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SODIUM DODECYL SULPHATE POLYACRILAMIDE GEL ELECTROPHORESIS OF CAMPYLOBACTER COLI

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***Campylobacter coli* were characterized using sodium dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE). The isolates were obtained from the faeces of diarrhoeic children with the age range of 0 t 36 months attending paediatric clinic at the Lagos University Teaching Hospital (LUTH) and Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. The 16 isolates studied were characterized into seven protein profiles based on their outer membrane proteins (OMPs). The flagella antigens of C. coli had the molecular weights of 24kDa and 84 kDa.**

The SDS-PAGE proves a reliable and rapid technique for typing strains from sporadic cases.

INTRODUCTION

Compylobacter jejuni and C. coli are one of the major causes of diarrhoea in the human populace (1) However, the organism is poised with a variety of problems concerning speciation as a result of its expanding allelic variation. Campylobacter jejuni and C. coli are a major cause of diarrhoea (personal communication). There are a variety of typing techniques for the genus Campylobacter, SDS-PAGE analysis of whole cell protein is one of the several approaches taken for identification of campylobacters (2) SDS-PAGE was first introduced by Pharmacia in Australia to find out usefulness of protein banding profile in the speciation of the genus Campylobacter. Pharmacia examined 14 reference strains of Campylobacter species and 50 test strain including 30 strains of hippurate negative Campylobacter. The electrophoretic patterns correlated well with existing biochemical tests and with available DNA homology data. In general, each species possessed unique an reproducible protein bands that are distinct for strains of that species (3) the aim of this study is to speciate our local strains of Campylobacter coli using the SDS-PAGE. This technique has not been done with our Nigeria strains.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains were obtained from Campylobacter Research Laboratory at the Lagos University Teaching Hospital (LUTH).

Preparation of sample protein

Twenty-four hour colonies were scraped into a homogeniser containing 100µ1 of sample buffer and homogenized for 5 min. The homogenate was transferred to a clean tube and 10µ1 of Tween 20 was added to solubilise the proteins. The pellet was obtained by centrifugation at 5,000 rpm for 10 min at 4 °C and the protein precipitated with cold enthanol. The pellets were then redissolved in sample reducing buffer and heated for 5 min at 100 °C. The standard protein markers were the treated the same way prior to loading on the gel.

Preparation of 16% and 4% resolving and stacking gels

16% gel was prepared by mixing 13.5ml of 30% acrylamide solution, 250µL of 10% SDS, 6.24 ml of 3M Tris-HC1, 5.13 ml of distilled water 12.5µL of undiluted TEMED and 150µL of ammonium persulphate. 4% was prepared by mixing 1.33ml of 30% acrylamide, 100µL of 10% SDS, 2.5 µL of 0.5M Tris-HC1 at pH 6.8, 6ml of distilled water, 5µL of undiluted TEMED and 100µL of ammonium persulphate.

Loading of samples and electrophorsis

Samples and standards were loaded and run by the use of Laemmli's method at 50V through the stacking gel and then 100V through the resolving gel. The gel was stopped when the tracking dye was close to the end of the gel. The stacking gel was cut off while the resolving gel was stained with 0.2% of coomassie brilliant blue solution for 2h. The gel was destained overnight in 7% acetic acid in 10% methanol and the photographed.

RESULTS

The sixteen strains of C. coli were characterized into seven protein profiles. The first group were made up of 6 (50%) of the stains. They are IF 33; IF 79, IF 34, LA 29, LA 12 and LA 1. This group had high outer membrane protein bands (OMP) with a molecular weight of 116 kDa. The second group was made up of 2 (16%) strains having both high and low OMP with bands of 14, 18 and 116 kDa. They are LA 14 and IF 4. The third group has only one strain (IF 27). This group is made up of 4 main OMP bands of 14, 18, 24 and 116 kDa respectively. The fourth group also has only one strain (IF 32), characterized by the presence of low OMP bands of 14, and 18kDa. Group 5 has only one strain (IF 3) characterized by the presence of high and low OMP bands of 116 and 22kDa. Group 6 comprising one strain (LA 4) was characterized by the presence of 3 low and high OMP bands of 18, 84 and 116kDa. The last group, also made up of one strain (IF 28) was characterized by the presence of high and low OMP bands of 18, 23 and 116kDa (Table 1). Group 3 to 7 constituted 8.3% of the isolates.

Corresponding Author

Group No.	No of Isolates	Sizes (kDa)
1.	6	116
2.	2	14,18,116
3.	1	14,18,24,116
4.	1	14,18
5.	1	22,116
6.	1	18,84,116
7.	1	18,23,116

Table 1: Table showing the groupings of 13 C coli isolates according to SDS-PAGE profiles.

Diagram: Picture

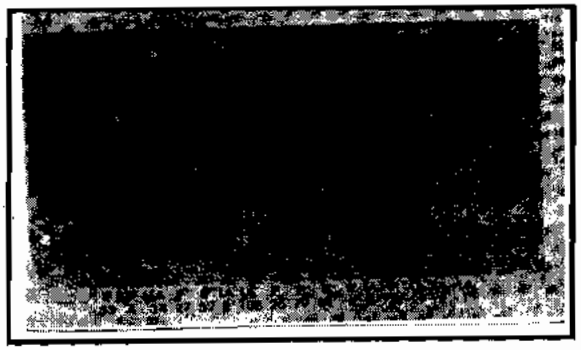

Fig. 1 Coomassie blue-stained SDS-PAGE of OMPs from 16 Campylobacter coli isolates. The numbers at the bottom identify the isolates. Lane M: molecular weight marker for the proteins in Kda

DISCUSSION

The ability of polyacrylamide gel elctrophoresis of whole cell proteins to identify Campylobacter spp has been established in several studies (4). Analysis of outer membrane proteins (OMP's) was performed by SDS-PAGE from 16 isolates of Campylobacter coli. Campylobacter coli were clearly differentiated into seven subgroups. The results of this work differ from that of Derclaye et al. (5), where twenty – two isolates were only grouped into two. In this study, the common bands seen were 116kDa, 18kDa and 14kDa respectively.

In a report by Derclaye et al. (5), the commonest bands were 37, 55kDa for C. jejuni reference stains, while 25 and 84 kDa were present for C. coli reference strain. In our study, only one strain had 84kDa while three strains had an OMP of 22, 23 and 24kDa. In another report by Penner et al. (6), approximately 60-62 kDa of protein bands were visualized in C. jejuni and C. coli. They explained that the protein bands visualized were flagella antigens by the use of acid-glycine extract in detecting serum antibodies that are common antigens associated with flagellin. Logan and Trust (7) reported that glycine extraction fraction contained flagellin antigen of approximately M_r 31 and 62kDa, while saline extraction was approximately M_r 22, 27 and 45 kDa. All these studies from previous workers showed a different molecular weight from our result possibly as a result of the different technique used (ethanolic and heat stressed) and also environmental variation amongst strains. The use of a probe to check for cross reaction within various isolates is suggested to see which of them share common antigenic determinants. The profile generated from SDS-PAGE is relatively simple and materials and equipments required are generally less costly than those needed for other genomic techniques. In addition the profiles are stable and reproducible, methodological differences between laboratories have title effect on identification.

SDS-PAGE is a valuable tool for the rapid identification of Campylobacter species in Nigeria, however, excellent results will be obtained when combined with serotyping as a confirmatory procedure, furthermore, in the developing countries where there are not much funds to carry out meaningful research it proves a reliable means for identifying Campylobacter species.

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THE DISCHARGING EARS IN ADULTS IN IBADAN, NIGEERIA CAUSATIVE AGENTS AND ANTIMICROBIAL SENSITIVITY PATTERN

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In an attempt to study the microbiology of discharging ears, ear swabs were taken from 347 adult patient with discharging ears in the University College Hospital, Ibadan between March 1995 and February 1997. The presumptive diagnosis and indication for ear swabbing were chronic suppurative otitis media (67.1%), acute suppurative otitis media (14.4%) and otitis externa (18.2%). Using standard microbiological methods, 82.4% of the patients had microbes in their ears. These were identified as Pseudomonas aeruginosa (34.6%), Staphylococcus aerus (19.4%), Klebsiella species (17.4%) and Proteus species (12.5%). Others were Candida albicans and Aspergillus species.

Susceptibility result showed that ceftazidime, azithromycin, ceftriaxone, cefuroxime and gentimicin were active against majority of the bacterial isolates and are therefore recommended as first line drugs, while the quinolones should be kept as reserve durgs in the management of these conditions. In addition antifungal cream should be used as wick in dressing, as well as systemic metrondazole to take care of the anaerobes.

INTRODUCTION

The discharging ear is a very common problem in the tropics. It is seen in all age groups but most prevalent in infants and children. Its decreasing incidence during and after adolescence is a result of the growth and development of he pharynx 1. Yet it is still one of the major problems of adults attending the Ear, Nose and Throat (ENT) clinics.

A discharging from the ear may arise from the external auditory meatus in otitis externa, or from the middle ear cavity in otitis media. There is very scanty information on the epidemiology of otitis externa, otitis media and otomycosis in the developing countries. In an attempt to further compliment the search for the most economically available antimicrobial agents which will prevent long term otological, audiological and neurological consequences, we studied the cases of adults presenting with discharging ears to the University College Hospital (UCH), Ibadan from March 1995 to February 1997.

PATIENTS AND METHODS

Adult patients presenting with discharging ears to the UCH between March 1995 and February 1997, whose wear swabs were sent for microbiological studies in the department of Medical Microbiology were recruited into the study. Routinely, each ear swab was inoculated onto blood, chocolate and MacConkey agars. Both the blood and chocolate agars were incubated in candle extinsion jar (microaerophlic), while the MacConkey agars were incubated aerobically at 37°C overnight. The isolates were identified to species level by standard microbiology methods and their antimicrobial sensitivities done by using Stoke's disc diffusion techniques.

RESULTS

During the study period, swabs were received from 347 consecutive patients. Of these, 304 (87.6%) were outpatients while 43 (12.4%) were impatients. 270 of the outpatients (88.8%) were from the ENT clinic and 14 (32.5%) of the in-patients were from the ENT wards. The distribution of age, sex and side of discharging ear overshown in table 1. The male to female ratio was 1:0:98. The side of ear discharge was not specified in 24.5% of these patients while 30.2%, 29.7% and 15.6% had right, left and Bilateral ear discharge respectively.

The presumptive diagnosis and indication for ear swabbing in these patients all shown in table II. Chronic supperative otitis media (CSOM) was the most frequent diagnosis (67.4%). This is followed by acute suppurative otitis media (ASOM) 14.4% and otitis externa 18.2%.

Of the 347 patients, 286 (82.4%) yielded positive culture from the ear swab, 232 (81.1%) of these yielded only one organism, 58 (20.3%) yielded a mixture of two organisms while (0.3%) had a mixture of three organism. This particular patient had CSOM. 45(77.6%) of these with two organisms had CSOM. 45 (22.7%) of the culture positive patients with CSOM had polymicrobial agents.

Table III shows the causative agents of discharging ears in the 286 adults. A total of 345 isolates were recovered *Pseudomonas aeruginosa* was the leading organisms (34.6%). *Staphylococcus aureus* with 19.4%, Klebsiella species with 17.4% and Proteus species with 12.5% closely followed this. Of the 233 cases of presumptive diagnosis of CSOM, 198(85%) yielded organisms. A total of 244 isolates were recovered from these patients. Of these Pseudomonas species was the predominant group of agents (38.5%), with *Pseudomonas aeruginosa* being the most prevalent organism 32%. Klebsiella spp 17.2%, *Staphylococcus aureus* 16.8% and Proteus spp 13.9% closely followed this. 5 cases (2.19%) has *Candida albicans* while 1 (0.4%) had Aspergillus spp. 45 of 198 (22.7%) culture positive cases of CSOM had mixed organisms.

Of the 50 patients with ASOM, 37 (74%) were culture positive. A total of 42 isolates were recovered Pseudomonas species was the predominant group of organisms with 33.3%. This was followed by Staphylococcus aureus, with 28.6%. 5 cases (13.5%) of ASOM had mixed organisms. These were mainly *Staphylococcus aureus*, Pseudomonas species and Klebsiella species. Of the 63 cases of

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Age Range (Yrs)	SEX		SIDE OF EAR DISCHARGE				Total	%
	Male	Female	Right	Left	Bilateral	Unspecified		
16-25	50	38	23	30	19	17	88	25.4
26-35	26	35	19	24	9	9	61	17.6
36-45	20	25	22	12	6	5	45	13.0
46-55	17	24	10	14	4	13	41	18.1
56-65	12	8	7	2	7	4	20	5.8
66-75	20	7	9	9	1	8	27	7.8
76-85	1	3	1	1	0	2	4	1.2
86-95	0	2	0	2	0	0	2	0.6
96	1	0	0	1	0	0	1	0.3
Not Specified	28	30	14	8	8	27	58	16.7
Total	175	172	105	103	54	85	347	100
%	50.4	49.6	30.2	29.7	15.6	24.5	100	

TABLE I – AGE, SEX AND SIDE OF EAR DISCHARGE IN ADULTS

Age Range (YRS)	OTITIS EXTERNA	ASOM	CSOM	POST-OP ABCESS	TOTAL
16-25	12	17	58	1	88
26-35	11	7	43	0	61
36-45	11	3	31	0	45
46-55	10	5	26	0	41
56-65	3	3	14	0	20
66-75	4	2	21	0	27
76-85	1	0	3	0	4
86-95	2	0	0	0	2
>=96	0	0	1	0	1
Not specified	9	13	36	0	58
Total	63	50	233	1	347
%	18.2	14.4	67.1	0.3	100

TABLE II: PESUMPTIVE DIAGNOSIS OF PATIENTS WITH DISCHARGING EARS

ASOM = Acute Suppurative Otitis Media
CSOM = Chronic Suppurative Otitis Media

DIAGNOSIS	PATHOGENS															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Otitis Externa	14	11	0	2	9	7	0	0	1	1	0	0	0	4	1	50
ASOM	11	5	0	3	8	5	0	0	3	1	0	1	0	0	0	37
CSOM	37	11	1	14	71	18	7	3	11	12	1	5	1	5	1	198
Post-op abcess	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Total	62	27	1	19	88	30	7	3	15	15	1	6	1	9	2	1
COMBINATION OF TWO OR MORE OGANISMS																286
Otitis Externa	0	0	0	0	0	3	1	1	1	1	0	0	0	1	0	8
ASOM	1	0	0	1	2	1	0	0	0	0	0	0	0	0	0	5
CSOM	4	3	1	2	7	10	2	2	6	4	0	1	0	2	1	45
Total	5	3	1	3	9	14	3	3	7	5	0	1	0	3	1	58

TABLE III: PATHOGENS FO DISCHARGING EARS IN ADULTS EARS IN IBADAN

KEY: The pathogens: 1= Staphylococcus aureus, 2= Staphylococcus albus, 3 = Streptococcus pyogenes, 4= Pseudomonas species, 5 = Pseudomonas aeruginosa, 6 = Klebsiella species, 7 = Klebsilla oxytocum, 8 = Kiesiella rhinoscleromatis, 9 = Proteus species, 10 = Proteus mirabilis, 11 = Proteus rettgeri, 12 = Escherichia coli,, 13 = Haemophilus influenzae, 14 = Candida albicans, 15 = Aspergilus species.

Bacterial Isolates	ANTIBIOTIC DISCS														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Klebsiella spp	N	38	38	38	40	38	40	40	40	40	40	40	35	35	38
	S	2	26	14	27	7	32	26	29	34	40	35	35	15	10
	(%)	5.3	66.7	35.9	67.5	18.9	80	65	72.5	85	100	90	100	100	25
Pseudomonas spp	N	80	89	NT	90	80	92	90	90	90	90	90	90	80	NT
	S	20	59		30	40	50	23	20	78	87	86	71	71	
	(%)	25	66.3		33.3	50	67	25.5	22.2	86.7	96.7	95.5	88.7	88.7	
Proteus spp	N	30	28	28	30	28	30	30	30	30	28	30	30	30	28
	S	8	0	14	17	6	25	23	24	24	28	30	30	30	1
	(%)	26.7	0	50	56.7	21.4	83.3	76.7	80	80	100	100	100	100	4
Escb. Coli	N	5	5	NT	5	5	5	5	5	5	5	5	5	5	4
	S	2	0		4	1	4	3	4	5	5	5	5	5	3
	(%)	40	0		80	20	80	60	80	100	100	100	100	100	60
Staph. Aureus	N	60	60	58	60	60	60	60	60	60	60	60	58	58	50
	S	9	30	38	52	39	52	48	33	50	60	60	58	58	15
	(%)	15	50	65.5	86.7	65	86.7	80	55	83.3	100	100	100	100	30

TABLE IV: DISC SENSITIVITY PATTERN OF THE BACTERIAL ISOLATES

Key: N = Number Tested, NT = Not tested, S = Number of strains that are sensitive
1 = Ampicillin, 2 = Amoxycillin, 3 = Augmentin, 4 = Azithromycin, 5 = Cotrimoxazole,
6 = Gentamicin, 7 = Ceftazidime, 8 = Ceftriaxone, 9 = Ceftazidime, 10 = ciprofloxacin,
11 = Ofloxacin, 12 = Amikacin, 13 = Streptomycin, 14 = Tetracycline

otitis externa. 50 (79.4%) yielded microorganisms. A total of 58 isolates were recovered. The predominant organisms was *Staphylococcus aureus* with 24.1%. This was followed by *Pseudomonas aeruginosa* 19%. Four cases had *Candida albicans* (65%) while one had Aspergillus species. Staphylococci, Pseudomonas and Proteus species coexisted with these fungi.

