate the drop out rate as well as missed opportunities. An EPI cluster method was used. Questionnaires were administered with WHO cluster form for infant immunization. Recall history and card records of immunization were the tools used. The result showed that 82 (39%) of the 210 children assessed were not immunized, 84 (40%) were partially immunized and only 44 (21%) were fully immunized. At one year of age, only 21 (10%) of the children completed their immunization. Observation of the follow up of vaccination showed that 65.5% of 127 children who started BCG vaccination dropped out as at the time of receiving measles vaccination. Reasons advanced for failure to immunize or complete immunization of the children included obstacles in 47.7%, lack of information 40.7% and lack of motivation in 11.6%. These factors contributed to missed opportunities. Only 9 (11%) of 82 children not vaccinated against measles attributed non vaccination to illnesses. Lack of health facilities and the terrain that is difficult to reach contributed to low coverage. Also, low literacy level, poor maternal health education, poor socioeconomic status and poor advocacy to community leaders and lack of commitment of health workers contributed to low coverage. For immunization coverage to improve in this area, these factors must be addressed

Keywords: infant, immunization, coverage, antigens, advocacy

INTRODUCTION

Immunization has been reported to be cost effective in reducing vaccine preventable childhood diseases (1). To achieve this, there must be high immunization coverage. Reports have shown that improved immunization coverage has promoted child health, reduced childhood diseases and death (2, 3).

Unfortunately, most reports of immunization coverage were low. Many factors have been identified to be impeding immunization. These include poor knowledge of immunization, lack of suitable venues, long waiting, transportation difficulties, non-medical facilities and poor motivation (1, 4, 5).

Apart from the social problems, the terrain of the environment has been reported to affect immunization coverage, especially where the terrain is difficult to reach (6). The poor knowledge of child immunization has caused missed opportunities in some communities. This has been found to cause a low coverage (7-9). The consequences of this low immunization coverage is the resurgence of diseases such as measles, tuberculosis and poliomyelitis (1, 6, 10).

Pedro village is a suburb coastal area of Lagos metropolis with a population of about 44, 000 people. The area is water terrain and difficult to reach. High morbidity (though low mortality) of measles in the village informed the need to assess the immunization coverage for all antigens of the children between the ages 12 and 23 months in the area.

The objectives of this study include; estimating coverage for Bacillus Calmette Guerin (BCG), Diphteria-Pertussis-Tetanus (DPT) and Measles antigens; determining the number of children fully immunized by one year of age; investigating reasons for failure to complete immunization; evaluating drop- out rate for antigens DPT 1-3 and OPV 1-3 and assessing missed opportunities.

MATERIALS AND METHOD

Study location

The study location, Pedro village, is a coastal suburb of Lagos metropolis. A major part of the village is water terrain that is accessible only by canoe and the remaining part is swampy land area that is accessible only by foot in summer. The people of the village are mostly fishermen. There is neither health facility nor any other infrastructural facilities such as piped borne water or electricity in the village. Due to the absence of health facility, a church yard is used as immunization centre.

Study design

The study was randomized using the standard Expanded Programme on Immunization (EPI) cluster method (11). With a random number and a sampling interval, housing units in a cluster were identified from which 7 children were evaluated for immunization coverage. The inclusion criteria are children 12 to 23 months of age and mothers who are able to confirm the date of child's birth and recall child's immunization history or produce child's immunization card. Thirty clusters of the community were evaluated and a total of 210 children were selected for the study.

Ethical issue

The concept of the study was explained to mothers of the children studied and only consenting mothers gave required information regarding children immunization. Ethical committee of Nigerian Institute of Medical Research gave consent for the study.

Information on immunization status

The mothers were interviewed by members of the investigating team comprised of nurses, social health workers and physicians using the WHO cluster form for infant immunization. The questionnaire was designed to contain information on immunization status and dates of receiving BCG, DPT, OPV and measles vaccines. Recall history of immunization by mothers and review of immunization cards were used as tools of assessment. The parents were asked the reasons for failing to immunize or complete immunization of their children.

Defined status of immunization

Children were defined as "not immunized" if at the time of interview, they have not received any vaccine dose. They were "fully immunized" if they receive BCG at birth, with DPT/OPV after receiving at least three doses of the vaccines and "fully immunized against measles" if they receive one dose of measles at 9 months. Children who did not complete three doses of DPT/OPV were taken as "partially immunized". Missed opportunity for immunization is defined as any visit by an eligible child to a health facility, which did not result in his or her vaccination.

RESULT

The age distribution of the children surveyed was 12-23 months. At age 12 to 23 months, children are presumed to have received the 5 antigens that were surveyed. The socio-demographic characteristics of the sampled population were the same as the population in the village (Tables 1 and 2). The mean age of the mothers was 29.4 years and 80% were illiterates.

Table 1: Demographic characteristics of children sampled in Pedro village

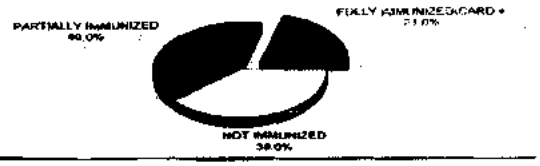
Sample size	210
Mean age	17.5 months
Median age	17.5 months
Children > 12 months	45.75%
Males	53%
Females	47%

Table 2: Sociodemographic data of mothers of children studied at Pedro village

Mean age	29.4 years
Median age	30 years
Level of education	
Never been to school	84%
Primary education	16%
Secondary education	0
Tertiary education	0

Using recall history and immunization card as tools of assessment, the result showed that 82 (39%) of the children were not immunized, 84 (40%) were partially immunized and 44 (21%) were fully immunized (Fig 1).

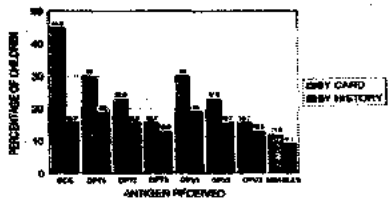
Fig 1 SUMMARY OF RESULTS FOR IMMUNIZATION COVERAGE OF 210 CHILDREN BETWEEN 12 TO 23 MONTHS OF AGE THAT WERE STUDIED



As at one year of age, only 21 (10%) were fully immunized with measles and were deemed to have completed their immunization.

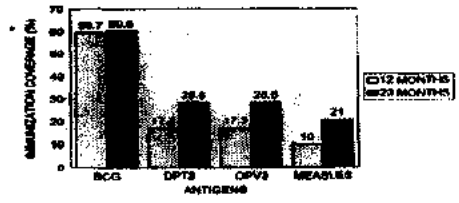
BCG recorded the highest coverage of the antigens by card with 44.8%, while measles was the lowest with 11.9%. DPT and OPV had 15.7% coverage. By recall history, DPT-1/OPV-1 coverage had the highest with 30% while measles had the lowest with 9.1% (Fig 2).

FIG. 2 IMMUNIZATION PROFILE OF CHILDREN STUDIED N=210 BY HISTORY AND BY CARD



A comparison made between children who received all antigens by 12 months and those who received all the antigens from 12-23 months revealed a significance difference with DPT/OPV antigens ($t = 3.297$, $p < 0.05$) and measles ($t = 3.241$, $p < 0.05$).

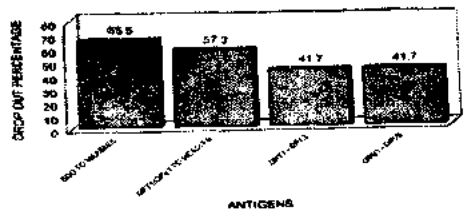
FIG. 3 COMPARISON OF IMMUNIZATION COVERAGE WITHIN 12 MONTHS AND 23 MONTHS



Drop out rate

Sixty five point five percent of 127 children who started BCG vaccination dropped out as at the time of receiving measles vaccination, 59 (57.3%) of 103 children who received DPT/OPV did not complete vaccination to the time of receiving measles vaccination. Also, 41.7% of the 103 children who started the DPT/OPV immunization did not complete the 3-dose regimen of the paired vaccines (Fig 4).