The disc sensitivity pattern of the isolates is shown in table IV. Ofloxacin, ciprofloxacin (ciprotab) had the best activity against the isolates. Ceftazidime, azithromycin, cefuroxime, ceftroxime, ceftriazone and gentamicin had good sensitivity against two third of the stains of all isolates. Ampicillin, amoxycillin, cotrimoxazole, streptomycin and tetracycline had poor activity against the bacterial isolates.

DISCUSSION

The epidemiology of both otitis externa and media is still not well charted, the etiology and pathogenesis are imperfectly understood, their treatment is controversial and subject to change particularly so little is known about middle ear infection(1). The results of our study have thrown some light upon some of these issues. Hence the pathogenic agents of these discharging ears are found to be polymicrobial in 20.3% of cases and monomicrobial (79.7%). The most frequent agents were Pseudomonas species, *Staphylococcus aureus, Pseudomonas aeruginosa*, Klebsiella species and Proteus species were the main causative agents of ASOM and CSOM. This finding agrees with reports of previous workers that *Haemophilus Influenzae* and *Streptococcus pneumoniae* do not play an important role in the pathogenesis of otitis media in the tropics (3). It is possible that the indiscriminate use of antibodies by most patients in our environment contribute to the selection of the Gram Negative bacilli found in our patients, majority of whom present late to hospital. This fact may also explain the culture negative results got in some of case of discharging ears.

Anaerobes were not routinely checked for because of technical problems associated with specimen collection and transportation. Subsequent studies will address this issue, as well as Tubercle bacilli as pathogenic agent of discharging ears.

With ceftazidime, azithromycin, ceftriazone, cefuroxime and gentamicin showing good activity against tow third of these isolates, we would recommend their use as the first line antibiotherapy of discharging ears. The quinolones should be reserved drugs while the penicillin cotrimoxazole and tetracycline are not useful. These chemotherapeutic agents should be combined with metronidazole to take care of anaerobes and the use of antifungal cream as wick in dressing for the fungi.

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INVITRO ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES FROM WOUND INFECTIONS IN UNIVERSITY OF ILORIN TEACHING HOSPITAL

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The outcome of 532 wound swabs received from patients with wound infections in different units of the University of Ilorin Teaching Hospital Ilorin, Nigeria, over a one year period (July 2000 – June 2001), and routinely processed by Gram staining and culture in the Microbiology Laboratory, is reported. 444(83.5) of all samples cultured positive for bacterial pathogens while 88 (16.5%) were bacteriologically sterile. 272 swabs yielded single isolate while 172 yielded a mixture of two or more organisms. Staphylococcus aureus predominates (35.8%), followed by Pseudomonas spp (21.8%), Escherichia coli (15.3%), Klebsiella spp (13.4%), Proteus spp (5.6%), Coagulase Negative Staphylococci (3.1%), Streptococcus faecalis (2.8%), Streptococcs pyogenes (0.9%), Group B β-haemolytic Streptococci (0.9%), and Acinetobacter spp (0.3%).

Both Gram-positive and Gram-negative organisms demonstrated moderate to high in vitro sensitivity to Ofloxacin and Ciprofloxacin (sensitivity rate 70-94%). In vitro sensitivity to Cloxacillin, Erythromycin, Azithromycin and Ceftazidime by Gram positive organisms ranged beween 55 and 90% while Gentamicin, Ceftrazidime and Azithromycin equally demonstrated moderate to high inhibitory effect on Gram negative organisms including Pseudomonas spp. (serisitivity rate 55-90%).

The Fluoroquino lones are the favoured antimicrobial agents nowadays, as demonstrated in this study. In our environment however, a combination of Cloxacillin and Gentamicin is an effective empiric alternative when cost is considered and this combination can be used. The need for continuous antimicrobial monitoring of clinical isolates of wound infection for drug resistance, which is of paramount importance in the empiric selection of antibiotics, is emphasized.

INTRODUCTION

Every individual carries a large resident microbial population on the skin surfaces, and in the openings of the hair follicles, sweat glands and sebaceous glands. This population comprises mainly Gram positive cocci of the genera Staphylococcus and Micrococcus, and Gram positive rods of the genera Propionibacterium and Corynebacterium together with the yeast, Pityrosporum (1). The skin is also host to a variable number of transient or contaminating bacteria. Although the resident flora produce antibacterial substances that provide some protection against colonization by potential pathogens, any breach in the skin surface, whether accidental or surgical, provides an open door for bacterial infection.

Surgical wound infection rates have been found to vary between 3 and 11% and wound, skill and burns are areas after genito-urinary tract, where no socomial infections tend to occur more commonly in surgical practice (2,3,4). The risk of infection increases with the degree of contamination and it has been estimated that about 50% of wound contaminated with bacteria become clinically infected. The prevalent organisms that have been associated with hospital – acquired wound infection include Staphylococcus aureus which from various studies have been found to account for 20-40% (3), and Pseudomonas aeruginosa 5-15% of the nosocomial infection, with infection mainly following surgery and burns. Other pathogens such as entercocci and members of he enerobacteriaceae have been implicated, especially in immuno-compromised patients and following abdominal surgery (3).

It is also known that aside surgical units, intensive care units, nurseries, operating room theatre, and recovery rooms are units where nosocomial wound infection frequently occurs (1,3). In the Accident and Emergency unit, accidental wound, clean or dirty, is one of the most common reasons for attendance by patients. In all these units, wound infection which are mainly due to nosocomial pathogens, tends to be associated with bacteraemia, septicaemia, shock and death in some patients, and prolong hospital stay in many others. This situation may be a serious matter for the patient and his family, as his maintenance in the hospital and treatment are expensive and meanwhile a bed space is occupied which might otherwise be used for other patients.

In view of this, there is a need for continuous monitoring of the hospital by infect control team, which should particularly be aware of not only nosocomial wound infection but the local prevalence of antibiotic resistant bacteria strains, as this varies greatly form place to place. The pattern of the bacteria pathogens isolated from wound swabs in this hospital and their antibiotic sensitivity pattern is intended to provide Clinicians and Surgeons valuable information upon which empiric antimicrobial therapy of wound infection can be predicated.

MATERIALS AND METHOD

This study was carried out over a period of one year (July 2000 – June 2001) at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. All wound swabs from different units of the hospital were received on the swab bench of the Microbiology laboratory and subjected to routine Gram staining and culture.

Gram staining was done according to the standard techniques (5). Swabs were inoculated onto Blood, Chocolate and MacConkey agar, and plates incubated aerobically at 37°C for 24 to 48 hours. Anaerobic culture was not done, as this is not a routine in our laboratory.

Corresponding Author

Growth on culture plates were identified by colony morphology, and confirmed by Gram staining reaction, standard biochemical and serological tests(5).

Antibiotic susceptibility of pure culture of confirmed isolates were performed on Diagnostic Sensitivity Test Agar by the Kirby Bauer disc diffusion method (6) using the appropriate Gram positive and Gram negative discs, and Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa as control strains. Isolate was considered sensitive or resistant by comparing zone diameter of inhibition to the zone diameter interpretive standard of the National Committee for Clinical Laboratory Standard (7).

Necessary Patient bio-data were obtained from the laboratory request forms and data were fed into EPI INFO version 6.0 computer with analysis done using the appropriate statistical methods where necessary.

RESULT

Of all the wound swabs received from 532 patients with clinical evidence of wound infection over the period of study, 346 (65%) were from in-patients, 132 (24.8%) from outpatients and 54 (10.2%) were from patients whose wards or clinics were not indicated on the request forms. The distribution of swabs and isolates by wards is shown in Table 1. Surgical wards accounted for the highest number of request and isolation rates, followed by outpatient units and lowest in Psychiatric and Obstetrics and Gynaecology units.

Of the 532 swabs, 444 (83.5%) cultured positive for bacterial pathogens while 88 (16.5%) were bacteriologically sterile. 272 (61.5%) of these yielded single, 152 (34.2%) yielded two while 20 (4.5%) yielded a mixture of three organisms (Table II).

The distribution of bacteria pathogens in pure and mixed cultures is as shown in Tables III and IV. A total of 642 bacterial isolates were obtained in all, 280 (43.6%) were Gram positive while 362 (56.4%) were Gram negative. Staphylococcus aureus was the predominant organism isolated accounting for 35.8%, followed by Pseudomonas spp (21.8%), Escherichia coli (15.3%), Klebsiella spp (13.4%), Proteus spp (5.6%), Coagulase Negative Staphylococi (3.1%), Streptococcus faecalis (2.8%), Streptococcus pyogenes (0.9%), Group B β-haemolytic Streptococci (0.9%), Acinetobacter spp (0.3%).

The antimicrobial profile of the pathogens is summarized in Table V and VI. The fluoroquinolones (Ofloxacin, Perfloxacin and Ciprofloxacin) showed increased activity against all the isolates. Cloxacillin, Erythromycin, Gentamicin and Azithromycin equally showed good activity against Staphylococcus aureaus, the predominant Gram positive isolate, with 77.4%, 87.8%, 93.9% and 96.5% of isolates sensitive. Caftazidime is the only Cephalosporin that showed moderate activity against Staphylococcus aureus with 52.2% of the isolates sensitive. Ampicilin and Penicillin G were ineffective against Staphylococcus aureus with only 18.3% and 16.5% of the isolates sensitive, but Streptococcus pyogenes and Group B β-haemolytic Streptococci are highly sensitive to these agents.

WARDS	SWABS	ISOLATES
Surgical (W2, W5, W8)	168(31.6)	144(32.4)
Outpatient (SOP/MOP/GOP)	132(24.8)	124(27.9)
Medical (W1, W4, W6)	52(9.8)	44(9.9)
Paediatric (W3)	52(9.8)	32(7.2)
Emergency (A/E & EPU)	50(9.4	36(8.1)
Obstetrics and Gynaecology	12(2.3)	10(2.3)
Intensive Care Unit	10(1.9)	8(1.8)
Psychiatric (W7)	2(0.4)	2(0.5)
Not Indicated	54(10.2)	48(10.8)
TOTAL	532(100)	444(100)

Key:
W = Ward
Number in parenthesis = Percentages

Table 1: Distribution of wound swabs and isolates by wards

Organism	No.	(%)
Staphylococcus aureus	230	35.8
Pseudomonas spp	140	21.8
Escherichia coli	98	15.3
Klebsiella spp	86	13.4
Proteus spp	36	5.6
CONS	20	3.1
Streptococcus faecalis	18	2.8
Group B β - haemolytic Strept	6	0.9
Streptococcus Pyogenes	6	0.9
Acinetobacter spp	2	0.3
Total	642	100%

Table 2: Distribution of Bacterial Pathogens isolated from 44 wounds swabs

Organism	Number	(%)
Staphylococcus aureus	116	(42.6)
Pseudomonas spp	58	(21.3)
Escherichia coli	46	(20)
Klebsella spp	24	(8.8)
Coagulase Negative *Staphylococci*	12	(4.4)
Proteus spp	6	(2.2)
Streptococcus pyogenes	4	(1.5)
Streptococcus faecalis	4	(1.5)
Group B β-haemolytic *streptococci*	2	(0.7)
TOTAL	**272**	**(100)**

No in parenthesis = Percentages

Table 3: Distribution of bacteria pathogens from wound swabs in pure cultures

Azithromycin, Gentamicin and Caftazidime respectively showed good activity against Pseudomonas spp, the most prevalent Gram negative pathogen, with 60%, 64.3% and 85.7% of isolates susceptible. Other Gram negative bacteria with the exception of Acinetobacter spp are equally susceptible to these antibiotics.

No in parenthesis = Percentages

Table 4: Mixed bacteria growth in wound swabs

ORGANISM	Number	(%)
Staphylococcus aureus, Escherichia coli	26	(15)
Staphylococcus aureus, Klebsiella spp	24	(14)
Psedomonas spp, Klebsiella spp	24	(14)
Staphylococcus aureus, Pseudomonas spp	22	(13)
Staphylococcus aureus, Proteus spp	16	(9)
Pseudomonas spp, Escherichia coli	12	(7)
Staphylococcus aureus, Psedo. spp, Klebsiella spp	8	(5)
Staphylococcus aureus, Streptococcus faecalis	4	(2)
Escherichia coli, Coagulase Negative Staphylococci	4	(2)
Strephylococcus faecalis, Proteus spp,	4	(2)
Staphylococcus aureus, Proteus spp, Pseudomonas spp	4	(2)
Staphylococcus aureus, Pseudomonas spp, E. coli	4	(2)
Pseudomonas spp, Proteus spp	4	(2)
Pseudomonas spp, Coagulase Negative Staphylococci	2	(1)
Klebsiella spp, Escherichia coli	2	(1)
Klebsiella spp, Proteus spp	2	(1)
Staphylococcus aureus, Streptococcus pyogenes	2	(1)
Escherichia coli, Streptococcus faecalis	2	(1)
Acinetobacter spp, Group B βhaemolytic Streptococi	2	(1)
Coagulase Negative Stapylococci, Strept. Faecalis	2	(1)
Pseudo spp, Group B βhaemolytic Strept /Klebs. Spp.	2	(1)
Streptococcusaecalis, Proteus spp, Pseudomonas spp	2	(1)
TOTAL	**172**	**(100)**

Organism	Penicillin 1unit	Ampicillin 10µg	Streptomycin 10µg	Gentamicin 10µg	Cloxacillin 5µg	Erythromycin 10µg	Chloramphenicol 10µg	Ceftriaxone 30µg	Cefuroxime 30µg	Ceftazimide 30µg	O-floxacin 10µg	Ciprofloxacin 10µg	Perfloxacin 10µg	Azithromycin 10µg
Staph aureus S R N230	38(16.5%) 192(83.5%)	42(18.3%) 188(81.7%)	148(64.3%) 82(35.7%)	216(93.9%) 14(6.1%)	178(77.4%) 52(22.6%)	202(87.8%) 28(12.2%)	160(69.6%) 70(30.4%)	148(64.3%) 82(35.7%)	176(77.4%) 52(22.6%)	120(52.2%) 110(47.8%)	214(93%) 16(7%)	194(84.3%) 36(15.7%)	210(91.3%) 20(8.7%)	222(96.5%) 8(3.5%)
CONS S R N20	0(0%) 20(100%)	0(0%) 20(100%)	12(60%) 8(40%)	10(50%) 10(50%)	6(30%) 14(70%)	14(70%) 6(30%)	16(80%) 4(20%	20(100%) 0(0%)	8(40%) 14(60%)	20(100%) 0(0%)	14(70%) 6(30%)	14(70%) 6(30%)	20(100%) 0(0%)	14(70%) 6(30%)
Strept faecallis S R N18	0(0%) 18(100%)	0(0%) 18(100%)	14(77.8%) 4(22.2%)	10(55.6%) 8(44.4%)	4(22.2%) 14(77.5%)	8(44.4%) 10(55.6%)	0(0%) 18(100%)	6(33.3%) 12(66.7%)	4(22.2%) 14(77.7%)	4(22.2%) 14(77.7%)	16(88.9%) 2(11.1%)	18(100%) 0(0%)	14(77%) 4(12.2%)	8(44.4%) 10(55.6%)
Gp Bßhaemo. S Streptococci R N6	6(100%) 0(0%)	6(100%) 0(0%)	4(66.7%) 2(33.3%)	6(100%) 0(0%)	6(100%) 0(0%)	4(66.7%) 2(33.3%)	4(66.7%) 2(33.3%)	4(17%) 2(33.3%	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)
Strep pyogenes S R N6	6(10%) 0(0%)	4(66.7%) 2(33.3%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	6(100%) 0(0%)	%) 0(0%)

Tables 5: Susceptibility pattern of Gram-Positive cocci isolated from wound swabs to antimicrobial agents

Organism	Ampicillin 10µg	Tetracycline 25µg	Streptomycin 10µg	Gentamicin 10µg	Cefuroxime 30µg	Ceftriaxone 30µg	Ceftazidime 10µg	O-floxacin 10µg	Perfloxacin 10µg	Ciprofloxacin 10µg	Azithromycine 10µg
Pseudomononas spp S R N 140	56(40%) 84(60%)	28(20%) 112(80%)	86(61.4%) 54(38.6%)	90(64.3%) 50(35.8%)	20(14.3%) 120(85.7%)	58(41.4%) 82(58.6%)	120(85.7%) 20(14.3%)	110(78.6%) 30(21.4%)	104(74.3%) 36(25.7%)	120(85.7%) 20(14.4%)	84(60%) 56(40%)
Escherichia S R N98	48(48.9%) 50(51.1%)	56(57.1%) 42(42.9%)	40(40.8%) 58(59.2%)	66(55.9%) 32(44.1%)	54(47.1%) 44(52.9%)	40(23.5%) 58(76.5%)	68(58.8%) 30(41.2%)	76(77.5%) 22(22.5%)	72(73.5%) 26(26.5%)	82(83.7%) 16(16.3%)	84(85.7%) 14(14.3%)
Klebsiella spp S R N86	52(60.5%) 34(39.5%)	36(41.9%) 50(58.6%)	70(81.4%) 16(18.9%)	56(65.1%) 30(34.9%)	42(48.8%) 44(51.2%)	40(46.5%) 46(53.5%)	62(72.1%) 24(37.9%)	78(90.7%) 8(9.3%)	68(79.1%) 18(20.9%)	74(86%) 12(14%)	74(86%) 12(14%)
Proteus spp S R N36	24(66.7%) 12(33.3%)	12(33.3%) 26(66.7%)	28(77.8%) 8(22.2%)	34(94.4%) 2(5.6%)	30(83.3%) 6(16.7%)	36(100%) 0(0%)	30(83.3%) 6(16.7%)	34(94.4%) 2(5.6%)	36(100%) 0(0%)	36(100%) 0(0%)	26(72.2%) 10(27.8%)
Acinetobacter ssp S R N2	0(0%) 2(100%)	2(100%) 0(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)	0(0%) 2(100%)

Table 6: Susceptibility pattern of Gram-negative rods isolated from wound swabs to antimicrobial agents

DISCUSSION

Bacteria contamination of wound is a serious problem in the hospital especially in the surgical practice where clean operations can become contaminated and subsequently infected (2,8). Although it has been argued that wound swabs from surface of intact or ulcerated skin for culture, provides little or no clinically useful information, because of lack of corretation between surface colonization and below-the-surface infection (9), it is nonetheless known that the degree of wound contamination from surface wounds become clinically infected (2). 83.5% of wound swabs in this study cultured positive for bacteria pathogens. If 50% of these were indeed from infected wound, then the wound infection are will be 41.3%. This figure is slightly higher than the 39% recorded in Lagos (10).

Surgical wards posted the highest number of request and isolation rates of organism. This is in agreement with the trend world wide (3,8,10,11), which is attributable to the fact that patients here are likely to undergo surgical operation, and more likely to have breaks in their local defense systems. The low rate of request and isolation rate in intensive care unit as against the normal trend may be due to the fact that this unit is quite small and requests are therefore correspondingly small. It may also be a reflection of strict hygiene and good nursing practice in this unit.

The common pthogens isolated are Staphylococcus aureus (35.8%), Pseudomonas spp (21.8%), Escherichia coli (15.3%), Klebsiella spp (13.4%), Proteus spp (5.6%), and CONS (3.1%). The preponderance of Staphylococcus aureus is in keeping with other studies (3,9,11,12,13). The organism is a normal flora of the skin in most people and can easily contaminate wounds. 56.4% of all isolates are Gram negative organisms against 43.6% Gram positive bacteria. This is similar to the observation in some other centres (10) where Pseudomonas spp, Klebsiella spp, Escherichia coli and Coliforms are the predominant pathogens responsible for wound and other nosocomial infections. This pattern is best understood in terms of selective pressure exerted on the organism based on the current antibiotic use. In our environment, the third generation Caphalosporins are increasingly ssssss being used.

The susceptibility pattern of the organisms heavily favours the Quinolones, particularly Ciprofloxacin, and the new macrolide, Azithromycin, which are effective but expensive antibiotics in the treatment of wound infections in this environment. Ciprofloxacin has to be used with caution in the paedatric age group. In the light of 74.4% sensitivity of Staphylococcus aureus to Cloxacillin and 87.8% to Erythromycin, and greater than 60% sensitivity of the predominant Gram negative organisms to Gentamicin, a cost effective empiric combination of Cloxacillin and Gentamicin or Erythromycin and Gentamicin may be favourably considered for wound infection in this environment.

It is recommended that in addition to using the above antimicrobial theraphy in the treatment of wound infection, adequate attention should be placed on preventive measures such as hand washing, disinfection, good nursing practice and good surgical techniques amongst others, to reduce bacterial contamination of wounds.

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A CORRELATION STUDY OF ULCER STATUS WITH BACTERIAL COLONIZATION AND INVASION

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Wound biopsy is a reliable way of diagnosing wound infection in patients with chronic ulcer of the limbs and in burn patients. The biopsy specimen is subjected to both histological and microbiological analysis. While wound swabs often cultured mixed contaminants, biopsy specimens usually reveal single organism growth. This is a prospective study of fifty patients with chronic leg ulcers attending surgical outpatient department over a period of 10 months. The ulcers were subjected to histopathology study. The clinical status of the ulcers were correlated with the histopathology result. There was both statistical and clinical significance between the ABDEFS' and HISTOPATHOLOGY scores. A clinician can therefore reasonably predict the degree of bacterial invasion of the ulcer based on the assessment of its clinical appearance and thus commence appropriate treatment before further complication sets in.

INTRODUCTION

Wound biopsy is a reliable way of diagnosing wound infection in patients with burn injury and those with chronic ulcers of the limbs. While wound swabs often culture mixed contaminants, biopsy specimens usually reveal single organism growth. The depth of bacterial invasion may be a pointer to some dangerous complications that can arise from chronic ulcer. For example, a perivascular invasion of the bacterial may be a pointer to an imminent speticaemia. Clinical status of an ulcer can be assessed and monitored by various wound severity scoring systems that have been devised by some workers. However, Oluwatosin et al (1) formulated a reliable and simple system called ABDEFS' scoring system in a study conducted at the University College Hospital, Ibadan. The scoring system was used in a study which showed a clinical correlation between the bacterial count of an ulcer and the clinical status of the ulcer. This study is performed as an extension of the previous one to see if the histology of the biopsy specimen has any correlation with the clinical status of the ulcer.

PATIENTS AND METHODS

Forty patients with chronic leg ulcers attending the surgical outpatient department of the University College Hospital, Ibadan were studied. Study period was ten months from December 1999 to September 2000. Patients with malignant ulcer as well as diabetics and patients with haemoglobingpathy were excluded from the study. The ulcers were assessed using ABDEFS' scoring system by a Registrar in the division of plastic surgery. The wound biopsies were taken after injecting the biopsy site with xylocaine and adrenaline local anaesthetic agents. The specimens were sent to histophathology laboratory of the hospital for analysis. The clinical status of the ulcer was correlated with the histopathology results. The analysis was performed using SPSS –9 for windows statistical package. Level of statistical significance was taken to be $P<0.05$ and a 95% confidence interval applied.

The ulcers were scored as follows:

		Scores
A. aetiology -		
	(i) Local	1
	(ii) Controlled systemic disease	2
	(iii) Systemic disease uncontrolled	3
	(iv) Malignancy	4
B. Base	(i) Soft, mobile	1
	(ii) Hard, fixed	2
D. Discharge		
	(i) Slight to moderate	1
	(ii) Compious, purulent	2
E. Edge	(i) Flat, shelving, punched out	1
	(ii) Undermined, raised	2
F. Floor	(i) Predominantly granulation	1
	(ii) Predominantly sloughy	2
S. Size	(i) <2.5cm in dimension	1
	(ii) >2.5cm in dimension	2

Total score was applied for each of the patients maximum score being 14. the histopathology resul was scored as follows:

(i)	No pathogens, only granulation tissue	1
(ii)	No pathgens but pus cells present	2
(iii)	Colonization, that is, organisms present in non-viable tissue	3
(iv)	Bacterial invasion of viable tissue	4
(v)	Perivascular invasion	5

RESULTS

Thirty four out of expected forty results were analyzed. The histopathology studies were carried out by three consultants in the pathology department of the hospital depending on who was on duty the day the specimens were processed.

The mean (sd) age of the patients was 40.76(18.16), most of the cases of the chronic leg

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ulcers were secondary to poorly treated traumatic ulcers. The mean (sd) ABDEFS score was 8.26(1.82) while the mean (sd) histopathology score was 2.10(1.11) as shown in table 1. This means that on the average there were no pathogens but presence of pus cells in the biopsed tissue. There were few cases of bacterial invasion of normal tissue and only in one case did the histopathology result reveal pervascular invasion.

	Mean	Standard Deviation	N
HISTOPATHOLGOY	2.10,	1.11	34
ABDEFS	8.21	1.82	34
AGE	40.76	18.16	34

TABLE I : **DESCRIPTIVE STATISTIC ON HISTOPTHOLOGY ABDEFS AND AGE**

		HISTOPATHLOGY	ABDEPS
Pearson correlation	HISTOPATHOLOGY	1.000	0.440
	ABDEFS	0.440	1.000
Singnificance(1-tailed)	HISTOPATHOLOGY	.	.005
	ABDEFS	.005	.
N	HISTOPATHOLOGY	34	34
	ABDEFS	34	34

95% CONFIDENCE INTERVAL FOR

Lower. Bano		Upper Bound
Constant	- 1.769	1.544
Coefficient of x	0.071	0.465

Linear Regression equation. Y = 0.44x
When y = Histopathology score
X = Abdefs score.

TABLE II: **CORRELATIONS BETWEEN HISTOPATHOLOGY AND ABDEFS SCORES**

Pearsons correlation results are expressed in table 2. it showed a P-value of 0.005 which is less than 0.05. The correlation coefficient ® value of 0.440 fell within the 95% confidence interval of values showed in table 2. There is therefore both statistical and clinical significance between the ABDEFS' and HISTOPATHOLOGY scores. The histopathology score, that is, the degree of bacterial invasion can be calculated by using the linear regression equation obtained in this study. y = 0.44x where y = HISTOPATHOLOGY and x = ABDEFS.

DISCUSSION

Various wound severity scoring system have been devised by different workers. David Kinghton et al[3] made use of wound scores based on general wound parameters, anatomic consideration and wound measurements. Some of these scoring systems are not easily applicable in this environment. ABDEFS scoring method is a simple and reliable means of evaluating ulcers[1,2] The current emphasis in medical statistics is to report results in a way that is not only statistically significant but also clinically meaningful[4,5] In this study the p-value of 0.005 and correlation coefficient ® value of 0.440 which falls within 95% confidence interval established both statistical and clinical significance between the ABDEFS' scoring system and histopathology result of an ulcer.

Since histopathology study is not readily available in some centers, it will be most appreciable if a clinician can predict the clinical state of an ulcer with respect to the degree of bacterial invasion. The depth of bacterial invasion may be a pointer to some dangerous complications that can arise from chronic ulcer. For example, a perivascular invasion by bacteria may be a point to an imminent septicaemia. Using the ABDEFS' scoring system, a clinician in peripheral and some general hospitals in Nigeria can predict the degree of bacterial invasion of the ulcer based on assessment of its clinical appearance and thus commence appropriate treatment before further complication set in. For example an ulcer whose appearance has been scored as eight will be expected to have histopathology sore of[3,5] This study constitute a beneficial additional adjunct to the previous study of the correlation of the clinical status of an ulcer with the bacterial count of the ulcer biopsy.

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BACTERIAL PATHOGENS ASSOCIATED WITH INFECTED WOUNDS IN OGUN STATE UNIVERSITY TEACHING HOSPITAL, SAGAMU, NIGERIA.

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A prospective study was conducted at Ogun State University Teaching Hospital (OSUTH) between August 1999 and July 2000 in the Orthopaedics, Obstetrics and Gynaecological units to identify the bacterial pathogens associated with infected wounds as well as their antibiotic sensitivity profile.

A total of 1670 patients were seen in these units, out of which 130 (7.78%) developed wound infections. There was a statistical difference (P < 0.05) between the septic wounds associated with the non-operative cases (11.9%) and those of post-operative cases (6.41%). Amongst the 186 bacterial agents isolated from all the samples examined, Klebsiella species (25.3%) accounted for the most common isolates while the least was Enterococcus faecalis (5.4%). Klebsiella species was observed to be most prevalent in the Obstetrics and Gynaecological wounds while Pseudomonas aeruginosa was the commonest in the Orthopaedic wounds.

The sensitivity profile of the isolates to the commonly used antibiotics including those used as pre-operative prophylactic agents ranged between 1.67-46.8%, the range for the aminoglycosides was between 61.8-75%, while the fluoroquinolones had a range of 82.8%-89.2%.

The high level of bacterial resistance to the common antibiotics in this study, re-emphasized the need to properly monitor the use of antibiotics including those used as pre-operative prophylactic agents in this country.

INTRODUCTION

Wounds are commonly encountered in clinical practice. They may arise post-operatively, following trauma, in association with haemoglobinopathy, or could be primarily of infective origin. The contamination or the mere presence of pathogenic organism in a wound without local or systemic tissue involvement may not result in infection. [1] Wound infection could be defined as the presence of pus in a lesion, as well as other general or local features of sepsis including pyrexia, pain, and induration. [2] Wound infection is an important cause of morbidity and mortality among surgical patient. Apart from causing discomfort to the patient, the effect may vary from being a simple nuisance; to a delay in wound healing and other major disasters, such as wound dehiscence, gas gangrene or tetanus. This often result in prolonged hospitalized, thereby adding appreciably to the cost of treatment and management of the patient. [3] Factor such as the type of wound, nature of surgery, the dose and virulence of infecting organism, host resistance and use of antibiotic have been reported to be important in the establishment of wound infection. [1,2,4] The bacterial agents often incremianted in wound infections include *Staphylococcus, Pseudomonas, Klebsiella, Proteus* species and *Escherichia coli* as well as anaerobes such as *Clostridium* and *Bacteroides species*. [4,5]

The management of infected wounds is a challenge in terms of rational antimicrobial use, especially with the presence of a wide array of antimicrobial drugs and their unrelenting promotion by pharmaceutical companies. Similarly, a lot of concern has been generated world-wide over bacterial drug resistance, in view of the fact that cheap drugs have to be replaced with more effective and expensive ones. [6] Sule and Olusanya [7] have reported the increasing prevalence of bacterial resistance to most of the commonly used antibiotics in this environment. Otokunefor et al [5], have also suggested the need for an in-depth knowledge of the current predominant strains of bacterial agents and their pattern of antibiotic sensitivity in hospital units, as such information could assist in the blind treatment of bacterial infections when facilities are inadequate for laboratory diagnosis.

This study is therefore aimed at determining the prevalence and type of bacterial pathogens in both post-operative and non-operative wound infections in this hospital. The antibiotic sensitivity profiles of the isolates is also to be determined so as to develop a policy for the chemotherapeutic management of wound infections.

MATERIALS AND METHODS

A prospective study was conducted at Ogun State University Teaching Hospital (OSUTH) Sagamu, between August 1999 and July 2000 in the Orthopaedics, Obstetrics and Gynaecological units to identify the bacterial pathogens associated with infected wounds. The sensitivity profile of such agents were also determined.

Patients seen in these units with clinically diagnosed cased of infected non-operative or infected wounds complicating a surgical operation were included in the study. All data on each patient examined were entered into a proforma used during the study. The information entered into the proforma included name, age, sex, whether the wounds were post-operative or non-operative, the size and site of the wound. Other information included admission and operation interval, type and duration of operative procedure as well as the type of pre-operative antibiotics used.

Two wound swab samples were collected from each patient. These were inoculated into Cooked Meat Medium and Glucose broth to preserve and maintain the anaerobic and aerobic organisms present respectively, during transportation to the

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Medical Microbiology Laboratory of Obefemi Awolowo College of Health Science, Ogun State Unviersity, Sagamu. On receipt in the laboratory, all the turbid broth cultures were subcultured immediately while the non-turbid ones were incubated for 18-48 hours at 37oC before being subcultured. Each Glucose broth culture was inoculated onto MacConkey an Blood Agar and Kanamycin Blood Agar plates, and were incubated aerobically and anaerobically (using Oxoid gas generating kit for the generation of hydrogen and carbondioxide in an Oxoid anaerobic jar) respectively.

All the plates incubated aerobically were initially examined for growth after 24hours, the ones without growth were further incubated for up to 48 hours, while those incubated anaerobically were examined after 48 hours. Each isolate of the different colonies from both the aerobically and anaerobically incubated plates were picked for microscopic, biochemical and serological identifications using standard methods.

Sensitivity testing was carried out on each identified organism by touching 4-5 well isolated colonies with a sterile straight inoculating wire. This was inoculated into sterile peptone water which was then poured onto a previously dried sensitivity test agar plate. The excess culture fluid was decanted into a discard jar containing disinfection. The inoculated plate was left to dry and antibiotic disc was applied with a sterile forceps, allowed to and for 10-15 minutes for pre-diffusion of the antibiotics and incubated at 37oC overnight. The zone of inhibition produced after incubation was read using a meter rule[9]. The antibiotics used in this study were commercially produced single and multodiscs obtained from Oxoid Ltd. Basingstore Hampshire England; Interpharma Ltd, Lagos and Abtek Biological Ltd. Liverpool. The concentration of the different antibiotics used, were as shown in Table III. Statistical analysis of all the data were done by Chi-square method.

RESULTS

Among the 1670 patients seen in the different units, 130 (7.78) developed wound infections. Out of the total patients examined, 1248 had post-operative wounds while the remaining 422 had non-operative wounds. Table 1, showed that 80 (6.41) of the post-operative patients developed wound sepsis while 50(11.9) of the non-operative patients had septic wound. Statistical analysis of the data showed that the difference between the post-operative patients with septic wounds and non-operative patients with infected wounds were statistically significant ($P<0.05$).

Category of Patients	No	Those with infected Wounds	Percentage (%)
Post-operative	1248	80	6.4
Non-operative	422	50	11.9
Total	**1670**	**130**	**7.78**

Table I: The Prevalence of Post-operative and Non-operative Wound Infections in Ogun State University Teaching Hospital.