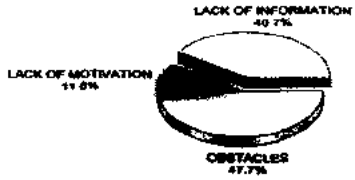
FIG. 4 DROP OUT RATES



Failure to receive immunization

The reasons advanced by mothers for failure to vaccinate or complete immunization of their children include; obstacles such as busy nature of mother schedule of work, most of whom are traders, long distance of outreach clinic to the village and unavailability of transport means such as canoes and boats to the vaccination centres. These accounted for 47.7% of the reasons for non vaccination. Lack of information about immunization accounted for 40.7% while lack of motivation and neglect of village accounted for 11.6% of failure to receive or complete immunization of children (Fig 5).

Fig 5 REASONS FOR IMMUNIZATION FAILURE IN CHILDREN



For missed opportunities, analysis of result showed that 14 (13.5%) of 103 children vaccinated against DPT/OPV missed the completion of the 3-dose regimen due to lack of information on the vaccination regimen. Of the 82 children not vaccinated against measles, 9 (11%) attributed illnesses as the reason for non vaccination, while 42 (51.2%) attributed lack of information and 31 (37.8%) attributed lack of transportation to vaccination centres.

DISCUSSION

Appraising immunization coverage is important in determining the strength and weaknesses of any system adopted for the programme. This could become necessary where resurgence of disease is emerging in people who are supposed to be protected (12-14). This informed the conduct of this survey. The EPI cluster sampling technique employed has been widely used and accepted especially in developing countries where birth and vaccination records are inadequate.

Our study shows that immunization coverage is low among the children in the village with only 21% of the study population fully immunized and only 10% were fully immunized with measles by one year of age. This is similar to the findings of Bosu and his group in a rural setting in Ghana (2). Poor knowledge of immunization is observed to affect compliance as shown in the trend of results obtained in this study. This is compounded by the very low literacy level of the mothers.

BCG ranked as the vaccination with the highest coverage rate by immunization card. This could be possibly due to many mothers delivering in the hospital where first immunization dose, BCG, is given before discharge. However, a set back in the follow-up vaccination was observed for the remaining antigens similar to the findings of Sokhey *et al* (5) in India.

Immunization coverage of DPT/OPV was very low with 15.7% coverage by card. Although, there is little surveillance report of pertussis, the coverage for its prevention is low. The low OPV coverage could be general in the State as wild polio virus infection is still being detected in Lagos State at the time of this survey (personal communication with core facilitator of National Immunization Day on Polio surveillance). Measles immunization is also very low with 11.9% coverage. This low coverage level can not possibly interrupts the spread of measles in any outbreak. If this low level is maintained, the mortality and morbidity associated with measles can not be reduced.

Reasons advanced for failure to immunize or complete child's immunization include obstacles in the way of the mothers, accounting for 47.7% of cases. This indicates that immunization has not been given any priority by the women and this can be attributed to their poor knowledge of immunization and belief about immunization.

Also, lack of information about details of the vaccination programme contributed about 40.7% to failure to complete vaccination. This was observed with the DPT/OPV vaccination where many mothers were unaware of the third dose and stopped receiving vaccination after the second dose.

All these factors could have accounted for the high dropout rate as well as missed opportunities observed in the study. This is a

reflection of poor maternal education about immunization in the area and is similar to reports from other poor or rural communities (1, 2, 15).

The reasons for missed opportunities in the community may be attributed to fear of water terrain by the health care providers and the poverty level of the people. The health care workers are often times afraid to walk on planks used as bridges to get to the village, while mothers equally lacked transport means to get to the vaccination centres.

CONCLUSION

It is recognized that low literacy level coupled with poor maternal health education, lack of structural and health facilities affected immunization coverage in this area. Also, poor socio-economic status, coupled with poor advocacy about immunization and poor commitment of health workers assigned to the village contributed immensely to the low immunization coverage.

There is therefore need for government of Lagos State to establish health facilities in the village and motivates health workers to work in this difficult-to-reach area of the State. Equally important is the need to strengthen maternal beliefs of the efficacy of immunization through health education. Advocacy about the importance of immunization to community leaders who hold cultural belief and opinion on any issue of interest such as immunization will need to be carried out, to ensure acceptability and mobilization of the people of this area for immunization of their children.

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TETANUS TOXOID IMMUNIZATION COVERAGE AMONG MOTHERS OF CHILDREN BELOW ONE YEAR OF AGE IN DIFFICULT-TO-REACH AREA OF LAGOS METROPOLIS

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A retrospective survey of tetanus toxoid immunization coverage was conducted among 196 mothers of children less than one year of age in a difficult-to-reach area of Lagos metropolis. This was to ascertain the status of coverage among the women presumed to have been immunized with minimum of two doses of tetanus toxoid during pregnancy, estimate drop out rates, investigate reasons for failure to be immunized and determine missed opportunities to get immunized. An interview was conducted by administering questionnaires using WHO cluster form for tetanus toxoid immunization. Recall history and immunization card review were taken as response. The results showed that with card/history criterion, 109 (55.6%) women received first dose of tetanus toxoid (TT), 80 (40.8%) received second dose and 22 (11.2%) received third dose while 87 (44.4%) were not immunized. Using the card criterion that only established valid immunization, 20 (10.2%) women received first dose and 14 (7.2%) received the second dose. With the card criterion analysis, 14 (7.2%) mothers were protected against tetanus and only 4 (2%) babies born of these mothers were protected against tetanus. A decline was observed in compliance with the regimen of vaccination. Thirty three (16.8%) of women studied missed the opportunity of getting vaccinated and 87 (79.8%) of 109 who started vaccination dropped out. Reasons advanced for failure to be immunized included lack of information accounting for 65.85%, lack of motivation was 20.4% and 13.8% as environmental factors. Poverty and lack of health facilities also contributed to the low level of immunization coverage. For TT immunization to improve in the area studied, factors impeding immunization must be addressed.

Keywords: Tetanus, immunization, coverage

INTRODUCTION

Immunization of pregnant women with tetanus vaccine is one major means of controlling neonatal tetanus (NT). Available reports of different studies indicate that the disease is still persisting. A community based study conducted in Ilorin local government of Kwara State in Nigeria showed a neonatal mortality of 11.9 per thousand live births (1).

Owa and Makinde (2) studied 52 cases of NT in Osun State of Nigeria and observed that 35 of the mothers did not receive tetanus toxoid (TT) immunization and 4 of the 52 of the babies with NT were from immunized mothers. Grange (3) also observed that in the 419 cases of NT studied in Lagos, Nigeria, history of maternal TT immunization was lacking for most mothers. A community based study in Rivers State of Nigeria on TT immunization status of parturient women showed a complete, partial or no coverage status of 41.2%, 17.0% and 41.8% respectively of women surveyed (4).

These records indicated low coverage of TT immunization among women of reproductive age, especially pregnant women. This low coverage was noted to be common in rural areas where the awareness of TT immunization is very low (4). Also, to date most of the women delivering at the facilities of traditional birth attendants (TBA) have no record of tetanus toxoid immunization (personal communication with TBAs).

It is this observation that informed our team to conduct a survey of TT immunization coverage in a coastal area of Lagos metropolis that is difficult to reach, to determine effect of terrain on immunization coverage. The objectives of the study are to determine the number of mothers of children less than one year of age who received a minimum of 2 doses of TT immunization during pregnancy; estimate

the drop out rates in the course of receiving the antigens, investigate reasons for not receiving TT immunization and determine the percentage of missed opportunities.

MATERIALS AND METHOD

Study location

The study location, Pedro village, is a coastal suburb of Lagos metropolis. A major part of the village is water terrain that is accessible only by canoe and the remaining part is swampy land area that is accessible only by foot in summer. The people of the village are mostly fishermen. There is neither health facility nor any other infrastructural facilities such as piped borne water or electricity in the village. Due to the absence of health facility, a church yard is used as immunization centre.

Sampling technique/Study population

The WHO cluster sampling technique (5) was used. A cluster in this case is a randomly selected group of housing units containing 7 mothers of children below one year of age to evaluate the TT immunization coverage. The survey covered 30 clusters. The inclusion criterion was mothers of children below one year of age living in Pedro village. Only 196 out of the targeted 210 mothers met the criterion and were recruited into the study. Each mother gave an informed consent at the beginning of the study.

Source of information on immunization status

The mothers were interviewed using the WHO cluster form with closed end questionnairea for tetanus toxoid immunization. The questionnaire was pre-tested and contained information on immunization status, dates of receiving TT antigen, number of doses received and sources of vaccine. Recall history and immunization card review were used as tools of assessment. The mothers were also asked to give reasons for failing to be immunized with TT or for not completing the immunization. Missed opportunity was defined as inability to receive vaccination when vaccine is available, such as being ill at the time of vaccination or refusal to come to vaccination centre when mass immunization is going on.

Data analysis

Data were analyzed using COSAS and EPI INFO version 6.04 (Centre for Disease Control and Prevention, Atlanta, GA)

RESULTS

Immunization

The WHO recommendation stipulates that a woman of reproductive age is required to receive 5 doses of tetanus toxoid in her life time (6). In this study, using the card/history criterion, 87 (44.4%) of women were not immunized (Table 1).

Table 1: Tetanus toxoid immunization coverage

TT immunization status	No of women (n = 196)	%
TT dose 1	109	55.6
TT dose 2	80	40.8
TT dose 3	22	11.2
TT dose 4	10	5.1
TT dose 5	7	3.6
No vaccine given	87	44.4
TT missed opportunity	33	16.8
Drop out TT1-TT2	29 of 109	26.6
Drop out TT1-TT3	87 of 109	79.8

No studied = 196, Information by card + history

Using only card criterion, 20 (10.2%) women received one dose of TT and 14 (7.1%) received two doses (Table 2).

Drop out rates

The record of response and compliance of the women to TT immunization showed a drop out rate of 26.6% when estimating the population that received TT1 and TT2. This drop out rate became 79.8% when the percentage of women who received TT1 to TT3 doses was analyzed (Table 1).

Missed opportunities

A total of 33 (16.8%) of the women studied missed the opportunity of receiving TT immunization (Table 1). This was based on the information from recall history (7) and immunization card review.

Status of protection from TT immunization

The analysis of TT immunization showed that 14 (7.1%) of women studied were protected against tetanus. This analysis was based on valid immunization as shown in the record card (Table 2). Babies born during the period of protection of the mothers were assumed to be protected against tetanus. If any mother received immunization 1-2 weeks before delivery, the baby is regarded as being unprotected. By this analysis, only 4 (2%) were protected against tetanus in the study (Table 2).

Table 2: Tetanus toxoid immunization coverage by card only

TT immunization status	No of women (n = 196)	%
TT dose 1	20	10.2
TT dose 2	14	7.1
TT dose 3	0	0
TT dose 4	0	0
TT dose 5	0	0
Women protected from tetanus	14	7.1
Babies protected from tetanus	4	2
No vaccine given	176	89.8

No studied = 196

Reasons for immunization failure

The reasons adduced for failure to receive immunization included lack of information which accounted for 65.8%, lack of motivation 20.4% and 13.78% for other reasons peculiar to the community. These included non commitment of the health workers, which is attributed to fear of drowning in the river, and refusal of mothers living on water to come for immunization on the land area of the village. Also, the struggle to fend for the families engaged the time of the mothers who did not remember to get vaccinated.

DISCUSSION

Immunization of women of reproductive age especially pregnant women is very important in preventing neonatal tetanus. For this to be effective, the coverage must be large and compliance with vaccine regimen must be encouraged. A contrast to expectation was observed in the results of this study. A low TT immunization coverage was observed with as many as 44.4% of mothers not immunized when the history/card criterion was used. This is high and shows that there is poor knowledge of immunization and its benefits among women in this area. The pattern of compliance among those who were vaccinated also showed that there was high rate of dropout. These observations no doubt indicate that newborns cannot be protected with this pattern of immunization (1, 8, 9).

Realizing that immunization card review is more valid than recall history, only card documentation of TT immunization was analyzed and it revealed that 14 (7.7%) of the 196 mothers were confirmed to have received 2 doses of TT. This analysis also showed that only 4 of the 196 babies delivered by the mothers studied were protected against tetanus. This value is very low indicating that it will take some time before occurrence of neonatal tetanus can be reduced in this area.

The dropout rate of 79.8% observed indicates poor knowledge of the mothers about immunization. This must have been responsible for the poor attitude of the mothers to TT immunization generally. This has been reported to be one of the main factors associated with high mortality of NT in Northern Nigeria (10). As many as 33 (16.8%) mothers missed the opportunity of getting vaccinated with TT. Long distance to

vaccination centres, poverty of the people with no means of transportation and the poor terrain of the community must have contributed to these missed opportunities (4, 9). In this study, the location of the vaccination centre is on the land part of the village, a site that was rejected by those living on water. Similar observation was made in a study conducted in Bangladesh (11). Compounding the situation was the absence of health facility in the community which made those on water to feel rejected.

Reasons adduced for failure to be immunized by the mothers were lack of information and motivation. The mothers were largely unaware of TT immunization and its regimen. This made many to be partially immunized. The attitude of the health care providers was not encouraging to mothers, as some of them alleged harassment and monetary extortion for syringes used for injection of vaccines, by some of the workers. These were lack of motivations for the mothers to get immunized. The health care workers were equally not well motivated by government and this affected their attitude to work in the village.

CONCLUSION

Poor awareness of TT immunization due to lack of maternal health education coupled with absence of health facilities affected immunization coverage in area studied. Also affecting the coverage was poor commitment of health workers assigned to the village.

Therefore, for TT immunization coverage to increase among women of reproductive age in this village, health care facilities and other infrastructures must be put in place (12). Maternal health education must be established to create awareness of the importance and benefits of TT immunization. This can be provided through counseling and social mobilization. Dhadwal and his groups (13) found this approach to be very useful. Reducing missed opportunities to promote immunization as well as targeted home visitation of women in need of additional immunization could be steps to further improve coverage (10). These measures will undoubtedly reduce the incidence and the mortality and morbidity associated with neonatal tetanus in the area.

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OPPORTUNISTIC INFECTIONS AND CLINICO-EPIDEMIOLOGICAL FACTORS IN HIV/AIDS CASES SEEN IN A TERTIARY CARE HOSPITAL IN NEPAL

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Opportunistic infections are the leading cause of morbidity and mortality among HIV/AIDS patients. The spectrum of opportunistic pathogens involved in such infections in Nepal is not well documented. A cross sectional (hospital-based) study was carried out at the AIDS clinic of Manipal Teaching Hospital, Pokhara, Nepal. A total of 404 clinically suspected cases of HIV/AIDS seen at the clinic between July 2001 and December 2002, were screened for HIV. Seventy four (18.3%) were sero-positive for HIV. Fever was the commonest presenting symptoms 48.6% followed by cough and dyspnoea 36.5%, weight loss 36.5% and pulmonary tuberculosis 21.6%. Fifty five of the 74 (74.3%) HIV positive cases were in the age group 20-39 years. Heterosexual mode of acquisition/transmission was seen in 60.8% and 21.6% were intravenous drug abusers (IVDA). A total of 45 opportunistic pathogenic isolates were recovered from the 74 patients. *Mycobacterium tuberculosis* was the commonest pathogen 60%, followed by *Cryptosporidium spp* 13.3% and *Candida spp* 11.1%. Four patients died during the period of study giving a mortality rate of 5.4%. This study shows that HIV/AIDS is rapidly becoming a grave concern in the Pokhara valley of Nepal. Intensive and effective health education programmes among the target population may be a cost effective method to curb the rising prevalence of HIV/AIDS in a developing country like Nepal. Also, further regional studies are required to establish more detailed epidemiological database of opportunistic infections in HIV/AIDS patients in Nepal.

Keywords: HIV/AIDS, Opportunistic infections, Nepal

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the most significant emerging infectious pathogen of the 20th century (1). HIV infection leading to acquired immunodeficiency syndrome (AIDS) is probably the most crucial issue with regard to its economic, cultural and social impact in the population worldwide. Since its recognition in 1981, it has reached pandemic proportions affecting the whole world (2).

In South-East Asia, about 216, 443 AIDS cases have been reported up to March 2002 and over 90% of them were reported after 1993 (3). Also, the estimated population living with HIV infection up till March 2002 was about 5.3 million (4). In 2000, the prevalence rate of HIV/AIDS infection in South-East Asia was more than 358 per 100,000 populations (5). HIV/AIDS was the leading cause of death in the age range 15-49 years in South-East Asia in 1998 and the number of all STD cases excluding HIV/AIDS was 3, 107, 007 (5). According to the latest reports published in June 2002, there are nearly 3.86 million HIV sero-positive cases and 39,742 full-blown AIDS cases in India (5).