Out of the 130 septic wound examined bacteriologically, 125 gave positive bacterial cultures while only 5 had no growth. Seventy three of the specimens were mono-bacterial while 52 were polybacterial cases two or three bacterial agents. A total of 186 bacterial agents were isolated from all the septic wound examined. *Klebsiella* species 47 (25.3) accounted for the most frequently isolated organisms in all the 3 units while *Enterococcus faecalis* 10(5.4) accounted for the least isolates (Table II). Interestingly, *Klebsiella* species was found to be the most common with wounds from Obstetrics and Gynaecological units while *Pseudomonas aeruginosa* was the most frequent in the orthopaedic wounds (Table II). No anaerobic organism was isolated in all the samples examined in this study.

Table II

THE BACTERIAL ISOLATES FROM DIFFERENT UNITS OF SURGICAL WARDS

WARD	TYPE OF ISOLATES	GRAM NEGATIVE				GRAM POSITIVE				TOTAL
		Klebsiella spp	*Pseudomonas aeruginosa*	*Proteus* spp	*Escherichia coli*	*Staph. aureus*	*Staph. epidermidis*	*Ent. faecalis*		
Obstetrics		21(32.8)	14(21.8)	9(14.1)	5(7.8)	9(14.1)	3(4.7)	3(4.7)		64(34.4)
Gynaecology		9(22.5)	2(5.0)	7(17.5)	4(10.0)	6(15.0)	7(17.5)	5(12.5)		0(21.5
Orthopaedics		17(20.7)	21(25.6)	9(11.0)	8(9.8)	17(20.7)	8(9.8)	2(2.4)		82(44.1)
Total		47(25.3)	37(19.9)	25(13.4)	17(9.1)	32(17.2)	18(9.7)	10(5.4)		186(100)

The sensitivity profile of the isolates to the different antibiotics showed a low susceptibility of the isolates to Ampicillin, Tetracylines, Penicillin and Cotrimoxazole (Table III). However, Gentamicin, Colistin and Tobramycin were more effective against gram negative organism while Chloramphenicol was also more effective against the gram positive organisms. Similarly, all the isolates showed a remarkably high in-vitro susceptibility to the Fluoroquinolones tested (Table III). The analysis of the proforma showed that

Antibiotics	GRAM NEGATIVE ORGANISMS					GRAM POSITIVE ORGANISMS				GRAND TOTAL
	Klebsiella Spp	Ps. aeruginosa	Proteus Spp	E.Coli	Total	Staph. aureus	Staph. epidermitis	Ent. faecalis	Total	
*Ampicillin 25 µg	3(6.38)	1(2.70)	10(40.0)	3(17.6)	17(13.5)	4(12.5)	1(5.56)	2(20.0)	7(11.7)	24(12.9)
*Tetracycline 25 µg	25(532)	13(35.1)	9(36.0)	4(23.5)	51(40.5)	16(50.0)	5(27.8)	5(50.0)	26(43.3)	77(41.4)
*Streptomycin 25 µg	30(63.8)	19(51.4)	14(56.0)	2(11.8)	65(51.6)	15(46.9)	3(16.7)	4(40.0)	22(36.7)	87(46.8)
Cotrimoxazole 25 µg	14(29.8)	3(8.11)	5(20.0)	1(5.88)	23(18.3)	11(34.4)	3(16.7)	6(60.0)	20(33.3)	43(23.1)
Getamicin 10 µg	29(61.7)	25(67.6)	16(64.0)	10(58.8)	80(63.5)	22(68.8)	9(50.0)	4(40.0)	35(58.3)	115(61.8)
Colistin 25 ug	40(85.1)	26(70.3)	14(56.0)	15(88.2)	95(75.4)	NT	NT	NT	NT	95(75.4)
Tobramycin 25 µg	34(72.3)	27(73.0)	16(64.0)	15(88.2)	92(73.0)	NT	NT	NT	NT	92(73.0)
Nalidixic acid 30 µg	38(80.9)	11(29.7)	17(68.0)	11(64.7)	77(61.1)	NT	NT	NT	NT	77(61.1)
Nitrofurantoin 200 µg	40(85.1)	9(24.3)	15(60.0)	14(82.4)	78(61.9)	NT	NT	NT	NT	78(61.9)
Penicillin 1 i.u.	NT	NT	NT	NT	NT	1(3.13)	0(0.00)	0(0.00)	7(1.67)	7(1.67)
Erythromycin 5 µg	NT	NT	NT	NT	NT	22(68.8)	9(50.0)	6(60.0)	37(61.7)	37(61.7)
Chloramphenicol 10 µg	NT	NT	NT	NT	NT	30(93.8)	11(61.1)	7(70.0)	48(80.0)	48(80.0)
Cloxacillin 5 µg	NT	NT	NT	NT	NT	11(34.4)	4(22.2)	1(10.0)	16(26.7)	16(26.7)
Pefloxacin 5 µg	41(87.2)	31(83.8)	23(92.0)	12(70.6)	107(84.9)	27(84.4)	14(77.8)	6(60.0)	47(78.3)	154(82.8)
Ciprofloxacin 5 µg	43(91.5)	34(91.9)	24(96.0)	12(70.6)	113(89.7)	29(90.6)	16(88.9)	8(80.0)	53(88.3)	166(89.2)
Norfloxacin 10 µg	41(87.2)	32(86.5)	23(92.0)	14(82.4)	110(87.3)	29(90.6)	13(72.2)	10(100)	52(86.7)	162(87.1)

NT = Not Tested

* 10 µg of these antibiotics were used for the Gram Positive. The concentration of others were similar.

( ) Percentage Sensitive

Table III

Sensitivity Profile of Organisms from Post-Operative and non-Operative Wound Infections

the prophylactic antibiotics used pre-operatively varied from a single regime to a triple regime. The prophylactic antibiotics used included Ampicillin, Flagyl, Gentamicin, Zinacef®, Ampiclox, Rocephin®, Floxapen®, and Profloxacin. It was evident that majority of the isolated in this study were resistant to most of the antibiotics used prophylatically.

DISCUSSION

Wound infection is an important determinant of the success or failure of surgery. The total wound infection rate of 7.78% obtained for all the units studied is within the range of 4.8-17% reported earlier from other countries[9]. However this rate is much lower than the 23.3% reported from Tanzania[2]. The difference between these two reports may be related to the relatively lower number of patients per wards in OSUTH, in view of the high number of beds allocated to the surgical wards, because of the hospital's strategic location at the T-junction of two express roads. Reports have shown that overcrowding of patients in a ward, may contribute significantly to the high rate of cross infections in an hospital setting[2]. A statistically significant to the ($P<0.05$) is observed between the non-operative and post – operative wound sepsis. The observed low prevalence of post-operative wound infection is not surprising because pre-operative prophylatic antibiotic are used in majority of the studied cases, which corroborates the previous reports that pre-operative prophylatic antibiotic may significantly reduce the prevalence of wound infections.[10]

Klebsiella species, *Ps. aeruginosa and Staphylococcus aureus* are the most common organisms associated with wound infections in this study, an this is similar to the previous report[4,5]. It is however, interesting to note that while Klebsiella species is more associated with the Obstetrics and Gynaecological wounds, Ps. Aeruginosa is the commonest pathogen in the Orthopaedics wounds. The prevalence of Ps. Aeruginosa in Orthopaedics wounds may probably be attributed to the contamination of the wounds with soil and other environmental microbes, as majority of the orthopaedics cases are traumatic wounds from road traffic accidents. Lowbury, et. Al[11]. have suggested that infections with *Pseudomonas* species is usually acquired from poor environmental sources.

The non-isolation of anaerobic organisms in this study is surprising because of the measures taken to recover these types of organisms from the septic wounds. Such measures include the inoculation of Cooked Meat Medium, a medium known to enhance the growth of the anaerobes, the subculturing onto selective medium as well as the incubation of the plates under anaerobic condition. The inability to isolate such organisms could however, be due to the use of dry swabs for the collection of the wound samples and probably a delay in the inoculation of these swabs into the cooked meat medium. It has been reported that wound specimens collected on swabs are usually subjected to drying. This study is shown that there is a need to improve the methods of collecting clinical samples especially when anaerobes could be among the implicating organisms.

The low level of susceptibility of the isolates of Ampicillin, Penicillin, Tetracyclines and Cotrimoxazole is similar to an earlier report in this environment and this have been attributed to the unrestricted use of these agents. However, the remarkable susceptibility of Gram – negative isolates to aminoglycosides and those of the Gram-positive to Chloramphenicol may be due to the lesser use of these antibiotics as a result of their toxic effect. The aminoglycosides have been reported to cause nephrotoxicity and damage to the eighth cranial nerve in human while chloramphenicol have also been reported to cause bone marrow toxicity. Although the fluoroquinolones are very effective against most of the organisms which are resistant to other antibiotics, it is however worrisome to note that most of the isolates that are resistant to the fluoroquinolones are multiresistant to other antibiotics including those used as prophylactic agents. These genes that specify resistance to a number of useful antibiotics have been located on transposons, thus providing possible explanation for the rapid evolution of R-Plasmids that posses a wide variety of antibiotic resistance determinant.

It is evident that most of the organisms isolates in this studies are multiresistant to majority of the common antibiotics including those used as pre-operative prophylatic agents. There is need therefore, to properly monitor the choice of antibiotics to be used as pre-operative prophylatic agents, if they are to serve the purpose of preventing post-operative sepsis.

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PATTERN OF DRUG INDUCED HYPERURICAEMIA IN NIGERIANS WITH PULMONARY TUBERCULOSIS.

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Thirty-one patients with newly diagnosed pulmonary tuberculosis were longitudinally studied between January 1997 and June 1998; each for 6 months to determine the pattern of drug induced hyperuricaemia. Biochemical indices determined were serum urate and 24 hours urinary output of urate, before and during treatment with antituberculosis therapy.

At the end of the 1st and 2nd months of therapy 16 (51.6%) and 15 (48.4%) of the patients respectively were hyperuricaemic. These were statistically significant when compared with the pretreatment data with P value of 0.001 and 0.002 respectively. At the end of the 6th months there was no significant difference in the incidence of hyperuricaemia observed as compared with the pretreatment level.

The pretreatment mean 24 hours urinary urate output was 4.83 mmol/24 hours, the corresponding values at the end of the 1st and second months of treatment was 3.38 mmol/24 hour and 3.74mmol/24 hours. These value are significantly lower than the pretreatment value with P value of P < 0.05 respectively. This however returns to the pretreatment range by the end of he 6th month of treatment with a value of 4.05 mmol/24 hours and P – value of 0.178.

We concluded therefore that while hyperuricaemia is a known cause of nephropathy, the pattern of drug induced hyperuricaemia that occurs in patients with pulmonary tuberculosis is self- limiting and should therefore not hinder us from optimizing the benefits of the drugs.

INTRODUCTION

In man, urate is the end product of catabolism of purine nucleoside, adenosine and guanosine. The elimination of urate from the body is mainly by renal excretion and to a lesser extent by intestinal uricolysis. Alterations in urate metabolism is one of the important complications of drugs used for the treatment of tuberculosis.

Three of the commonly used anti-tuberculous drugs: Ethambutol, Para-aminosalicylic acid (PAS) and Pyrazinamide have been shown to have effects on renal clearance of urate. Pyrazinamid is one of the first line drugs in the current antituberculosis drug regimen is used world wide, and it has remained the most powerful agent causing urate retention. Pyrazinamide exert its effects by suppresing normal tubular secretion of urate in the urine. By this action pyrazinamide becomes the most potent agent causing hyperuricaemia. This inhibition of tubular secreation also leads to reduction in renal elimination of urate by pyrazinamde.

The fact that hyperuricaemia causes renal damage is well established, this established fact informed our decision to examine the pattern of drug induced hyperuricaemia in patients with pulmonary tuberculosis.

MATERIALS AND METHODS

A total of 50 consecutive adults with newly diagnosed pulmonary tuberculosis from the chest clinic of the University of Ilorin Teaching Hospital were admitted to the study. After a detailed medical history and thorough clinical examination, to exclude people with evidence of renal impairment, urinalysis was carried out on every patient in order to define pretreatment renal function.

Patients that were included in the study were sputum positive on direction smear by Zheil Nelson stain for acid fast bacilli: a supportive chest x-ray was also mandatory. Individuals with any of the following conditions were excluded from the study; those with arthrits or findings suggestive of gout, those on uricosuric agent (like oestrogen, phenylbutazone or salicylate) those on hyperuricaemic drugs (like diuretics, salicylate, Nicotinic acid ethanol, L-Dopa and cytotoxic drugs), patients with myeloproliferative disease and those that have been previously treated for tuberculosis.

These patients had the six months, short course anit-tuberculous drug regime. This consist of isoniazid at 15mg/kg body weight,Rifampicin at 20mg/kg body weight. Ethambutol at 20mg/kg body weight and pyrazinamde at 25mg/kg body weight. Pyrazinamide and Ethambutol were used only for the first 2 months of the therapy.

Thirty one age and sex matched healthy controls were recruited (because of the 31% default among patients) also for the study. 5ml of blood was taken on the first day of visit before commencement of therapy. Subsequent samples were collected from the patients at the end of the 1st, 2nd, 4th and 6th months of therapy. Serum was separated from the blood sample and freezed at – 20°C unit assayed. Blood sample was taken from the control subjects for defining the reference range for the study.

Both the patients and the control subjects were also given one clean 2 litre plastic container for 24 hours urine collection. The volume was later recorded and an aliquot taken. Urate concentration both in the serum and in the urine was determined using the modified Caraway 1955 method. Prior to the assay, serum sample was allowed to thaw completely and to adjust to room temperature, while the urine sample was heated to 60°C to allow all urate precipitate to dissolve.

Statistical Analysis

Statistical analyses were carried out in an IBM compatible Personal Computer using EPI Info

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version 6.1. Which is a database and statistical software developed by the Centre for Disease Control, Atlanta, Georgia, United State of America. The percentages of those that developed hyperuricaemia was determined at the end of the 1st, 2nd, 4th and 6th month of therapy. The paired student t-test was used to determine the level of significance of mean urate values as compared with those of controls. The mean 24 hours urinary urate output at various stages of treatment was similarly assessed using paired student t-test.

RESULTS

The study which is longidinal lasted for 18 months One patient died, 4 requested for transfer letters while out of the remaining 45 patients, 31 (69%) completed the study while 14(31%) were lost to follow-up. 31 age and sex matched controls were also studied.

Serum Urate Level

Details of mean value of serum urate level and 24 hours urinary urate output of patients and controls is displayed in table 1. The table shows that the means serum urate level of controls subjects was 0.273 mmol/L (SD =0.777, range 0.119 – 0.427 mmol/4). The mean serum urate levels for the patients before the commencement of treatment, at the end of 1st month, end of 2nd month, end of 4th month and end of 6th month of treatment were: 0.311 mmol/L, 0.454 mmol/L, 0.510 mmol/L, 0.336 mmol/L and 0.330 mmol/L respectively.

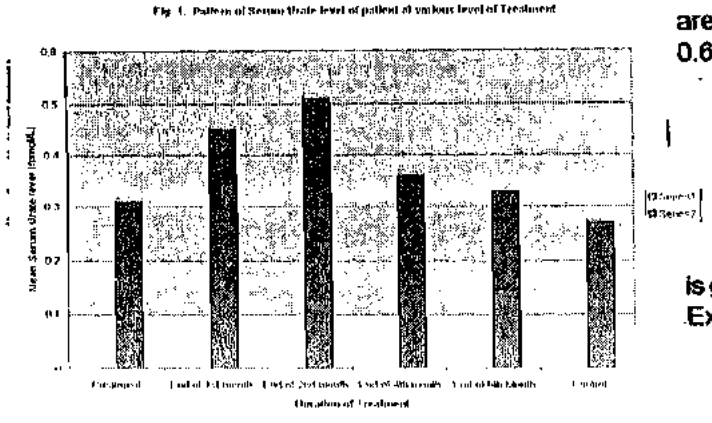
VARIABLE	CONTROL	PATIENTS means (SEM)				
	Mean (SEM)	Pretreatment	End of first month	End of second month	End of fourth month	End of sixth month
Serum Urate mmol/L	0.27 (0.014)	0.31 (0.017)	0.45 (0.022)	0.51 (0.31)	0.36 0.019)	0.33 (0.014)
24 hours urinary urate excretion	3.40 (0.213)	4.83 (0.3349)	3.38 (0.326)	3.74 (0.402)	3.86 (0.407)	4.05 (0.467)

Table 1 Mean values of serum urate level of 24 hours urinary urate output.

Figure 1 shows that the mean serum urate level ncreases from pretreatment level with commencement of antituberculosis therapy and reaches its peak at the end of the second month. However, by the end of the 6th month it has fallen to the pre-treatment range. Using the mean serum urate level of the control population plus 2SD to define the upper limit for 95% of control subjects. Table II shows the percentage of patients with hyperuricaemia at various stages of treatment. Form the table it can be seen that 2(6.5%) subjects amongst the controls and 3(9.7%) amongst the patients before the commencement of treatment had hyperuricaemia. There is no significant difference between these 2 percentage P > 0.89.

However, by the end of the 1st month of antituberculosis therapy, the number of patients with hyperuricaemia has increase to 16(51.6%). This percentage hyperuricaemia is statistically significant (P < 0.001) when compared with the controls. Also 15 (48.4%) patients were hyperuricaemic at the end of the second month of therapy. Again, this is statistical significant when compared with the control group (P < 0.02).

At the end of the 4th and 6th month of therapy the number of patients with hyperuricaemia had dropped to 5(16.15), and 2 (6.5%) respectively. These values are not significantly different from the control (P > 0.65 asd P > 0.89) respectively.