The first reported case in Nepal was in July 1988 (6). A growing trend has been observed in the number of reported HIV/AIDS cases after 1998 (7). According to the National Centre for AIDS and STD Control of Nepal (NCASC), established in 1986, seropositivity among voluntarily tested individuals in 1997 was 3.58% and this figure increased to 6.1% in December 1998 (8). The number of HIV infected persons in Nepal, as of June 1999, was 25,000 with a prevalence of 66 per 100,000 populations (3). The estimated number of cases up to 2002 was about 34, 000 and the expected number of deaths from AIDS in year 2002 is close to 3, 000 and this figure is projected to more than double in 2005 (9).

This exponential growth rate of HIV/AIDS in the recent years in Nepal can

be attributed to increasing number of new HIV/AIDS infection, more awareness of the disease and availability of serological and diagnostic tools for testing (10). Despite observing an increasing trend in the reported HIV/AIDS cases, the spectrum of opportunistic infections in these patients is not well documented. Also, there are no available epidemiological data on the regional distribution of these opportunistic infections in the country.

In an effort to document the clinical and the epidemiological profiles of the disease, a study was undertaken at Manipal Teaching Hospital (MTH), Pokhara, Western Development Region, Nepal to determine the proportion of seropositivity in clinically suspected cases of HIV/AIDS; to evaluate the spectrum of clinical presentations of HIV/AIDS cases; to determine the epidemiological factors responsible for transmission of HIV/AIDS and to identify the commonly occurring opportunistic pathogens associated with HIV infection. This knowledge will serve as guides in the antimicrobial therapy of opportunistic infections in HIV/AIDS patients and in formulating control measures to reduce transmission of HIV in Nepal.

MATERIALS AND METHOD

Study design/area

A hospital based cross-sectional study was conducted in HIV/AIDS clinic at Manipal Teaching Hospital, Pokhara, Western Region, Nepal, during the period July 2001 and December 2002. Manipal Teaching Hospital is a tertiary care center located in Pokhara sub metropolitan with the population of about 1.79 hundred thousand (as per Census 2001).

Subjects

All clinically suspected cases presented with symptoms such as prolonged fever, chronic cough and dyspnoea, chronic diarrhea, progressive weight loss, disseminated tuberculosis, oral thrush, herpes zoster and unexplained lymphadenopathy. All patients attending the in-patient and out-patient departments during this study period and belonging to Pokhara sub metropolitan city were included in the study. A total of 404 clinically suspected cases of HIV/AIDS were screened. Informed consent was taken and pre-test and post-test counseling was routinely done for all the by trained experts.

HIV testing and confirmation

HIV infection was confirmed when at least two of the following tests were positive; HIV 1 and 2 (Tri-Dot ELISA, J. Mitra & Co., Ltd., New Delhi, India), HIV-Spot (Eli scan, Ranbaxy, New Delhi), or HIV 1 and 2 Western blot (Gene Lab Diagnostics, Singapore) as recommended by WHO strategy – II of 1993.

Subject bio-data/Post test counseling

A total of 74 patients were found to be HIV positive. Information on age, sex, and educational status, mode of acquisition/transmission and clinical profiles of the HIV positive patients were obtained using a questionnaire. Based on the National Center for AIDS and STD Control (NCASC) guidelines, all the HIV positive cases were counseled and educated regarding different modes of transmission as this is helpful in controlling the spread infection.

Statistic analysis

The EPI-INFO soft ware package programme was used for the statistical analysis.

RESULTS

Table 1 shows that out of 404 suspected cases, 74 (18.3%) tested positive for HIV, with majority (74.32%) in the age group 20-39 years. Fifty nine (79.7%) of the HIV positive patients were males and 15

(20.3%) were females. The odds ratio calculated for HIV positivity in relation to sex was 3.16 (with CI 1.66 - 6.08) and p < 0.001 showing a strong association between HIV positivity and sex, with male preponderance.

In Table 2, out of 404, 219 (54.2%) cases were illiterate. The proportion of illi-terate among HIV cases was 28.6% and proportion of cases with primary level education was 55.4%. Association between literacy and HIV positivity was found to be significant ($X^2 = 24.93$, $p < 0.001$).

In Table 3, the proportion of sex workers or clients of sex workers in HIV positive cases was found to be 60.8% and the proportion of intravenous drug users (IVDUs) was 21.6%. The proportion of vertical transmission from infected mother to the baby was found to be 4.05%. Association between factors responsible for transmission and HIV positivity was significant ($X^2 = 25.70$, $p < 0.001$). Table 4 shows that the commonest presenting symptom in HIV positive cases was fever (48.6%), followed by weight loss (36.5%), cough and dyspnoea (36.5%), chronic and recurrent diarrhoea 18.2%, and 28.4% and 21.6% for pneumonia and pulmonary respectively. The proportion of patients presenting with tuberculous meningitis (TBM) and disseminated TB was 5.4%. Slim's disease was seen in (5.4%) cases. Out of 74 HIV positive cases, 4 (5.4%) cases died giving a mortality rate of 5.4%.

Table 5 shows the distribution of infectious agents in the different types of clinical specimens obtained from patients in

Table 1 Distribution of HIV Positivity according to age and sex

Age group	HIV positive			HIV negative			Total
	Male	Female	Total (%)	Male	Female	Total	
< 19	03	02	05(6.76)	06	05	11	16(3.96)
20-39	47	08	55(74.32)	72	94	166	221(54.70)
40-59	07	04	11(14.87)	74	30	104	115(28.47)
> 60	02	01	03(4.05)	31	18	49	52(12.87)
Total	**59 (79.73)**	**15 (20.27)**	**74(100) (18.32)**	**183**	**147**	**330 (81.68)**	**404(100) (100)**

$x^2 = 19.52$, $p < 0.001$, Odds Ratio=3.16, Confidence Interval (CI) = 1.66 - 6.08

Table 2: Distribution of HIV Positivity according to literacy status

Literacy status	HIV Test		Total (%)
	Positive	Negative	
Illiterate	21(28.38)	198	219(54.21)
Primary	41(55.41)	96	137(33.91)
Secondary & above	12(16.21)	36	48(11.88)
Total	**74(100)**	**330**	**404(100)**

$x^2 = 24.93$, $p = <0.001$

Table 3: Distribution of the factors influencing modes of transmission of HIV

Factors	HIV Test		Total (%)
	Positive	Negative	
Commercial sex workers or clients of sex workers	45(60.81)	103	148(36.63)
Blood product recipients	1(1.35)	22	23(5.69)
IVDUs	16(21.63)	113	129(31.93)
House wives	9(12.16)	84	93(23.03)
Mother to child	3(4.05)	8	11(2.72)
Total	**74(100)**	**330**	**404(100)**

$x^2 = 25.70$, $p = < 0.001$

the study. A total of 45 microbial isolates were recovered from the patients.

Table 4: Clinical profile of HIV positive patients

Clinical symptoms	HIV Test		Total
	Positive (N=74) (%)	Negative (N=330)	(N=404) (%)
Fever	36(48.65)	189(57.27)	225(55.69)
Weight loss	27(36.49)	107(32.42)	134(33.17)
Cough & dyspnoea	27(36.49)	113(34.24)	140(34.65)
Pneumonia	21(28.38)	97(29.39)	118(29.21)
Pulmonary TB	16(21.62)	49(14.85)	65(16.09)
Skin & mesothelial infection	13(17.57)	27(8.18)	40(9.9)
Recurrent diarrhea	12(16.22)	101(30.61)	113(27.97)
PGL	05(6.76)	1(0.3)	06(1.49)
TBM & Disseminated TB	04(5.41)	1(0.3)	05(1.24)
Extra-pulmonary Tubercular lymph node	07(9.46)	03(0.91)	
Depression	03(4.05)	17(5.15)	20(4.95)
Lymphoma	02(2.7)		
Slims disease	04(5.41)	1(0.3)	05(1.24)
Death	04(5.41)	1(0.3)	05(1.24)

[NB: PGL-persistent generalised lymphadenopathy, TB -tuberculosis, TBM- tubercular meningitis]

Mycobacterium tuberculosis constitute the majority with 60%, followed by *Cryptosporidium spp* 13.3%, *Candida albicans* 11.1%, *Cryptococcus neoformans*

Table 5: Pathogens isolated in HIV infected patients (n = 45)

Organism	Specimen	No of patients (%)
Mycobacterium tuberculosis	Sputum	16 (35.5)
	Lymph node	7 (15.5)
	CSF	4 (8.8)
Cryptosporidium spp	Stool	6 (13.3)
Candida albicans	Oesophageal brush	5 (11.1)
Cryptococcus neoformans	CSF	3 (6.7)
Isospora belli	Stool	1 (2.2)
Pneumocystis carinii	Broncho-alveolar lavage (BAL)	1 (2.2)
Strongloidis stercoralis	Stool	1 (2.2)
Cyclospora spp	Stool	1 (2.2)
		45

6.7% and 2.2% each of *Isospora belli*, *Pneumocystis carinii*, *Strongloides stercoralis* and *Cyclospora spp*.