The changes in the percentage hyperuricaemia is graphically shown in figure 2. 24 hours Urinary Urate Excretion.

Fig. 2. Percentage Hyperuricaemia Against the Duration of Treatment

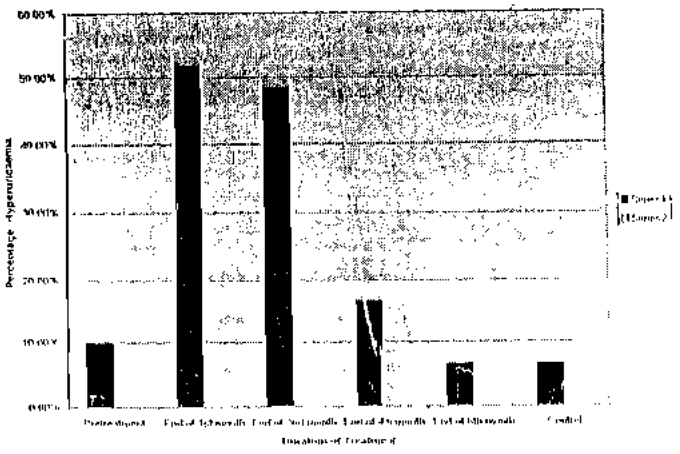


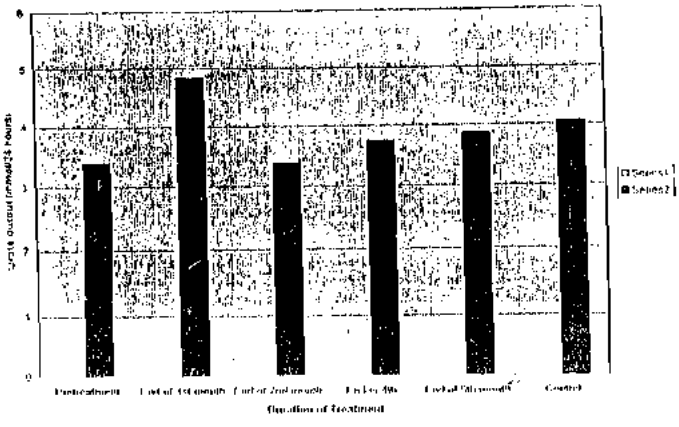
Table 2 shows that the mean 24 hours urinary urate excrtion in the control group was 3.40 mmol/24 hours (SEM = 0.215), while the pretreatment value for the patients was 4.83 mmol/ 24hours (SEM = 0.350). Using student t-test for these two mean values (P > 0.005), there difference is statistically significant. Upon commencing anti-tuberculosis therapy the mean 24 hours urinary excretion reduced to 3.38 mmol/24 hours (SEM = 0.325) and 3.74 mmol/24 hours (SEM = 0.400) at the end of the 1st and 2nd months respectively. These mean values were significantly different from the pretreatment mean value with (P < 0.05 and P < 0.05) respectively. This however returns to the pretreatment range by the end of he 6th month with corresponding values of 4.05 mmol/24 hours (SEM = 0.475) and P – value of 0.178.

Duration of treatment	No of patients with value >0.427 mmol/L	n	% of patients with hyperuricaemia	P-value
Pretreament	3	31	9.7%	0.8946
End of 1st Month	16	31	51.6%	0.0001
End of 2nd month	15	31	48.4%	0.0002
End of 4th month	5	31	16.15%	0.655
End of 6th month	2	31	6.5%	0.8946
Control	2	31	6.5%	

Table II
Scrum Urate Level of patients compared with that ot controls

Figure 3 shows the relative proportions of the 24 hours urinary urate output.

Fig. 3. Pattern of Mean 24 Hours Urinary Urate Excretion in patient on Treatment



DISCUSSION

DeCock et al and many other workers revealed that the prevalence of tuberculosis is increasing in sub-saharan Africa. As Gizybowski put it that "in order to reduce the tuberculosis problem, we must reduce the risk of tuberculosis infection; this is best achieved by finding cases of tuberculosis and curing them permanently with approprite chemotherapy". The six-month short course regimen using isioniazid, rifampicin, pyrazinamide and ethmabutol (or streptomycin) is the common drug regime in use globally now. However, the problem of poor drug compliance remains a very difficult one to solve, in fact Houston et al in their review concluded that "very poor compliance is the rule rather than the exception in operational surveys of tuberculosis programmes". The present study recorded a 31% default rate, a value that is an agreement with the summation of Houston et al.

In addition to poor compliance, the problem of side effects and biochemical derangements, mot importantly hyperuricaemia has been well documented. The use of pyrazinamide in the treatment of pulmonary tuberculosis was first reported by Yeager et al in 1952. They noted the occurrence of pain and

restricted joint motion without redness, in one-fourth of the patients they treated. Also Zierski and Bek reported that 56% of patients on pyrazinamide developed hyperuricaemia. Our study with 51.6% of the patients developing hyperuricaemia is in agreement with above mentioned works on the prominence of hyperuricaemia, as a drug induced problem in patients with tuberculosis.

However, the pattern of hyperuriaemia as revealed by this study that 9.7%, 51.6%, 48.4%, 16.1% and 6.5% of the patients have hyperuricaemia before treatment at the end of the 1st, 2nd, 4th and 6th months of therapy respectively is very instructive: While it confirms the earlier finding that hyperuricaemia is derangement of high frequency, it however shows also that the serum urate level returns to normal by the end of the sixth month of therapy.

This study recorded significantly higher 24 hours urinary urate output by patients before treatment when compared with the control group. This could possibly result from the diseased state impairing the extrarenal pathway of urate excretion or enhancing tubular secretion of urate like nephrotic syndrome does to creatinine secretion. However, because of inhibition of tubular secretion of pyraziamide there was a significant reduction in the 24 hours urinary urate output. This finding is in agreement with the findings of Elland and Haslam. They also observed a significant decrease in the 24 hours urinary urate output n patients on pyrazinamide. The lower 24 hour urinary urate excretion at the end of the 6th month of therapy when compared to the pretreatment 24 hours urinary urate excretion has been attributed to activation of extrarenal routes by the hyperuricaemia associated with the treatment.

We therefore conclude that the drug induced hyperuricaemia seen in patients with pulmonary tuberculosis is transient and also helps in opening up the extrarenal pathway of urate excretion blockd by the disease itself before treatment. We however suggest that further studies be done to assess the effect (if any) of the transient hyperuricaemia on the renal function in patient with pulmonary tuberculosis on treatment.

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GENITAL ULCER DISEASE IN ILORIN, NIGERIA

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This is a review of 32 consecutive cases of patients with genital ulcers or who were repeatedly reactive to serological tests for syphilis (STS) at the Venereology Clinic of the University Teaching Hospital, Ilorin, Nigeria, between January 1993 and April 1995. The criteria for diagnosis of the various conditions included the history, clinical presentation and the results of laboratory investigations. The commonest cause of genital ulcers was chancroid, accounting for 6(18.7%) of the 32 cases. Other common causes were lymphogranuloma venereum (LGV), gential herpes and primary syphilis, each acounting for 12.5 percent of the cases. An unusual presentation of oro-genital aphthosis, with hyperkeratosis and paraesthesia of a localized area on the palm, in addition to the usual genital and oral lesions was reported. Also reported were cases of perigenital cutaneous onchocerciasis and a case of leprosy presenting as chronic biological false positive (BFP) to STS. Patients with chancroid responded favourably to treatment with ceftriaxone (Rocephin) and so was the hyperkeratosis of oro-gential aphthosis to topical treatment with flumethasone pivalate/salicylic acid ointment (Locasalen). The importance of histological technique for making the diagnosis of some tropical conditions affecting the genitals was highlighted, and the exercise of caution in interpreting the results of STS was advocated.

INTRODUCTION

There have been various reports on the prevalence of the various sexually transmitted diseases (STDs) from different centres in Nigeria (1, 2, 3). With the setting up of new University Teaching Hospital (UITH) with a Venereology Clinic at Ilorin, Nigeria, a lot of attention was initially devoted to Public Health Education Programmes on STDs on the local radio and television channels. This made it easy for people to seek medical attention for these conditions. The report of our preliminary experience has been published elsewhere (4). The present communication is on the aetiology, clinical manifestations and management of genital ulcer disease at the University of Ilorin Teaching Hospital, Ilorin, Nigeria.

MATERIALS AND METHODS

All patients attending the venereology Clinic of the University Teaching Hospital, Ilorin, Nigeria between January 1993 and April 1995 with a complaint of genital ulceration, or found to be repeatedly reactive to serological tests for syphilis (STS) were included in the present report.

The criteria for the diagnosis of the various conditions were as follows:

(a) Chancroid was dagnosed on the basis of the clinical presentation and on the demonstration of gram–negative coccobacillary forms in "Schools of Fish" appearance by the method described by Kraus and associates (5).

(b) Lymphogranuloma venereum (LGV), genital herpes, condyloma acuminatum and oro-genital aphthosis were diagnosed on the basis of their history, clinical presentation and negative STS.

(c) The diagnosis of painless indurated ulcers, with spirochaetes on dark-ground microscopy; or repeated positive STS with or without genital sores at the time of examination provided other causes of biological/ false positives (BFP) were eliminated.

(d) Other conditions affecting the gentialia like enchocerciasis and Hansen's disease were diagnosed by means of histology slides on properly taken biopsies.

Management of cases

Patients with chancroid were treated with either double strength trimehoprim/ sulphamethoxasole for 3 weeks or with a single injection or ceftriaone (Rocephin) 1gm given intramuscularly or inravenously. Patients with primary syphilis were treated with daily injections of procaine penicillin 500,000 units for 15 days with 1gm probenecid orally. Those with post-primary syphilis were treated with injections of Benzathin Penicillin, 2.5 mega units followed by twice weekly injections of 1.4 megaunits for 3 weeks.

Patients with LGV were treated with either Sulphadiasine 2gm daily in 4 divided does alone, or in combination with daily injections of streptomycine1gm for 10days. Herpes genitalia was treated local applications of Saline water, and when secondarily infected, single strength trimethoprim/ sulphamethoxazole was given for one week.

The treatment of oro-genital aphthosis was with tetracycline, 2gm in 4 divided does, daily, for one week with Vitamine B complex tablets; their hyperkeratotic conditions were treated with topical flumethasone pivalate/salicylic acid ointment (Locasalon). The treatment of Onchocerciasis and Hensen's disease were those of the systemic conditions.

RESULTS

32 patients were found to have genital ulcer disease during the 28 month-study period: 28(87.5%) were males and 4(12.5%) were female. They were aged between 15 and 54 years, but 25(78.1%) were aged between 20 and 38 years (Table 1). Chancroid (18.7%) was the most common cause of genital ulceration in Ilorin.

*Corresponding Author

Age (Years)	No. of Patients			
	Male	Female	Total	%
15 – 19	1	1	2	(6.3)
20 – 24	8	1	9	(28.1)
25 – 29	6	2	8	(25)
30 – 34	5	-	5	(15.6)
35 – 39	3	-	3	(9.4)
40 and above	5	-	5	(15.6)
TOTAL	28	4	32	(100)

Table 1:
Age Distribution fo Patients with Genital Ulcer Disease in Ilorin, Nigeria

(Table 2). Other causes were primary syphilis, genital herpes, lymphogranuloma venereum and condyloma acuminatum, each responsible for 4(12.5%) of the 32 cases. Oro-genital aphthosis accounted for 3(9.4%) of the cases. A patient presented with a rash in the suprepubic region: the biopsy showed it was due to *Onchocerca volvulus*: while another patient with apersistently positive STS was found by ear-lobe biopsy to be suffering from Hanson's disease.

Patients with chancroid presented with a short incubation period of 1 to 7 days. 4 of the 5 patients with chancroid were treated with ceftriaxone with a very favourable response.

Of the 4 patients with primary syphilis one had gonorrhoea as well, he came because of the urethral discharge and dysusia.

Figure 1 is the primary chancre from this patient.

One of he 3 patients wit oro-genital aphthosis had paraesthesia and hyperkeratosis in a localized area on his right palm in addition to the usual genital and oral lesions.

Figure 1: Showing Primary Chancre on the Penis

Clinical Diagnosis	No. of Patients			
	Male	Female	Total	(% Total)
Syphilis:				
Primary	4	-	4	(12.5%)
Post primary	2	1	3	(9.4%)
Chancroid	5	1	6	(18.7%)
Herpes genitalis	4	-	4	(12.5%)
Lymphogranuloma Venereum	4	-	4	(12.5%)
Condyloma acuminatum	2	2	4	(12.5%)
Oro-genital aphthosis	3	-	3	(9.4%)
Others	4	-	4	(12.5%)
TOTAL	28	4	32	(100%)

Table II
Genital Ulcer Diseases in Ilorin, Nigeria

1 patient each presented with cutaneous onchocerciasis, candidal balanitis, chronic BFP due to Hansen's disease, and multiple infection from gential herpes and chancroid.

DISCUSSION

This report has shown that Chancroid is he commonest cause of genital ulcer disease in Ilorin, Nigeria, being responsible for 18.7 percent of all diagnosed cases. Primary syphilis, genital herpes, lymphogranuloma venereum and condyloma acuminatum occurred at an equal frequency of 12.5 percent. The actual prevalence of condyloma acuminatum in Ilorin should be higher than the present figure obtained from the Venereology Clinic, because many women with such complaints reported at the gynacology clinic for management.

The method described by Kraus and associates (1) has made easy the identification of Haemophilus ducreyi from chancroid ulcers. Ceftriaxone (Rocephine) has also been found to be effective for the single –dose treatment of chancroid.

It is uncommon in other centres in Nigeria for patients to be seen with the primary chancro of syphilis and hence the resort to sero-epidemiologic surveys (6, 7). This is because syphilitic ulcers are painless and heal without leaving during the course of the present study because of multiple infections and secondary bacterial infections leading to painful ulcers. Also the prominence given to sexually transmitted diseases by the local television station in Ilorin helped to educate the public on the need to seek medical attention.

Three cases of post-primary syphilis and a case of chronic biological false positive (BFP) due to Hansen's disease were diagnosis on the basis of serological and histological tests. One has to interpret the results of STS with great caution because of the presence of BFPs, a subject that has been discussed by other authors (8, 9, 10).

Genital herpes is a condition that has not been much reported in Nigeria because of the death of virology culture techniques. However, Sogbetun and associates (11) demonstrated that a significantly high proportion of children and young adults in Ibadan have Herpes simplex type II antibiodies in their blood. The condition is easily diagnosed by the presence of vesicles with erythematous base and superficial ulcers. Patients with LGV usually attend the clinic because of the painful inguinal bubo formation, although rarely the primary chancres may b seen at the first visit.

Balanitis is not common in Nigeria because most males are circumcised. Only one case was encountered during the present study and it was in an uncircumcised patient. The only case of perigenital cutaneous enchocerciasis reported here was only conclusively diagnosed by histological techniques. Adeyemi – Doro and associates (12) have similarly reported a case of pergenital cutaneous schistosomiasis in Ibadan. Although chancroid was found to be the commonest cause of genital ulcerations in Ilorin, primary syphilis, genital herpes and LGV were also common and occurred at equal frequencies. An unusual presentatio of oro-gential aphthosis with hyperkeratosis and paraesthesia of a localized area on the palm in addition to the usual genital and oral lesions was also reported. The hyperkeratosis responded well to local application of flumethasone pivalae/salicylic acid ointment. The importance of histological techniques for making the diagnoses of other tropical conditions like onchocerciasis, schistosomiasis and Hansen's disease that may localize around the genitalia was lightlighted; and the correct use and interpretation of STS was advocated.

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INTESTINAL HELMINTHIASIS AMONG MALNOURISHED SCHOOL AGE CHILDREN IN PERI-URBAN AREA OF IBADAN, NIGERIA

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This study was carried out between November and December 1999 in a peri urban area of Ibadan in Lagelu Local Government Area to determine the prevalence rate of intestinal helminth infection among malnourished school children. Stool samples and finger prick blood samples were respectively collected from pupils in form 3 to form 6 for analysis. The relationship between infection and their nutritional status was determined using such parameters as weight, height, age, sex, arm to head circumference. The haematocrit value and worm density in subjects were determined to rate level of infectivity in the individual.

The study shows that there are three common intestinal worms in the area Ascaris lumbricoides has the highest prevalence rate of 40.7% followed by Tribchuris trichiura (4.8%) and hookworm (4.4%).

Age and sex gender made no significant difference in the distribution of infection (P >0.05). however, there was a significant effect on weight and height by worm burden (P <0.05). Worm density impact negatively on the blood level in body thereby precipitating anaemia in the children. Epidemiological factors affecting the infection among the subject is discussed.

The strategies for control of the infection are discussed. It is recommended that the public be adequately health educated on the epidemiology of the infection through the mass media and community health talks. The academic curriculum in schools should include epidemiology and control of parasitic infestation. Periodic mass treatment of children is advocated.

INTRODUCTION

Parastic disease are common in the developing countries and are of major health hazard because of their high prevalent rate and their effect on both nutritional and immune status of the population (1) Intestinal parasitic infections mainly affect the parasitic and mental development of children who are most vulunerable, (2) Intestinal parasitic infections are distributed throughout the world. Ascariasis, hookworm infection and trichuriasis are among the most common infections in the world: other parasitic infections like abdominal angiostringyliasis, intestinal capillariasis and stronglodiasis are of public health concern (1,3).