DISCUSSION

In this study (hospital based cross-sectional study), 18.3% of a total of 404 patients screened for HIV were confirmed positive, with majority in the age group 20-39 years and with male preponderance (79.7%). These findings are consistent with otherstudies in Nepal (11) and elsewhere. The age group 20-39 years is an established high risk group for HIV infection world wide because this is the most sexually active group in any population.

In the study, literacy was significant -ly associated with HIV positivity ($x^2 = 24.93$, $p < 0.001$), with over 50% of those positive being literate above primary school education. This is similar to the finding of Sharma *et al* (12). The more literate a person is, the more sexual adventure he is likely to undertake, with a tendency to keeping multiple sexual partners, which is an identified high risk sexual behaviour.

The main mode of transmission in this study is predominantly heterosexual contact (60.8%) as attested to by a high proportion of sex workers and clients of sex workers testing positive. This agrees with reports of the study by Gurubacharya *et al* (13). One hundred and twenty nine (32.9%) of the 404 patients screened were intravenous drug users (IVDUs), out of which 16 (3.9%) were HIV positive. Published data (14) have indicated that intravenous drug abuse is a problem in many parts of Nepal. About 22% of the HIV positive patients in this study were IVDUs. This definitely calls for urgent health education campaign to these vulnerable youths on the danger of using unsterilized needles or sharing of needles when injecting drugs.

About 4% of cases seen were children who acquired the infection through vertical transmission. HIV infection in children born to symptomatic sero-positive mothers is yet another deplorable situation and remains the submerged stem of iceberg in the vast population of Nepal. This figure tallies with other studies done in other Asian countries (15).

The non-specific symptoms such as fever, weight loss, cough and dyspnoea seen in our patients were not different from those seen in other studies conducted in neighboring countries like India (16). HIV and TB synergy has a devastating impact in the developing world where 95% of the people live with dual menace of tuberculosis and HIV infection (17). The HIV surveillance in patients with tuberculosis in Nepal during 2001 and 2002 reveals a 2.4% prevalence of HIV in TB patients. HIV prevalence in TB patients continues to rise and has increased by 4-fold in the last 6 years. Moreover, 84 % of cases are in men aged above 25 years and in women all cases occurred in the under 25-year age group (18). In this study, pulmonary tuberculosis was found in 16 (21.6%) of HIV positive cases while *Mycobacterium tuberculosis* was isolated from lymph node and CSF as extra-pulmonary tuberculosis in 24.44%. These finding are comparable to similar studies conducted in Thailand (19) as well as in many other developing countries as reported by Raviglione *et al* (20).

Despite better understanding of the pathogenesis and clinical significance of weight loss in Slim's disease, not much has been done to improve the nutritional status of these patients during early stage of the disease. This is attributed to variety of constraints namely parasitic diarrhoea, depression, anorexia (drug or disease induced) and inescapable poverty (21). In the present study, we found 4 (5.4%) HIV positive cases with Slim's disease. This is the same picture in Zaire and Uganda where for the first time in early 1980's, Slim's disease, characterized by profound weight loss and chronic parasitic diarrhea, was recognized and where it was difficult to improve the nutritional status in the early stage for purely economic reasons (22).

The opportunistic pathogens and the infections they caused in our patients in this study have been described for most patients with HIV infection elsewhere in the world, and these infections are more pronounced at the terminal stage of the disease.

CONCLUSION

In this study, we found HIV positivity to be high in the 20-39 year age group, the group that is made up of the most economically productive unit of any society. We also found main mode of transmission to be heterosexual. Therefore, health education emphasizing practice of safe sex, avoidance of multiple sexual

partners, premarital and extramarital sex would go a long way in preventing the spread of HIV infection in the community. This can be achieved through mass media, use of video films, charts and posters.

To prevent mother to child transmission, it is necessary to educate mothers and adolescent females regarding benefits of antenatal care and making HIV screening mandatory in antenatal clinics, so that sero-positive mothers can have a choice of keeping or terminating the pregnancy.

The present study documents that tuberculosis, cryptosporidiosis and candidiasis are the most predominant opportunistic infections in HIV infected patients in the Pokhara valley (Western Development Region) of Nepal. To the best of our knowledge, it is the first study of its kind in the region highlighting the variety of opportunistic infections in HIV patients.

Most of the studies on HIV carried out in Nepal are mainly hospital based due to limited resources. These do not give accurate information of the incidence and distribution of opportunistic infections by geographical or regional location. It is therefore necessary to conduct community-based studies, especially in inaccessible rural areas of Nepal where the situation is still not clear. It is also necessary to organize quarterly or half-yearly health campaign in the remote rural areas and encourage voluntary screening.

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ORIGINAL ARTICLE

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AIDS KNOWLEDGE, ATTITUDE AND BEHAVIOURAL PATTERN AMONG HIGH SCHOOL STUDENTS IN SOUTHWESTERN NIGERIA

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An evaluation of knowledge, attitude and behavioural patterns of high school students in Oyo and Osun States, Southwestern Nigeria, towards HIV/AIDS was undertaken. A structured questionnaire was administered to respondents from six secondary schools that were selected by systematic random sampling method from the two States. The questionnaire focused on specific aspects of knowledge, attitude and behavioural patterns related to HIV/AIDS, its mode of transmission and preventive measures. Results from the study showed that 362 (73%) of the 496 respondents had correct knowledge of the causative agent of AIDS, 69% had correct knowledge of the mode of transmission, 83.2% had correct knowledge of the people at risk and 80.2% had good knowledge of methods of prevention. Attitude toward AIDS victims was however relatively poor with 21% believing that AIDS patients should be isolated and avoided and additional 7% believing that people should not eat or share utensils with AIDS patients. Only 57.7% believed that people should relate freely with AIDS patients. This study showed an improvement in the knowledge and attitude towards HIV/AIDS over a previous one carried out over a decade ago in the same locality, highlighting the importance of mass media campaign programme embarked upon by the States over the years. However, there is need to further increase the awareness campaign especially as it relates to attitude towards AIDS patients and also on information dissemination, which should be more detailed and formal. Incorporating sex education into the curriculum of secondary schools will be a welcome development in stemming the tide of this dreaded disease.

Keywords: Knowledge, attitude, sexual behaviour, HIV, AIDS.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is a disease that affects primarily young adults (1-2). The scourge of HIV/AIDS epidemic has continued worldwide but the impact is most felt in developing countries especially Africa. The Joint United Nation Programme on HIV/AIDS estimates of December 2001 shows that globally, about 36.1 million people are living with HIV/AIDS (3). By 2002, the number had increased to 40 million, with new infection occurring at a rate of 16,000 per day (4) and by 2003, the total estimate was put at 60 million people infected, with about 20 million dead from AIDS related illnesses (5).

In Nigeria, the epidemic has continued unabated and presently health authorities establish that about 4 million adults and 800,000 children may be infected with the virus. In Oyo State, Southwestern Nigeria, the national sentinel survey put the level of infection at 4% in 2001, which increased to 10% in 2003, with most of those infected in the 15-24 years age group (6).

Adolescents and young adults are recognized as a group of concern in the continued spread of HIV/AIDS, not only for their unprotected sexual habits but for their propensity to have multiple sexual partners (7, 8), which are high risk behaviours. This group therefore represents a major target for AIDS prevention education. Although, HIV/AIDS awareness campaign at both national and state levels in Nigeria have been on for some years, they have not been found to sufficiently change the behavioural pattern and attitude of these youths who are at risk of contacting this infection (7-11). Health education therefore needs to be intensified among these youths. This approach has been applied in developing countries to reduce the transmission among homosexuals, intravenous drug users and prostitutes (12).

A study of high school students carried out in Ibadan, Oyo State, Southwestern Nigeria in 1988 revealed that

70% were unaware of HIV/AIDS and 90% did not know that it can be spread by sexual intercourse (13). Sixteen years after, we assess the knowledge, attitude and behavioral pattern of secondary school students toward HIV/AIDS in the same geographical zone, in order to determine to what level the awareness campaign have reached and identify areas that needs to be improved upon.

MATERIALS AND METHOD

Study area

The study was conducted in Oyo and Osun States, Southwestern Nigeria in May 2004. Osun State was created out of the old Oyo State in 1992. The States occupy latitude 7°48N and longitude 4°37E. The estimated population by the 1991 census in the country put the figure for the two States at 5.7million. There are about 630 secondary schools (public and private) in the two States.

Study population/selection

The study population consisted senior secondary school students (SSI, SSII and SSIII) from 6 schools in the two states selected by systematic random sampling technique. The schools included those run by the government (public) and private individuals. The estimated sample size determination was done using the sentinel sero-prevalence rate of 10% for each of the states and twice the number of questionnaires distributed in case of non-response and improper filling of the forms.

The students were randomly selected and the forms were filled during school hours by each student independently. Students who objected to filling the forms in the selected classes were excluded. In all, a total of 500 questionnaires were administered. We sought the co-operation and help of the class teachers and headmasters of the schools in selecting the students and administering the questionnaires.

Method

The questionnaires on HIV/AIDS were self-administered to respondents after a pre-test. The questionnaires focused on specific aspects of knowledge of HIV/AIDS, its mode of transmission and prevention, attitude towards AIDS patients and sexual behaviour of students in spite of the knowledge.

Demographic data of the students were also obtained and these include name, age, sex, religion, and type of schools. Other variables include weekly income of students, level of parents' education, willingness to undergo HIV testing and source of information about AIDS.

Statistical analysis

Analysis was done on IBM computer with EPI INFO version 5.0 software (14). Test of significance was done using Chi square and p value of < 0.05 was taken as significant value.

RESULTS

Demographic characteristics

Four of the questionnaires were not properly filled and were therefore excluded from analysis. The final sample available for analysis was 496 questionnaires. This represents a total response rate of 99.2%. Two hundred (40.3%) students were from secondary schools in Oyo State while 296 (59.7%) were from secondary schools in Osun state. Three hundred and forty-six (69.8%) students were from public schools while 150 (30.2%) were from private schools. The mean age of the students was 17.6 years. The male to female ratio was 0.9: 1. Most (71.8%) of the students were Christians and a few (0.8%) were married.

Table 1: Demographic characteristics of respondents

Data	Public school	Private school	Total (%)
Age: < 15years	72	62	134 (27)
> 15years	274	88	362 (73)
Sex: Male	165	63	228 (46)
Female	181	87	268 (54)
Religion: Christian	249	107	356 (71.8)
Muslim	97	40	137 (27.6)
Others	–	3	3 (0.6)
Class: SSS I	37	63	100 (20.2)
SSS II	259	76	335 (67)
SSS III	50	11	61 (12.3)
Weekly: <N100	134	56	190 (38.3)
Spending < N500	128	62	190 (38.3)
> N500	84	32	116 (23.4)
Marital: Married	3	1	4 (0.8)
Status Single	343	149	492 (99 .2)

Table 2: Knowledge on causes, person at risk, transmission and prevention of HIVAIDS

a.) Causes	Overall%	Public school N (%) N=346	Private school N (%) N=150	X^2 value	P value
Virus	73.0	229 (66.2)	133 (88.7)	26.75	p < 0.005
Malnutrition	9.3	36 (10.4)	10 (6.7)	1.71	0.05 < p< 0.975
"Juju"	2.6	8 (2.3)	5 (3.3)	0.44	0.05 < p< 0.975
No idea	15.1	73 (21.1)	2 (1.3)	31.87	p < 0.005
b.) Persons at risk					
Prostitutes	58.3	205(59.2)	84(56.0)	0.44	0.05 < p< 0.975
Hemophiliacs	21.8	86(24.9)	22(14.7)	6.41	0.01 < p< 0.025
Blood Transfusion	49.6	192(55.5)	54(36.0)	15.89	p < 0.005
Homosexuals	8.1	27(7.8)	13(8.7)	0.1	0.05 < p< 0.975
Promiscuous individuals	30.2	118(34.1)	32(21.3)	8.11	p < 0.005
No idea	17.7	74(21.4)	14(9.3)	10.37	p < 0.005
c.) Routes of transmission					
Sexual intercourse	59.3	191(55.2)	103(68.7)	7.88	p = 0.005
Unsterilized needles/objects	37.7	156(45.1)	31(20.7)	26.63	p < 0.005
Blood transfusion	50.4	175(50.6)	75(50.0)	0.0000	p > 0.995
Kissing	20.0	75(21.7)	24(16.0)	2.05	0.05 < p< 0.975
Hugging	0.4	2(0.6)	0(0)		
Handshaking	0.2	1(0.3)	0(0)		
Mosquito bite	4.8	21(6.1)	3(2.0)	3.8	p = 0.05
No idea	5.6	27(7.8)	1(0.7)	10.05	p < 0.005
d.) Preventive measures					
Condom use	45.8	174(50.3)	53(35.3)	9.34	p < 0.005
Avoid contact with victims	19.8	79(22.8)	19(12.7)	6.76	0.005 < p<0.01
Avoid transfusion of unscreened blood	31.9	117(33.8)	41(27.3)	2.01	0.05 < p< 0.975
Have just one partner	31.0	111(32.1)	43(28.7)	0.56	0.05 < p< 0.975

Knowledge about the cause of AIDS

The term HIV/AIDS was familiar to 99.6% of the students. Table 2 shows the students knowledge of the cause of AIDS. The most common response was that a virus was responsible in 66.2% of the students from public schools and 88.7% of students from private schools. Only 15.1% of the students had no idea of the cause of AIDS, and this is worse among the students from public

schools ($X^2 = 31.87$, $p < 0.005$). Their source of information included newspaper (29.6%), radio (35.9%), hand bills (31.3%), friends (19.8%), parents (33.5%), teachers (33.5%) and television (60.5%) (Table 3)

Table 3: Source of information about HIV/AIDS

Sources	Number	Percentage
Television	300	60.5
Radio	178	35.9
Newspaper	147	29.6
Textbook	37	7.5
Distributed handbills	155	31.3
Parental communication	118	23.8
Friends	98	19.8
School teacher	166	33.5

Knowledge of people at risk/ mode of spread

As regards the people at risk of being infected, 58.3% of the students believed that prostitutes are most likely to be infected, followed by those who receive blood transfusion (49.6%), and those with multiple sexual partners (30.2%).

On the mode of HIV/AIDS transmission, 59.3% believed that it can be transmitted by sexual intercourse (68.7% of the students from the private schools and 55.2% of students from the public schools). Other means of transmission mentioned include blood transfusion in 50.4% of students, and use of unsterilized needles in 37.7%. Twenty percent of the students believed that the virus can be transmitted through kissing while 5.6% of the students had no idea of how the virus can be transmitted (Table 2).

Knowledge of preventive measures

In response to the question on how HIV/AIDS can be prevented, 45.8% of the students believed condom can be used, 31% believed that having just one partner, while 31.9% believed avoiding transfusion of unscreened blood are preventive measures. Only 19.8% are of the opinion that prevention is by avoiding contact with AIDS victims. (Table 2)

Attitude towards AIDS patients

Twenty one percent of the students said AIDS victims should be avoided and isolated and 7% believed people should not eat or share utensils with AIDS patients. However, 57.7% are of the opinion that people should relate freely with AIDS patients (Table 4).

Table 4: Attitude towards HIV/AIDS patients

Attitude	Number	%
Patient should be isolated and avoided	104	21
Never eat with AIDS victim	35	7.1
Relate freely with AIDS victim	286	57.7
Take victim to doctor	446	89.9
Take victim to herbalist	24	4.8
Take victim to church	5	1

Sexual behaviour/HIV screening

Concerning HIV screening, 40% felt they could not have AIDS and are therefore willing to be freely screened for HIV while 59% are afraid to have the screening exercise (Table 5).