Intestinal parasites have been shown to cause poor appetite, intestinal abnormalities, poor absorption or increased loss of nutrient, which may result in protein-energy malnutrition (4). Chronic parasitism in a population will only not jeopardize their health, it will also render them susceptible to other diseases, weaken them, make them less effective thereby reducing their productivity level and academic performance (2) and as a rule this may lead to low contribution in moving the nation forward. Therefore, regular monitoring of the prevalence of parasitism in such area is essential as a prelude to effective management and control of these infections.

Thus, this study is designed to determined the prevalence of intestinal helminthic infection among school children in a peri-urban community of Ibadan, Nigeria to form a baseline data for evaluation and control of the infection.

MATERIALS AND METHODS

Study area: the study was conducted in November and December 1999 in a peri-urban community situated 10 kilometers northeast of the Ibadan metropolis in Lagelu LGA of Oyo State Nigeria in the rain forest zone. The community has a population of about 7,000 (Nigeria census of 1991) of predominantly peasant farmers, though some are engaged in distributive trade and civil service work.

Sample Selection and collection: For the purpose of this study all the five primary schools in the area were enlisted. Only pupils in primary 3 up to primary 6 were selected as recommended by WHO. The consent of parents was taken before sampling. A total of 248 pupils of both sexes were examined in the 5 schools – 27, 34, 66, 41 and 80 respectively. All samples were collected with the full cooperation and assistance of teachers pupils and parents.

The subjects were given a stool receptacle on the eve of the day of examination with specific instruction to collect in the morning while blood samples were taken by finger prick into heparinized capillary tube sealed with a plasticine.

Other data collected included age, sex, class, schools, weight and height and arm circumference.

Sample Analysis

The faecal samples were examined for parasites using the method described by WHO (5): Direct level saline preparation of stool smear was examined for ova of parasite under the microscope. Negative samples were subjected to concentration method as described (5).

Haematocrit value of patient was determined using the microhaematocrit method described by Dacie and Lewis (6).

The data analysis was done by using computer with SPSS package to determine correlation coefficient, chi-square were applicable.

RESULTS

Intestinal helminthiasis among school age children by age and sex in peri-urban Ibadan is shown in

Corresponding Author

Table 1 Result shows that peak infection (58.8%) occurred in the age group 9-11 years old followed by those in age bracket 15-17 years old (72.20%). Data revealed that there is no significant difference in prevalence by age (P >0.05). Similarly there is no statistical difference in sex of pupils as regard disease prevalence (P>0.05) The most prevalent parasite is *Ascaris* (40.7%) followed by *Trichuris* (4.8%). Data reveal significant difference in the occurrence rate of parasitic disease among schools (P <0.05).

AGE	SEX	No Exam	A. Lumbricoides No Infec.	% Infect.	Hookworm No. Infect.	% Infect.	T. trichiura No. Infect	% Infect.	E. vermicularis No Infect.	No Infect.	S. stercoralis %	%	Total No Infect.	%
6-8	M	9	3	15.7%	0	0%	0	0%	0	0%	0	0%	3	36.8%
	F	10	4	20%	0	0%	0	0%	0	0%	0	0%	4	
9-11	M	55	20	16.5%	5	4.30%	2	1.7%	0	8%	0	0%	27	58.80%
	F	64	34	28.60%	4	3.40%	4	3.4%	1	0.8%	0	0%	43	
12-14	M	38	16	17.40%	1	1.10%	0	1.1%	0	0%	1	1.1%	18	38%
	F	94	14	15.20%	1	1.10%	2	2.2%	0	0%	0	0%	17	
15-17	M	12	6	33.30%	0	0%	2	1.1%	0	0%	0	0%	8	72.2%
	F	6	1	22.2%	0	0%	1	5.6%	0	0%	0	0%	5	

Table 1: PREVALENCE OF INTESINAL HELMINTH INFECTION AMONG HE SCHOOL CHILDREN BY AGE AND SEX

Prevalence of helminthiasis by schools is shown in Table 2. The difference in the rate of parasitic infections by school is statistically significant (P <0.05). The highest prevalent rate in a school was 85% followed by 79% while one of the schools recorded 18.5%.

	SEX	Number Exam	A.lumbricoidses No.(%) Infect	Hookworm No. (%) Infect.	T. trichiura No. (%) Infect.	E. Vermicularis No. (%) Infect.	NO.(%) Infect.
St. Stephen Anglican Primary School 1 Alegongo (Peri Urban)	M	13	3(11.1)	0(0)	0(0)	0(0)	3(18.5)
	F	14	2(7.4)	0(0)	0(0)	0(0)	2
St. Stephen Anglican Primary School 2Alegongo	M	20	12(35)	2(6)	0(0)	0(0)	16(79)
	F	14	8(24)	2(6)	0(0)	0(0)	11
Ebenzer Anglican Primary School Orunde Ahua (Rural Area)	M	30	18(27.3)	3(5)	1(2)	0(0)	24(85)
	F	36	24(36.4)	5(7)	0(0)	0(0)	32
IDC School Akobo (Peri Urban)	M	24	7(17)	0(0)	0(0)	0(0)	7(46.3)
	F	17	12(29)	0(0)	0(0)	0(0)	12
IDC School Akobo (Peri Urban)	M	38	10(16)	0(0)	0(0)	1(1)	13(28)
	F	42	5(8.3)	0(0)	0(0)	0(0)	15
Total			248(40.7)	12(8.05)	1	1	107(43)

TABLE 2: PREVALENCE OF INTESTINAL HELMINTHIC INFECTION AMONG THE SCHOOL

Table 3 showed the degree of weight difference in both male and female school children compared with a marked depreciation due to the rate of infection. The weight loss affects all the children, with a marked depreciation due to the rate of infection. The weight loss increases along the age of the children but well pronounced in the age group 15-17 years old.

Generally there is a weight loss compared to the standard and this is linearly related to the rate of infection by sexes and ages with no significant difference (P >0.05), which implies that many children are nutritionally unstable. However the data analysis show significant difference in the weight loss and the infectivity rate (P <0.05), which are inversely proportional.

AGE	SEX	No. Exam.	Working Weight Mean value (kg)(W1)	Standard Weight Mean value	Weight Difference ($W_1 - W_2$)	No. Infect.	% Infectivity
6-8	M	9	20.3	23.14	2.84	3	33.3%
	F	10	19.4	23.3	3.9	4	40%
9-11	M	55	29.1	32.4	3.3	27	49%
	F	64	29.3	33	3.7	43	67%
12-14	M	38	27	45	18	18	47%
	F	94	32	48	16	17	32%
15-17	M	12	33	60	27	8	67%
	F	6	35	60	25	5	83%

Table 4 shows the degree of height difference with standard for both sexes. Result shows that infection retards growth rate of the children irrespective of the sex – an indicator of nutritional instability ($P < 0.05$).

AGE	SEX	No. children Exam.	Working Height (CM) Mean value	Standard Height (CM) Mean value	Difference	Total No. infect.	Infectivity
6-8	M	9	41	48.1	7.1	3	33.3%
	F	10	40.24	48	7.8	4	40%
9-11	M	55	43.2	55	12	27	49%
	F	64	43	55	12	43	67%
12-14	M	38	47	61	14	18	47%
	F	54	47	62	15	17	32%
15-17	M	12	46	69	23	8	67%
	F	6	48	64.03	16.02	5	83%

TABLE 4: RELATIONSHIP OF AGE, SEX AND WEIGHT COMPAED WITH THE STANDARD MEAN HEIGHT/AGE DISTRIBUTION OF WITH COMPARED WITH THE STANDARD.

The result of the mean haematocrit value of both male and female school children (Table 5) shows that the higher the disease prevalence the lower the haematocrit value of the child ($P<0.05$).

AGE	SEX	NO. Exam.	Mean Heamatocit value %	No. Affected	% Infectivity.
6-8	M	9	34	3	33.3%
	F	10	32	4	40%
9-11	M	55	31	27	49%
	F	64	33	43	67%
12-14	M	38	32	18	47%
	F	54	36.	17	32%
15-17	M	12	33	8	67%
	F	6	33	5	83%

TABLE 5: DISTRIBUTION OF MEAN HEAMATOCRIT VALUE BY AGE

The prevalence and intensity categories of intestinal helminth is shown in table 5 of the 40.7% with *Ascaris* 40.6% had light infection with only 0.1% having moderate shown that all the 4.4% of the population infected with. Hookworm infection had light infection while 4.5% afflicted by *Trichuris* had moderate infection.

PARASITE	ASCARIS LUMBRICOIDES	HOOK WORMS	T TRICHURA
PREVALENCE	4.70%	4.40%	4.80%
INTENSITY (EPG)	-	-	-
NEGATIVE	59.30%	95.60%	95.20%
LIGHT	40.60%	4.40%	0.30%
MODERATE	0.10%	0.0%	4.50%
HEAVY	0.%	0.0%	0%

TABLE 6: PREVALENCE AND INTENSITY CATEGORIES OF INTESTINAL HELMINTH

A. Lumbricoides
Light – 1-4999epg
Moderate – 5,000 –49999epg
Heavy – 50,000 + epg

Hookworm
Light – 1-999epg
Moderate – 2,000 –9999epg
Heavy – 50,000 + epg.

Trichuris trichiura
Light – 1-999epg
Moderate – 1,000 –9999epg
Heavy – 10,000 + epg.

DISCUSSION

Morbidity due to soil transmitted helminthiasis has remained major problem in the study area with an over all prevalence rate of 43%. This study has shown that *Ascaris lumbricoides, T. trichiura,* hookworm, S. *stercoralis E. vermicularis,* were the commonest parasites isolates. This report is not significantly different from some previous records (7, 8). The high prevalence is not unconnected with the fact that poor sanitation, lack of knowledge on health care in the study area, compared with poor personal and environmental hygiene practice.

The commonest human gastro-intestinal parasite among the study population was *A lumbricoides* with a prevalence rate of 40.7% that is significantly lower than 43.7% infection rate among subjects in Oluyole L.G.A., Oyo State of Nigeria (9) but accord well with the 40.0% prevalence rate reported among school children in Ilorin (10). The high morbidity due to *Ascaris* is a reflection of environmental contamination and unsanitary life style in the study area. This is a dangerous trend as intestinal parasites have been shown to impact deleterious effect on children especially those of school age (2,11, 12). *Ascaris* has been implicated with nutritional states of the patients. For instance, woodruff (12) observed that the presence of *Ascaris* in children is often associated with poor nutritional states. Gupta (11) believed that *Ascaris* contributed significantly to malnutrition among India children. He submitted that control of such infection could be a valid and practical method of nutritional intervention in communities with high prevalence of both malnutrition and intestinal helminthes.

Our study has shown that light infection is more common among children with an average of 2000 epg, a clear reflection of chronic infection characterized by low number of composted eggs without prejudice to the actual burden (2). This light infection may not be unconnected with (demure) during abuse among the study population as earlier observed by Adeyeba and Akinlabi 92). The subjects confirmed that the local health official once carried out a dowering flurry on the children I not too a distant past.

The relatively low rate of hookworm (4.4%) and Trichuris (4.8%) infection respectively is constant with the report of Agi (13) – 3.5% and 5.0% respectively in higher Delta area of Nigeria. It was observed that virtually all the children in the study render them more vulnerable to soil transmitted helminthic infection – hookworm and strongyloides –as they are throne to constant contact with the soil contaminated with infection stage of the parasite.

Although the study population had light hookworm infection the impact on their health cannot be overlooked. According to Stortzfius et al (17), light intensity infection are related to a loss of less then 2mg of heamoglobin per gram of faeces in African children who are infected heartily with *Necator americanus.* As the entire study population positive for strongyle egg were infected with this parasite specie, as shown in this study, then the infected individual suffers a loss of about 2g% haemoglobine per gram of faeces and by implication they are clinically aneamic. This assertion has been confirmed in this study as the subjects were found to have very low haematocrit vlaue (15,16).

This study has shown that the weight of the subject has been adversely affected by the parasitic infection that has given a picture of low weight compare to their height and age, a clear indication of adverse effect of infection on the subject. Therefore it appears from this study that the number of worm harbourd by individual ahs directly affected nutritional status of subject. There is an adverse effect on normal growth using weight and height as growth marker. Our inference has been supported by Stephenson (17) who also reported that worm antagonize the child metabolism and diminished appetite which reduces the weight that children with heavy warm infection are usually of substandard weight and height and shows common symptoms of malnourishment. For this category of people, case management should include the treatment of positive cases, an information, education and communication (IEC) strategies has a great impact and should be extensive implemented.

The prevalence of the third commonest parasite, Trichuris trichiura was 4.8%. This finding conformed tot he previous report of 5% in the Niger Delta area (13). It was observed that the subjects in the area of study are fond of eating unwashed fruits picked from the soil that may have been contaminated with the infective stage of this parasite. It is a common sit to see children and adult like eating food wrapped with papers, leaves etc picked from doubtful sources: the practice which may promote the transmission of this parasitic infection. The worm burden in subject revealed a moderate infection using the criteria of HWO (18) with the attendant adverse effect on the normal growth of the subject, which may manifest clinically as malnutrition.

There is a general picture of high prevalence of low haematocrit value among the subject. Some other workers (8) also think likewise that the parasite infection is related to severe anaemia which present clinical picture like weight loss, occasionally recta prolapse with worms embedded in the mucosal and extreme cachexia.

The prevalence of *Strongyloides stercoralis* (0.4%) and *Engterobius vermicularis* (0.4%) were very low. The low prevalence of *Strongyloides* may not be unconnected with the climate and weather at the time of the study – the ground was so dry which may have contributed immensely to the unlivable infective stage which were unable to penetrate the unbroken skin the portal of infection.

This study has shown that more females than males were infected with intestinal parasites, though differences was not significant. Our report also shows

that the age difference has no significant effet on prevalence despite this fact, we observe a gradual decrease in prevalence rate with increasing age group. This might be due to change in attitude, habits and more awareness towards personal hygiene and knowledge of health education.

It has been shown that intestinal parasites have deleterious effect on nutritional status of the subject through competition for nutrients, pathological changes, poor utilization of macro and micro nutrients, malabsoption of nutrient loss, altered metabolism, diminshed appetite, lowered immunity, sub-standard weight and height given sign of malnutrition (2, 4, 17) in the children, there is growth retardation and reduced learning ability (19,20).

This study has shown that intestinal helminthiasis among malnourished school age children in health problem in the area and Nigeria at large.

In view of the considerable morbidity and the public health significance of these parasitic infections, coupled with the fact that children are the future of any nation, it then becomes necessary as a matter or urgency to control these infections in the community. It is therefore suggested that only well organized health education programmed on personal hygiene and community health and adequate supply of portable and safe water in addition to the provision of basic sanitation facilities like toilet, shall bring a long lasting solution to the menacing problems of the infection. The community leaders head of schools, the staff, the pupils and also the local authority have a vital role to play in the rescue operation. In accordance with the recommendation of the WHO (21) a mass treatment of the entire study population is to be advocated in view of the magnitude and scope of the infection. Periodic deworming of children should form part of child care in the area.

Effort should be geared up to improve the nutritional status of the children by parents and the authority concerned as nutrition play an important role in infection by parasites and in severity of the disease produced. The interaction of infection, nutrition and immunity suggest the reciprocal for example the intensification of the worms malnutrition and immunosupression.

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ONCHOCERCIASIS IN COMMUNITIES IN FOREST ZONE, SOUTH WEST NIGERIA: PREVALENCE AND DIAGNOSTIC METHOD FOR RAPID ASSESSMENT

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To determine the prevalence of onchocerciasis and diagnostic method for a rapid assessment of the disease in two Local Government Area (LGA) of Osun State, Nigeria.

Method: the study area was randomly selected using lottery method. The study subjects are from all works of life of both sexes and not below the age of 10. Structured questionnaire was administered to obtain vital epidemiological information from study subjects. Skin snip as standard method of diagnosing onchocerciasis was done using method as described and was compared with other potential diagnostic indicators. The methods of sample analysis are described. Data were analysed by using correlation coefficient, Duncan multiple range test, and analysis of variance where appropriate.

Results: of the 240 subjects examined, 35.4% were skin snip positive. Whereas infection increases with age of subjects (P<0.05), the difference in the infection among male and female subjects is not significant (P > 0.05). Of all the methods of diseases assessment, only nodule palpation method correlate well with the standard diagnostic method – skin snip.

Conclusion: the merit of nodule palpation and criteria for the determination are discussed. Nodule palpation assessment method (NPAM) was recommended as an alternative rapid assessment method of large scale surveillance of onchocerciasis in Nigeria. NPAM could be used for monitoring and evaluation of the current programme of mectizan distribution in the country.

INTRODUCTION

Onchocerciasis is a major public health problem in West Africa. Although the onchocerciasis control programme (OCP) in the Volta River Basin areas and Nigeria have reported success, the problem persists in neighbouring countries. In Nigeria, onchocerciasis is nation wide in terms of geographical spread and importance (1).

Although ochocrciasis has existed in Nigeria for centuries, it was not until 1908 that the first report was published (2). Many authors (1,3,4) had since contributed to the existing knowledge of its natural epidemicity. Inspite of this, the distribution of the infection and its vector in Nigeria are far reach than currently known. For several poorly accessible areas of high endemicity exist in different geographical locations of this country where numerous infected rural resident were unidentified, undiagnosed and untreated (4).

The merits of the use ivermectin (mectizan) in the control of onchocerciasis are many, as many communities in Nigeria have benefited. The distribution of the mectizan is blindly guided by assumption based on previous knowledge of the disease in the country. The principle and procedure in disease control – preintervention and post intervention surveillance are more often than not ignored or carelessly done in some quarters. It is essential to monitor and evaluate the control. This study is designed to determine the alternative rapid assessment method of diagnosis of onchocerciasis in Iwo LGA of Osun State, a forest zone, South West Nigeria. In doing this the work report the prevalence of onchocerciasis in the area.

MATERIALS AND METHODS

Study Area: the study was carried out in nine communities in Iwo Local Government Area (LGA) of Osun State, Nigeria between November and December 1999. The study area is in the rain forest zone with a major river – Oba passing through. The major occupation of the people, which are predominantly Yoruba speaking is farming.

Sample selection method: the LGA was randomly selected from the 30 LGA in Osun State using lottery method. Nine villages were subsequently selected randomly. These villages were then paid advocacy visit to enhance success of the study in the area. The Local Health officials served as guides and helps.

Sample collection: in all, 240 subjects of both sexes whose age is not below 10 years old were enlisted. Skin snips and urine samples were aseptically collected from subjects using the method described by Basile (6). Care was taken in ensuring that blood did not contaminated sniped skin. Subjects were examined for the presence of leopard skin, nodules, such information on purities, excoriation, sex, age, occupation, history of infection were obtained by the administration of structured questionnaire.

Sample Analysis: Basically, samples were analysed using the methods described by Basile et al (6).

Skin snip: Blood lancet and razor were used to snip skin (1.5mm bite) from the iliac crest after swabbing with 70% alcohol. This was placed in saline preparation and later the aspirate was placed on microscope slide with cover slip applied and examined under compound microscope. Counts of microfilariae (mf) were made and mf density was calculated by dividing the number of mf counted by 0.65 (the average weight of 49 skin snip specimen pooled and weighed after blotting dry on filter paper). Intensity of infection for each individual was calculated as the average of two snips divided by 0.65 as described by Sokal and Rohlf (7). The community

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microfilarial load (CMFL) was determined by using the natural logarithm of the mean individual intensity plus 1 (i.e. log x + 1). The CMFL includes both positive and negative subjects.

Nodule assessment: each subject was palpated from head to toe using both hands with special attention to ribcage, iliac crest, greater trochanters, knees and scapular for nodules.

Leopard skin/Depigmentation assessment: this was recorded as present or absent after examining the lower limb for the presence of characteristic depidgmentation on the skin.

Urine examination: Urine sample was collected in 50ml capacity bottle and allowed to stand for 2 hours to 7 hrs. the sediments were examined microscopically for the presence of mf.

Degree of puritus: this was confirmed from subjects and reported as present or absent.

Excoriation: this obvious self-inflicted scratch marks explained by Basile (6) and reported as present or absent with special attention to trunk, buttocks and the lower limbs.

Data generated in each of the parametric methods were correlated with those of skin snip (the standard predictor of infection). The method that correlated with skin snip was deemed to be the method of choice is alternative on the field.

Data analysis: this was done using correlation coefficient, analysis of variance and Duncan multiple range test.

RESULTS

Prevalence on onchocerciasis in Iwo LGA

Table 1 shows the prevalence of onchocerciasis using sking snip method by sex and age of the 240 subjects examined, 35.4% were positive. Result shows that infection rate increases with age. The difference in infection rate between age group is statically significant ($P<0.05$). The results also shows that more males than females are infected but eh difference is statistically insignificance ($P<0.05$).

Prevalence of Onchocerciasis by village and method and of Assessment

Table 2 shows prevalence of onchocerciassis in each of the 9 villages by assessment method. Skin snip positivity shows that disease is unevenly distributed among the villages. Data reveals the intensity of infection (0.8-1.2) as reflected by community microfilaria load (CMFL) in the community.

TABLE 2: PREVALENCE OF ONCHOCERCIASIS BY VILLAGE AND METHODS OF ASSESSMENT

VILLAGE	TOTAL EXAM	SKIN SNIP (%)	LEOPARD SKIN (%)	NODULES (%)	PRURITUS (%)	EXCORIATION (%)	MICROFILARIURIA	CMFL
1. Agberire	138	33.3	11.6	13.8	49.3	4.4	16.2	0.9
2. Obajoko	22	22.7	27.3	13.6	45.5	13.6	9.1	0.8
3. Eleko	18	38.9	33.3	16.7	14.4	5.6	11.1	1.0
4. Otogunebi	28	35.7	17.9	7.1	39.3	7.1	3.6	0.9
5. Idiroko	10	30.0	30.0	30.0	50.0	20.0	10.0	1.0
6. Asipa Oniyangi	2	50.0	50.0	50.0	0	0	0	1.2
7. Adana	6	50.0	16.7	33.3	50.0	0	16.7	1.1
8. Asolo Isale	2	50.0	50.0	50.0	50.0	0	0	1.2
9. Joluegan	14	42.9	7.1	28.5	42.9	21.4	7.1	1.0

*CMFL = Community Microfilarial Load (it include both negative and Positive subject).

Age Group (Year)	MALE No. Exam.	MALE No Pos	MALE % Pos	FEMALE No.' Exam.	FEMALE No Pos	FEMALE % Pos	TOTAL No. Exam.	TOTAL No Pos	TOTAL % Pos
10-20	27	3	11.1	22	1	4.5	49	4	8.2
21-30	12	4	33.3	14	2	14.3	26	6	23.1
31-40	9	1	11.1	29	9	31.0	38	10	26.3
41-50	33	15	45.5	21	15	71.4	54	30	55.6
51-60	21	10	47.6	15	5	33.3	36	15	41.7
>60	24	12	50	13	8	61.5	37	20	54.1
All	126	45	35.7	114	40	35.1	240	85	35.4

TABLE 1: PREVALENCE OF ONCHOCERCIASIS USING SKIN SNIP POSITIVITY IN IWO LGA BY SEX AND AGE

Mean and range values of study methods and correlation with skin snip and CMFL: Data in Table 3 show that skin snip and CMFL correlate. Nodule assessment also correlate most with both skin snip and CMFL (P>0.05) though followed by Leopard skin (LS) which does not correlate with the former (P<0.05). The other parametric methods do not correlate with the standard predictor method – skin snip.

The parametric assessment and correlation with skin snip and CMFL by village is shown in Table 4. there is varied correlation pattern of assessment method to the standard skin snip positivity in the villages. Generally nodules assessment correlates with both skin snip positivity and CMFL.

METHODS	MEAN	RANGE	SKINSNIP r	p	CMFL r	p		
Skin snip								
Positivity (%)	39.3	22.7-50	-		-		0.9010	<0.0009
CMFL (ml/mg)	1.01	0.8-1.2	0.9010		<0.0009		-	-
Leopard Skin(%)	27.10	7.1-50	0.3269		0.3575		0.6226	0.0734
Nodules (%)	27.0	7.1-50	0.7447		0.1214		0.9249	<0.0004
Pruritus (%)	52.60	39.3-94	0.0692		0.8336		0.1527	0.7181
Excoriation (%)	12.02	4.4-2.4	0.0030		0.09956		0.3079	0.5528
Microfilarial (%)	10.61	36-16.7	0.2810		0.5415		0.3570	0.4319

Table 3: MEANS AND RANGE VALUES OF STUDY METHODS AND CORRELATION WITH SKIN SNIP AND CMFL

DISCUSSION

Onchocerciasis a tissue parasitic disease is caused by the filarial worm of the Genus Onchocerca volvolus and transmitted by black flies of the genius Simulium (8). Several African countries have recognized the disease and have embarked on preparation for control (9).

This study has shown a prevalence rate of 35.4% and a low intensity rate of infection (CMFL) that is far less than what is typically reported from other regions of forest onchocerciasis (10,11). The low prevalence of disease in Iwo LGA could be due to the control effort already put in place. Most of the study subjects have been treated or immunized with Mectizan. The different result is also party due to the difference in geographic area of study.

Many different criteria have been used for defining the level of endemicity of onchocerciasis in a population. According to Mcmahun et al (11), the onchocerciasis programme classified levels are sporadic, hypo, meso-or hyperendemic – on the basis of the standardized mf prevalence being <10%, 10-29%, 30-59% and 64% respectively. Therefore Iwo LGA is deemed to be classified as mesoendemic in view of the prevalence rate of 35.4%. It has been shown that infection increases with age of subject in the area. This means that able – bodies men in their productive years are bound to be afflicted with the attendant dwindling fortune at old age. The pattern of infection distribution in this study conforms to other reports (6). The highest rate was found among age 41 – 50 with 55.6%. That gender factor has no effect on distribution pattern is an indication that there is no discriminatory infectionbased on sex as both sexes are equally vulnerable by reason of exposure to the bite of the vector. The low infection rate among certain age group in the area is largely due to in and out pattern of living in the area just as a student on vacation. It was discovered that majority of subjects in the age group with low infection rate are not permanent residents on the farmhouses. They merely come to the far for a brief period and return to the city. This predisposed them to infrequent bite by vector. There is a need to put in place a virile control programme in conformity with the principle and practice of disease control which component include monitoring and evaluation through periodic epidemiological survey.

Based on the analysis of large volumes of epidemiological data on onchocerciasis from West, Central and East African, it was recommended among other things that pigmentation of the skin and palpation for nodules may be used as alternative methods for community diagnosis of onchocerciasis (12).

Although the demonstration of skin snip for microfilarial is the most common and reliable, the procedure demand among them, and the use of a microscope, a razor blade or preferably a sclero punch and the availability of trained personnel. Skin snipping is often frightening there by requiring persuasion and sometimes incentives to secure the cooperation of the villagers (1). The method is not acceptable for long scale mapping of onchocerciasis because the method is costly, time consuming and could introduce serious risk of transmitting agents such as human immunodeficiency virus and hepatitis virus B. Therefore the development of alternative method has become imperative. This study has shown that nodule palpation assessment method NPAM is an alternative rapid assessment method of diagnosing the disease. The NPAM has been shown to correlate well with skin snip method. This report accords well with the finding of some authors (5,6,11,13). The cheering news of this methods is that all the disadvantages of the skin snip method are taken care of. Therefore NPAM is strongly recommended for monitoring and evaluation of the current control programme of mectizan distribution.

This study has also shown that the use of pruritus and excoration may mislead diagnosis since different factors may be responsible for pruritus. For example, insect bite (other than that of *Simullum)* and reaction to some antigenic substances and also idiosyncrasy of individuals may provoke pruritus.

It has been shown that urine examination method is not sensitive as diagnosis could be missed even among individual whose skin snip is positive. These have been confirmed statistically as the method failed to correlate with the conventional skin snip.

In conclusion therefore, we recommend the use nodule palpation as a rapid predictor of onchocerciasis in large scale monitoring and evaluating the disease control programme.

Other methods of assessment of onchocerciasis are not recommended for use in the study area since they do not correlate well with the conventional standard method – skin snip. It is to be noted that the correlation pattern of the different assessment method to skin snip varies by village. For example, in one village the methods did correlate with skin snip ($P>0.05$), while others show confusing pattern. In essence, the method of choice is still NPAM which correlate at all times with skin snipping.

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PUBLIC HEALTH IMPORTANCE OF LASSA FEVER EPIDEMIOLOGY, CLINICAL FEATURES AND CURRENT MANAGEMENT REVIEW OF LITERATURE

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The public health importance of Lassa fever can not be over emphasized if one considers the high infectivity and mortality rates associated with the disease. This study dealt extensively on the epidemiology, clinical features and current management of Lassa fever through literature review. The aim of this study is to sensitise the public on what it needs to know on Lassa fever as well as updating the knowledge of health workers on current management of the disease and important precautive measures to take when handling a patient with Lassa fever. Strict barrier nursing, isolation, use of protective devices are important preventive measures when managing a patient with Lassa fever infection. As Lassa fever may have a long incubation period (Up to 20 days), it is possible that travellers from endemic areas may be incubating the disease. However, one case of Lassa fever entering a non-endemic area should not cause fear of an epidemic as long as correct infection control procedures are followed.

INTRODUCTION

Lassa fever is an acute viral illness caused by Lassa virus a member of the arena virus family of viruses. The disease was first described in the 1950's, although the virus was not isolated until 1969. Lassa fever is confined to West Africa from where it was first recognized in 1969 in Lassa, Northern Nigeria, when an American missionary died from it. Since then small outbreaks have occurred in Zarie, Liberia, Paragua Tongo in Sierra Leone and cases have occurred in various parts of Nigeria. It has been shown in Sierra Leone and cases have occurred in various parts of Nigeria. It has been shown in Sierra Leone and Nigeria that the infection occurs widely in communities as a major illness or unapparent infection. Many people in endemic areas have antibodies, for example, 35% in an area in Sierra Leone (1), and have had mild or unapparent infection.

Epidemics and deaths are particularly associated with hospitals and poor hygienic practices. Hospital personnel appear particularly vulnerable. Every year there are about 100,000 cases in West Africa and about 5,000 deaths (2). A distressing report on mosocomial Lassa fever in two hospitals in Nigeria highlights the problems that the health system in large pats of Africa is facing: poor formal education and training, unqualified personnel, lack of resources and materials as well as lack of supervision (3). This report was just a limited out break in two small private hospitals with 34 patients, 22 of whom died, indicating at 65% fatality rate. Among the deaths were 3 doctors, six nurses and a son of a patient. The fatality among the doctors and nurses was probably the reason while this epidemic came into light. Most patients were exposed to the disease in hospital. The staff was infected during emergency surgery and while caring for nosocomically infected patients.

EPIDEMIOLOGY

Lassa fever is transmitted to humans from wild rodents (the multimmamate rat, *mastomys natalensis*). In rodents, the infection persists and the virus is shed throughout the life of the animal. *Mastomys natalensis* was identified in Sierra Leone in 1972 as a rodent reservior of Lassa virus (4). Disease transmission is primarily through direct or indirect contact with excreta of infected rodents deposited on surfaces such as floors or beds, or in food or water. Infection can also occur by inhalation of tiny droplets (aerosols) of virus – laden rodent excreta. Exposure may also occur during occupational activities such as agricultural works or mining.Person-to-person and laboratory infections occur, especially in the hospital environment, through direct contact with blood (including inoculation with contaminated needles), pharyngeal (throat) secretions or urine of a patient, or by sexual contact. Person to person spread may occur during the acute phase of fever when the virus is present in the throat. Studies have shown that person-to-person spread of virus is common; it contributes less than rodent contact to human infection (5). Infection rtes in families are significantly higher in households with rodents that have antibody to Lassa virus (undoubtedly reflecting persistent infection) and where food is stored indiscriminately. Spread of virus from rodents to humans is strongly associated with a large household rodent population as well as practices such as catching, cooking, and eating rodents. Person-to-person transmission in a household is associated with direct contact or care of someone with a febrile illness, as well as sexual contact with a partner during the incubation or convalescent phase of illnesses. This virus may be excreted in the urine of patients for three to nine weeks from the onset of illness. Lassa fever can be transmitted via semen for up to three months. Nosocomial transmission of Lassa fever was well described during the outbreaks that occasioned the discovery of the virus more than three decades ago. Study has suggested that this is not a frequent event, and that basic barrier nursing methods (gloves, gowns, and masks) are highly effective in reducing the risk (1).

Lassa virus transmission is the most consistently endemic of all the arena viruses. More patients are admitted to hospital during the dry season of

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February to May, but cases occur in every month of the year. It is possible that the increased stability to Lassa virus at low relative humidity periods range from 7 to as long as 20 days (**6**). From 1969, about 12 Lassa fever outbreaks had occurred in Nigeria. These cases were repored from Jos –1969/70, Onitsha 1970, Zonkwe 1974,. 1975, 1976 and 1977, Vom- 1975, Aboh-Mbaise and Owerri 1989, Lafiya 1992-1993, Ekpoma 1990 and 1992. Other countries in Africa have reported Lassa outbreaks and these include Central Africa Republic, Liberia and Sierra Leone (Fig. 1).

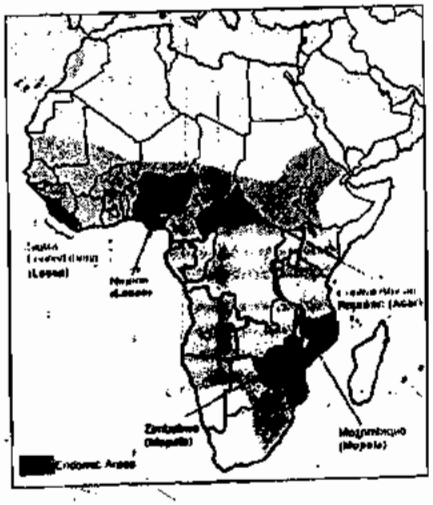

Fig. 1 Geographical locations of Lassa and related viruses from Africa. The stippled area represents the distribution of the single mouse isolate Soft (*Mastomys* (*praomys*) *natalensis*) in this continent

(Fig. 1). Serological evidence of human infection has been found in Democratic Republic of Congo, Guinea, Mali and Senegal. Seroconversion rates ranged from 5 to 20 percent in prospective studies of Lassa fever in susceptible (seronegative) populations of Sierra Leone villages (**5, 6**). The highest were in crowded, highly mobile populations with lower rates in traditional agricultural villages. These same studies demonstrated disease-to-infection ratios of 10 to 25 percent, and the proportion of febrile illness associated with seroconversion to Lassa virus was 5 to 14 percent. All age group are susceptible to Lassa fever infection as shown by serological surveys as well as studies of hospitalized patients (7). Age and sex specific antibody prevalence shows that the proportion of the population with antibody increases with age and thee is no significant difference in antibody prevalence between sexes.