In response to sexual behaviour, 14.3% admitted to having sexual partners, 10.3% admitted to have had sexual intercourse in the past, and 66% of those who have had sexual intercourse in the past before used condom.

Table 5: Sexual behaviour of students

Sexual behaviour	Public school (%)	Private school (%)	Overall %
Have sex partners	65(18.8)	6(4.0)	14.3
Have no sex partner	281(81.2)	144(96.0)	85.7
Have had sexual intercourse before	40(11.6)	11(7.3)	10.3
Have used condom during sexual intercourse	33(9.5)	1(0.7)	6.8
Volunteer for HIV screening	141(40.8)	58(38.7)	40.1

DISCUSSION

Over the years, a lot of awareness campaign and propaganda on HIV/AIDS have been carried out at both national and state levels in Nigeria through the mass media especially radio and television. In this study therefore, majority of the students had good

knowledge of the deadly scourge better than it was in 1988 when over 70% of high school students were unaware of AIDS and 90% did not know that the infection can be spread through sexual intercourse (13). Although the knowledge of the causative agent was high in this study, many (62.3%) still did not know that the virus can be transmitted by using unsterilized needles and sharp objects, while about 25.4% believed that the virus can be transmitted by kissing, hugging, handshaking and by mosquito bite, and about 5.6% had no idea of mode of transmission. These erroneous beliefs, premised on inadequate or improper information, can adversely affect societal attitude towards AIDS patients and further aggravates stigmization and discrimination against these patients, as can be deduced from the response of some of the students. These are areas the awareness campaign must focus on.

The students also demonstrated poor understanding of the preventive measures, as less than half knew the use of condoms as a protective measure. A similar finding was reported in a Tanzania study (15) where only 39% of the study population knew condom use as a preventive measure. This finding is also an indication of inadequate or improper information dissemination about HIV/AIDS transmission, prevention and control.

Students from private schools were better informed about HIV/AIDS than those from public schools ($X^2 = 26.75$, $p < 0.005$). This may be a reflection of the fact that, students from private schools are likely to be from parents in middle to high socioeconomic class and with good educational background and therefore have more access to information from their parents, teachers, mass media and other means.

With regard to sexual behaviour, 14.3% of the students, in spite of their AIDS knowledge, agreed to having sexual partners, 64.8% of whom have had at least one sexual experience and 71.9% of these had used condom during intercourse. This finding is similar to those from Ghana (16) and South Africa (17). It highlights the sexual networking going on among the youths and shows that sexually experienced students are at risk of exposure to HIV and other sexually transmitted infections

Most of the students in this study got their information through the mass media indicating that, to a large extent, the mass media campaign has been of tremendous benefit in sensitizing the populace. However, information needs to be more accurate and detailed. Only 23.8% of the students were informed by their parents, which implied that parents do not actively communicate with their children about adolescent sexuality and sexually transmitted diseases. In our society, there are traditional norms that inhibit discussions of sexual issues with parents, particularly among the inquisitive female youngsters. Many studies (16, 18-20) have shown a strong association between family communication about HIV/AIDS, sexual behaviour and condom use. Therefore, family communication about sexual issues with their children needs to be encouraged in our society.

The prospect for HIV/AIDS control largely depends on recognizing the scale of threat and implementing policies to counter it. It is not enough to increase the awareness of high school students about HIV/AIDS but also to impact accurate knowledge on the modes of transmission and methods of prevention in other to successfully control the epidemic among the adolescents. This may mean changing the overall approach to the dissemination of HIV/AIDS information and making it more detailed instead of being haphazard and informal.

Emphasis should also be placed on promoting moral attitude of the youths, encouraging abstinence and avoiding premarital sexual intercourse. In achieving a successful AIDS education programme in this group, there will be a need to review the curriculum of secondary schools to include adolescent sexuality, sex education and sexually transmitted diseases. This school based intervention programmes must involve parents, teachers and the Ministries of Health, Education, and Social welfare in creating supportive environment to strengthen the school effort.

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CASE REPORT

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LEISHMANIASIS PRESENTING AS SEVERE ANAEMIA IN AN ADULT FEMALE NIGERIAN

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Visceral leishmaniasis is a rare cause of anaemia. We report a case of visceral leishmaniasis presenting as severe anaemia and pyrexia of unknown origin in an adult female Nigerian. The objective was to highlight the importance of exhaustive investigations in the diagnosis of anaemia and pyrexia of unknown origin in our environment.

Keywords: visceral leishmaniasis; chronic anaemia; pyrexia of unknown origin; Nigeria.

INTRODUCTION

Leishmaniasis is a zoonotic infection caused by the protozoa belonging to the genus Leishmania. It is an intracellular parasite that infects the reticulo-endothelial system (RES) and which is manifested by gradual onset, spectrum of disease such as localized ulcers or wildly disseminated progressive lesion of the skin, mucus membrane or the entire RES (1). The reservoir of infection in African leishmaniasis includes the rodents and domestic animals such as dogs; and is transmitted to man through the bite of sandflies (*Phlebotomus* and *Lutzomyia species*).

Although leishmaniasis is not a common disease in Nigeria, exhaustive investigations in the diagnosis of anaemia and pyrexia of unknown origin in our environment may be rewarding as in this case report.

Case Report

Miss A. A, a 20-year-old nursing student, presented with a three weeks history of fever, progressive body weakness and headache. Fever was high grade, intermittent and associated with chills and rigors. Four days before presenting in the hospital, she developed abdominal pain that was associated with vomiting and passage of non-bloody, non-mucoid watery stool. She also gave a history of watery, non-mucoid and non-foul smelling vaginal discharge; but denied any previous sexual exposure. She used several anti-malaria drugs without any sustained remission in fever.

Physical examination revealed an acutely ill-looking, febrile, and markedly pale but anicteric young lady. There was no significant peripheral lymphadenopathy or oedema. There was tenderness in the right iliac fossa but rebound tenderness was not elicited. Spleen was enlarged 6cm below the left costal margin; there was no hepatomegaly.

Serial full blood count showed haematocrit of between 12 and 26% (median = 22%), platelet count slightly reduced at a range of 55 – 131 x 10^9/L (median = 79 x 10^9/L) and essentially normal white cell count (with differentials) of 1.9 – 4.3 x 10^9/L (median = 3.1 x 10^9/L). The reticulocyte counts were consistently < 1%. Bone marrow aspiration

showed erythroid hyperplasia and negative iron store. Serum biochemical parameters showed hypoproteinaemia of 59g/L with reversal of albumin/globulin ratio.

Repeated septic work-up yielded no growth and stool was free of ova and parasites. Screening for human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B surface antigen (HBsAg) were negative. Abdominal ultra-sonography did not reveal any pathology either intra-abdominally or in the enlarged spleen.

Fever remained high despite anti-malarial, antibiotics, anti-fungal and anti-viral agents. She had 7 units of packed red cells while on admission. The spleen increased in size to 14cm during admission and the liver also became palpable at 7cm. Liver biopsy undertaken a month into admission confirmed the presence of Leishmania-Donovan (LD) bodies in the Kupffer cells (Fig 1). A diagnosis of leishmaniasis was made.

Fig1: Liver histology [x640] showing enlarged Kupffer cell

DISCUSSION

Reports on leishmaniasis in Nigerians are very scanty. The few reported cases presented with cutaneous lesions (2, 3), and only one case presented with visceral type (4) as seen in this patient. In a review of dermatological lesions seen over a period of nine years in a tertiary hospital in Nigeria, leishmaniasis accounts for about 0.1% of all cases (2). Similarly, in a serological investigation carried out in two hospitals in Nigeria, antibody to leishmania antigen was detected in only 9.5% of cases compared to 100% in malaria (5).

Anaemia featured prominently in this case, as she was blood transfusion dependent. The patient had a total of 7 units of packed red cells during her 24 weeks of hospitalization. Factors such as splenic sequestration (6, 7, 8), ineffective erythropoiesis (7), hypersplenism and haemodilution (6); and immune-mediated red blood cell destruction (9, 10) have been linked with the pathogenesis of anaemia in visceral leishmaniasis. This patient presented with massive splenomegaly and hepatomegaly, both of which may sequester and/or destroy significant red cells. The persistent reticulocytopaenia of < 1%, depleted iron store and hypoproteinaemia also suggest hypoproliferative anaemia in this patient. The presence of all these factors in this patient suggests that the mechanism of anaemia in visceral leishmaniasis could be multi-factorial.

The recurrent high fever seen in this patient could not be attributable to neutropaenia as reported in other cases (7), as the patient had normal white cell count including the differentials. However the T cell response depression and the disturbances in cytokine networks associated with viscera leishmaniasis (11) could cause several opportunistic infections that may be responsible for the recurrent episodes of fever recorded in the patient.

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CASE REPORT

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CRYPTOCOCCAL MENINGITIS IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION: REPORT OF THREE CASES

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Although Cryptococcus is an infrequent pathogen in patients without AIDS, it is known to be a major cause of meningitis in those with the disease. In recent times, the incidence of cryptococcal meningitis in patients infected with HIV has increased worldwide mainly because of the increased awareness by both the physicians and clinical microbiologists. We report here three cases of cryptococcal meningitis in HIV patients treated at the Port-of Spain General Hospital in one year. The clinical manifestations in these patients included prolonged and severe headache, neck stiffness and blurring of vision. The patients were treated with amphotericin B. Two patients died a few days after receiving the drug while one patient survived. We suggest that any HIV patient with prolonged headache should be promptly investigated for cryptococcal meningitis

INTRODUCTION

Cryptococcus is one of the most common community acquired opportunistic fungal agents causing serious infections (1). Cryptococcal disease is the most frequent life threatening and third most common fungal infections in patients with AIDS (1, 2). The incidence of cryptococcal infection in AIDS ranges from between 6% to 10% in the United States and 15% to 30% in sub-Sahara Africa (2, 3). *Cryptococcus neoformans* var *neoformans* is the most frequent species affecting the central nervous system and meningitis is most commonly observed (1, 2, 4-7).

The clinical manifestations of cryptococcosis have been extensively reviewed both in patients with or without AIDS, and there is practically no difference in presentation (8). Amphotericin B, with or without 5-fluorocytosine, remains the drug of choice (1, 4). The effective dose and duration of treatment with these drugs has been the subject of controversy and some authors have recommended lifelong therapy (9, 10).

We present the clinical features and course of cryptococcal meningitis in three HIV infected patients diagnosed in the course of one year at the Port.-of-Spain General Hospital, Trinidad and Tobago, West Indies.

CASE REPORTS

Case 1 (R.S., POSGH No. 624006)

A 22 year old male of African descent presented with headache of two months duration with progressive worsening during the two weeks to admission. This was associated with intermittent fever, vomiting and weight loss after receiving treatment from his doctor. On the day of admission, he had suffered a brief period of loss of consciousness, which prompted him to seek medical attention. History of HIV seropositivity was also obtained and confirmed at the sexually transmitted diseases clinic.

Examination revealed febrile patient with generalized body wasting. He had neck stiffness and positive Kernig's sign. There were

no lateralizing signs. Haemoglobin was 102 gm/L, WBC 0.9 X 10^9/L, platelet 89 X 10^9/L, Basal Urea Nitrogen (BUN) 4.67 mg/L, creatinine 109.09 µmol/L, sodium 132 mmol/L and potassium 3.7 mmol/L. Lumbar puncture yielded clear and colourless CSF with no cells, protein level of 4 mg/L and glucose concentration of 2.11 mmol/L (simultaneous blood glucose level was 6.22 mmol/L). An India ink preparation was positive for Cryptococcus.

Despite treatment with combined intravenous amphotericin B and oral fluconazole, he deteriorated progressively and died 6 days after admission.

Case 2 (L.F., POSGH No. 13672)

A 32 year old male of African descent who received treatment from a health center was admitted with weight loss, progressive worsening headache, intermittent vomiting and blurring of vision of two months duration.

On admission, he was wasted and febrile, with oral candidiasis, axillary lymphadenopathy and mild neck stiffness. Ophthalmoscopy showed slightly blurred disc margins and mild retinal venous distension. No localizing signs were evident on neurological examination. Haemoglobin was 93 gm/L, WBC 5.4 X 10^9/L and platelets 155 X 10^9/L. BUN was 2.5 mg/L, creatinine 90.91µmol/L, sodium 131 mmol/L and potassium 5.1 mmol/L.

Lumbar puncture yielded turbid CSF containing no white cell but numerous yeast cells. CSF protein was 0.306 mmol/L and CSF glucose was 0.167 mmol/L (simultaneous blood glucose was 7.67 mmol/L). CSF India ink stain was positive and cryptococcal antigen titre was reported as > 1/1256. On culture, *Cryptococcus neoformans* was isolated. Serological testing for HIV antibodies was positive by ELISA and Western blot method.

Patient was treated with intravenous amphotericin B (660 mg over 13 days) followed by oral fluconazole 400 mg on day 15 and 200mg daily thereafter. There was slow improvement during the period of hospitalization and he was discharged after 17 days on admission. A follow up appointment at the STD clinic was continued with further supply of oral fluconazole.

Case 3 (D. K., POSGH No. 987902

A 27 year old man of East Indian descent, treated by a general practitioner was referred to Port-of-Spain General Hospital because of persistent night sweats, cough, weight loss, fever and severe headache of three months duration.

Physical examination revealed a dehydrated, wasted male patient with fever (temperature 38.2°C), generalized lymphadenopathy, oral candidiasis and mild neck stiffness. Haemoglobin was 89 gm/L, WBC 3.4 X 109/L, BUN 9.17 mg/L, creatinine 118.18 µmol/L, sodium 125 mmol/L and potassium 4.1 mmol/L. The CSF was cloudy and contained numerous yeast cells but no white cells, protein concentration was 60 mg/L and glucose level was 2.78 mmol/L (simultaneous blood glucose was 5.22 mmol/L). CSF India ink stain was positive and culture yielded Cryptococcus. Cryptococcal antigen titre in the CSF was 1/256. A positive HIV test on his blood was confirmed by the Public Health Laboratory.

Treatment with intravenous amphotericin B daily was begun but the patient deteriorated rapidly and died six days after admission.

DISCUSSION

Cryptococcus neoformans is an opportunistic fungus that causes life threatening infections in human but especially

in immuno-compromised patients (8, 11). The infection is the most commonly diagnosed fungal infection of the CNS with meningitis being the most common manifestation (12). It ranked third among infectious agents causing neurological disease in AIDS patients (1, 7). Cryptococcal disease is common in patients with HIV (4) and in the United States of America and the countries of the sub-Saharan Africa, the incidence ranges from between 6-10% and 15-30% respectively (2, 3).

In the three cases presented, which were seen in the year 1996 at the Port-of-Spain General Hospital, several features associated with poor prognosis in cryptococcal meningitis as previously reported by Diamond and Bennet (14), were seen. These include severe leucopaenia, demonstration of the organism on India ink stain, high titre of cryptococcal antigen in the CSF, hyponatraemia and prior treatment with corticosteroid.

High mortality has also been reported in patients with cryptococcal meningitis who were diagnosed very late (8, 15), and the high mortality in our series was therefore not unexpected as they presented after 8-12 weeks of symptoms. Also, as cryptococcosis is an insidious and slow infection, asymptomatic infection could occur and the actual period of the infection before treatment might have been longer (16). The prognostic factors associated with high mortality may also be obscure in some cases (9) as was the case in the one patient who survived in our series.

As previously demonstrated by others (17, 18), our cases showed that cryptococcal infection in AIDS patients is associated with high mortality even when treated with amphotericin B with or without flucytosine, the drugs of choice (19). The effective dose and duration of treatment with these drugs has been subject of much controversy and some authors have advocated life-long therapy (9, 10).

The only patient who survived in our series received several antimicrobial agents from private doctors before reporting to the hospital. He was transfused two units of whole blood after receiving amphotericin B for 6 days and was found to improve clinically with the haemoglobin rising to 6 mg/dl. The antifungal drugs and fresh whole blood received could have helped to reduce the severity of infection and also improve the immune system in this patient as Liss and Rimland (20) have reported that both neutrophil and cell mediated immunity are important host defenses for this infection in AIDS (16).

From the cases reported, we suggest that sexually active individuals with fever and prolonged headache, especially those who are immuno-compromised or at high risk of contacting HIV infection, be promptly investigated for meningitis, as early diagnosis still offers the best chance for successful treatment.

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