Epidemic investigations of Lassa fever should include assessment of rodent infestation and infecion in conjunction with a careful history of human disease. The role of human-to-human transmission is clearly more important and this must be kept in mind when investigating a case or cluster of cases in an endemic area. Investigation in a non-endemic area should center on possible human contacts or recent travel to an endemic Lassa fever area.

The main methods of control are isolation of cases, disinfection, surveillance of contacts and rodent control. In hospital, barrier nursing, strict procedures for handling of both body fluids and excreta should be maintained. Patient relatives should not be allowed to handle sercretions, urine or excreta of the patient. Disinfectants such as 0.5% sodium hypochlorite solution. 0.5% phenol with detergent, heating and bleach solution are effective for controlling transmission. Identify all close contact (people living with, caring for, or testing laboratory specimens of patients) within three weeks of onset of illness. Close surveillance of contact should be established by conducting body temperature checks at least two times daily for three weeks after exposure. In case of temperature greater than 38.8°C, hospitalize immediately in isolation facilities. The place of residence of the patient during the three weeks prior to onset should be determined and a search initiated for unreported or undiagnosed cases. Prophylactic oral Ribavirin should be considered in a person who is known to have had a close contact with a confirmed case of Lassa fever during 2 weeks prior to the onset of symptoms, while symptomatic or during the 8 weeks after recovery. Although it is not clear how long this drug need to be given to abort the infection (**8, 9**). Unintentional ecological manipulation, introduction of crop rotation using soybeans with corn, may be responsible for the reduction of human disease(**10**).

CLINICAL FEATURES

Most Lassa fever infection probably occurs as a result of viral contact with exposed membranes or skin abrasions. Patients with Lassa fever enter hospital 2 to 4 days after onset of symptoms. At this time viraemia may be absent or present in widely different concentrations. Persistent high viraemia is a significant predicator of outcome of illness. It is not unusual to encounter patients with vireamia on admission to hospital that also have high levels of both IgM of IgG immunofluorescent antibody (IFA). In fact there is no correlation between the vireamia level and that of the IFA for Lassa fever (**11**).

Neutralizing antibodies to Lassa virus are almost never detectable in the serum of patients at the beginning of convalescence, and in most people they are never detectable. In a minority of patients some low-titer serum neutralizing activity may be observed but only several months after resolution of the disease and clearance of the virus (**11**).

Lassa fever begins 7 to 8 days after the primary infection with sublet onset of fever, headache, and malaise (**7**). Fever is sustained with peaks of 39 to 41oC, usually in early morning and early evening. Aching in the large joints and lower back pain develop in more than half of hospitalized patients by the third or fourth day of illness. The physical examination shows these patients to be toxic and anxious. Unless the patient is in shock the skin is usually moist from diaphoresis. There is an elevated

respiratory rate, and the pulse is usually commensurate with elevated body temperature. The systolic blood pressure ranges from > 100 to > 110 with a mean of 103. There is no characteristic skin rash. Petechiae and ecyhmosis are not seen, nor is jaundice a feature of Lassa fever. Conjunctivitis occurs in about one-third of patients; conjunctive hemorrhages are occasionally seen and portend a poor prognosis. Seventy percent of patients have pharyngitis with diffusely inflamed and swollen pharynx and tonsils, but few if any petechiae. In over half of the patients the pharyngitis is exudative, with yellow patches, primarily on the tonsils, and rarely with distinct ulcers. The pharyngeal pain associated with Lassa fever is extraordinarily severe, and it is common to see patient expectorate on saliva in a cup because swallowing is so painful. Bleeding occurs in only 15 to 20 percent of all patients. It occurs most often in the gum and nose, but also occurs as gastrointestinal o vaginal bleeding, it is of course associated with severe disease. Oedema of the face and neck are commonly seen also in severe disease, without peripheral oedema – suggesting capillary leakage, rather than cardiac dysfunction and impaired venous return oedema and bleeding may occur together or independently. About 20% of patients have pericardial or pleural rubs, presumably associated with effusions, which though rarely present on admission, develop in early convalescence occasionally in association with congrestive cardiac failure.

The ECG may be abnormal, particularly with elevated T-waves and evidence of pericarditis and myocarditis, but there is no correlation between the T-waves abnormalities and the presence of pericardial rub or other evidence of pericarditis. The abdomen is diffusely tender in under half of the patients but there are no localizing signs and bowel signs are usually active. Neurological manifestations may be absent in acute Lassa fever or there may be a range of abnormalities from unilateral to bilateral deafness, with or without tinnitus, to moderate or severe diffuse encephalopathy with or without general seizures. The encphalopathic complications generally carry a poor prognosis, while deafness usually occurs just as recovery is underway. Manifestations during the acute phase range from mild confusion and tremors to grand mal seizures and decerebrate coma. Focal fits are not seen. Cerebrospinal fluid specimens usually show a few lymphocytes but are otherwise normal and virus titers are low. Other than deafness, focal neurological signs rarely occur. Nerve deafness, sometimes permanent occurs in 25 percent of all Lassa fever infections.

The mean white blood cell count on admission is 6 x 10^9/L with early lymphopenia and in a few severe cases late neutropenia (12). A circulating inhibitor of platelet function has been detected in the plasma of severe cases in humans. The haemetrocrit in Lassa fever patients is often elevated (mean 50.1) due to dehydration. Proteinuria is common, occurring in two-third of patients. The blood urea nitrogen may be moderately elevated probably due to dehydration. Lassa fever is also a pediatric disease-affecting children of all ages (6). The disease appears to be difficult to diagnose in children because its manifestations are so general. In very young babies, marked oedema may be seen, associated with very severe disease. In older children the disease may manifest as diarrhoea, as pneumonia, or simply as un-explained prolonged fever. The case fatality rate in children is 12 to 14 percent. The clinical course of Lassa fever in children is as diverse as it is in adults, ranging from mild febrile illness to severe fulminating disease. Lassa fever is highly variable disease with a broad range of manifestations and many degrees of severity. This makes it difficult to distinguish clinically, especially in the early stages, from infuenza or other upper or lower respiratory viral infections, as well as from other causes of general febrile illness or from febrile gastroenteritis. Typhoid fever is a common misdaignosis. There are no firm clinical predicators or pathognomonic signs of Lassa fever. Although it is classified as a hemorrhagic fever, it is not frequently a cause of overt bleeding. A case control study of the clinical diagnosis or prognosis of the disease (7). There are several significant complications of Lassa fever, which add to the overall burden of the disease in the poor rural populations of many West Africa countries. One of these is the adverse effect of Lassa fever durign pregnancy (13). Limited data suggest that Lassa fever may be a common cause of maternal mortality in many areas of West Africa. Another important complication of Lassa fever is that of acute VIIIth nerve deafness. Nearly 30 percent of patients with Lassa fever infectio suffer an acute loss of hearing in one or both ears. Other complications, which appear to occur much less frequently, are uveitis, pericarditis, orchitis, pleural effusion and ascities (7). Renal and hepatic failures are not seen.

The simplest and most common methods of diagnosis are serological tests on paired sera by Immunofluorescent Antibody (IFA) or enzyme-linked immunosorbent assay (ELISA) to detect an increase in antibody titer or an elevated titer (at least 1:32), and presence of specific IgM (11,14). Lassa virus produces sustained vreamias so that virus isolation is studies of South American hemorrhagic fever, particularly infection, show that vireamia is also a consistent feature, although probably not at the same levels as with Lassa fever(1). Thus virus isolations is an alternative diagnostic method in the absence of paired sera. Ideally, a method of rapid diagnosis would help with early identification and isolation of the patients both for therapy and prevention of transmission; however, no such method has yet been developed. The diagnosis of Lassa fever by IFA on fixed tissue using monoclonal antibodies to Lassa virus makes possible postmortem diagnosis in situations where methods of collection and storage of specimens are limited (14). For virus isolation, serum

should be separated from a clotted specimen when possible, although whole blood may be used. Ideally, the specimen should be stored at -60°C but specimens stored at 4°C for several days will still yield the virus. Lassa virus can be cultured from urine, spinal fluid, breast milk pharyngeal secretions and tissues like spleen, liver and lymph node. Specimens should be placed in dry ice or liquid nitrogen as soon as possible; storage at -20°C will maintain virus viability for several days.

CURRENT MANAGEMENT

1. *Drug Treatment*: the only known specific treatment for Lassa fever is Ribavirin. After having made the diagnosis of Lassa fever Intravenous Ribavirin treatment should start as soon as possible.
   - First give a single loading does of 33mg per kg body weight.
   - Then every six hours give 16mg per kg body weight for four days
   - Then every eight hours give per kg body weight for six days.
   - Total treatment period is ten days.
   - A treatment chart (attached0 should be completed for each individual patient clearly laying out correct amount to give for each does.
   - Once started, a Ribavirin treatment should not be discontinued until the ten-day course is complete.
   - Each ampoule of Ribavirin contains 100mg in 1ml. Ribavirin does not need to be diluted for administration and there are no contraindications to Ribavirin.

• Abnormal bleeding (e.g. gums mouth, nose)
• Red eyes or conjunctivitis
• Spontaneous abortions
• Swollen neck and or face
• Low blood pressure (systolic BP < 100mmHg) or shock.

BOX 1: MAJOR DIAGNOSTIC CRITERIA
Source: FMOH – Abuja.

2. *Supportive Therapy*: Many patients arrive in a moderately dehydrated state with elevated packed-cell volume (PCV), and require fluid require fluid replacement. No data are available on specific electrolyte or acid-base imbalances. The major crisis to overcome is the sudden and profound hypotension, which may occur between the fifth and the fourteenth day of illness. For those patients with severe anemia, whole blood or packed cells may be helpful (15). In situation where there is no whole blood, plasma or haemacel can be used. Whenever possible, fluid, electrolyte, and osmotic imbalances should be corrected in anticipation of the development of clinical shock. Other supportive therapies may include:
   - Analgesic e.g. Paracetamol for pains
   - Quinine injection especially in malaria endemic regions
   - Nasogastric-tube feeding when necessary.
   - Remember to protect yourself, your staff and the patient's relatives when treating Lassa fever. Simple protective measures such as non-disposable gowns, gloves, and masks as used by hospital personnel are effective in preventing excess risk of Lassa virus infection.
   - Strict barrier nursing should be maintained.

• Headache
• Sore throat
• Leucopenia (<400/mm/.)
• Nausea and Vomiting
• Diarrhoea
• Cough
• Pleural effusion or ascities
• Swollen lymph nodes
• Body weakness
• Proteinuria.

BOX 2: MINOR DIAGNOSTIC CRITERIA
Source: FMOH – Abuja

3. *Isolation:* the degree to which patient isolation is accomplished depends on the hospital where the patient is admitted. The patient should be placed in a room with a single entrance, preferably through an adjoining room. The room should contain the materials necessary for patient care and staff protection, including gowns, gloves and masks. The entrance room should also contain hand-washing facilities and decontaminating solutions (antiseptics). Persons entering the patients room should wear gowns, gloves, and masks (non-disposable ones may be decontaminated after use and reused). Feet should be covered, and protective eyewear should be worn by the staff if patient is disoriented and combative or if procedures likely to produce vomiting or bleeding are performed (i.e. nasogastric tube or arterial line). Protective clothing should be put on and removed in the entrance room, and only essential hospital personnel and immediate family members should be allowed in the room.

Laboratory tests should be carried out in high containment facilities. If there is no such facility, specimen handling should be kept to a minimum and performed only by experienced technicians using all available precautions, such as gloves and bio-safety cabinets.

- Isolate the patient
- Restrict access to the isolation area
- Only hospital staff and useful family caregivers should have access into the isolation room.
- People with open cuts or wounds should not look after patient with Lassa fever.
- Wear protective clothing e.g. gown, gloves, masks, eyeglasses etc.
- Handle specimens carefully and safely
- Wash hands with antiseptic soap and water after contact with patient or his/her body fluids.
- Sterilize all equipments/instruments used for patient
- Use of invasive procedures should be ver minimal.
- All wastes from patient should be disposed of carefully
- Strict barrier nursing.

BOX 3: IMPORTANT HINTS FOR SELF PROTECTION WHEN HANDLING LASSA FEVER PATIENT

CONCLUSION

Since there are no firm clinical predicators or pathognomonic signs and symptoms of Lassa fever, it is therefore recommended that a high level of suspicions should be maintained when dealing with a patient with persistent fever and any of the major diagnostic criteria or two minor diagnostic criteria as well as history of contact with Lassa fever case. Good history taking with emphasis on exposure to rodent either at home or during occupational activities may give clue to making a diagnosis.

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SURVEY OF ANTIBODIES TO NEWCASTLE DISEASE VIRUS IN APPARENTLY HEALTHY ADULT NIGERIAN INDIGENOUS CHICKENS *(Gallus domesticus)* IN IBADAN USING ELISA

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The prevalence of antibodies to Newcastle disease virus (NDV) in Nigerian indigenous chickens raised in Ibadan was surveyed using the indirect enzyme-linked immunosorbent assay (ELISA). Sera from 161 chickens from 3 are as of Ibadan viz University campus, Agbowo and Oremeji were analysed. The prevalence rate obtained ranged between 52.5% and 83.4% with an overall prevalence of 73.3%. The extent and implication of NDV activity in the Nigerian indigenous chicken as well as the advantages, sensitivity and usefulness of ELISA in serological investigation were discussed.

INTRODUCTION

Newcastle diseases (ND) is an acute infectious and highly contagious viral disease of poultry avian species, (1). ND was first diagnosed in Eastern Nigeria in 1951 and confirmed Hill et al; (2) in 1953 and since then, the disease has continued to be the most devastating disease of poultry in Nigeria. (3,4,5). Despite the availability of locally produced vaccines and the protective immunity conferred by recommended vaccination regime,(6), the number of reported outbreaks of ND remain high. (7). Cases of outbreaks in vaccinated birds have also been reported (5) and these were attributed to antigentic differences between the vaccines used and the field ND viral strains and the resultant insufficient immunogenic protection (8).

Previous serological survey of ND virus haemagglutination inhibition (NDVHI) antibody in exotic birds in parts of Nigeria showed that 22% had detectable (NDVHI antibodies (6). Adene and Njoku (9) reported low NDVHI antibody titre (0-10) for imported day-old exotic chicks, while Abdu and Garba (10) reported higher titres for those hatched in one of the hatcheries in the country.

According to Akinwunmi et al (11), 124 million of 134 million chickens in Nigeria are indigenous local ckicken. They are usually kept on free range management system and are normally not vaccinated (12). Adene (13) also reported that the rural poultry account for over 70% of Nigerian poultry, hence they are very important in the epidemiology of poultry diseases. To properly evaluate their role, several studies had been done on many important poultry diseases which affect these birds such as Marek's disease (Adene 14), infectious bursal disease (15,16,17, 18), Newcastle disease (4,12, 17,18), egg drop syndrome (18), Fowl typhoid (19, 20) and brucellosis (21,22).

In most of the studies done on the seroprevalence of Newcastle disease in Nigerian indigenous chickens, haemagglutination inhibition technique were employed. In order to properly evaluate the prevalence, there is need to employ other diagnostic techniques hence the use of ELISA technique which has been found to be efficient, accurate, easier and more sensitive in the diagnosis and seromontoring of poultry diseases (23, 24). The following study was thus undertaken to investigate the extent of NDV activity among Nigerian indigenous chicken in Ibadan and also to determine the usefulness of ELISA technique in the detection of humoral antibodies to Newcastle disease in the indigenous chicken.

MATERIALS AND METHODS

The test sera were obtained by jugular venopuncture of adult indigenous chickens kept on free range management by small holder/backyard rearers in 3 areas of Ibadan. 3-4 samples were collected per household in these areas, and all the chickens had no history of any vaccination but they were apparently healthy. A total of 161 samples were randomly obtained. Sera were heat inactivated at 56oC for 30mins and stored at 20oc until analysed.

The Newcastle disease virus (Lasota strain) was used as antigen in the ELISA test the lyophilized virus was obtained from the Nigerian Veterinary Research Institute, VOM and used at a protein concentration of 5.0mg/ml following the determination of the viral protein concentration as described by Warburg and Christian (25). The virus was diluted in carbonate – bicarbonate buffer pH 9.6.

Rabbit anti-chicken IgG horseradish peroxidase labeled conjugate (Zymed Inc. California) was used as conjugate. It was diluted 1:2,000 in PBS containing 0.5% Tween 20 and 1% Bovine serum albumin (PBST-BSA) following a chequer board titration.

The substrate/chromogen was prepared by dissolving 0.82g of sodium acetate in 100ml of distilled water and adjusting the pH to 6.0 with 0.5m citric acid. This solution was divided into 25mls aliquots. To each 25mls aliquots, 4il of 30% hydrogen peroxide and 250il of tetramethy lbenzidine (TMB) in dimethylsulphoxide (10mg/ml) were added prior to use.

The ELISA procedure was conducted essentially by adaptation of the method described by Oyejide et al (26) for infectious Bronchitis with some modifications. Following the determination of the optimal working dilutions for antigen, serum and conjugate by chequer-board titration, polystyrene micro ELISA

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plates with 96 flat bottom wells were coated with 100ll of NDV antigen of 5.0mg/ml protein concentration in carbonate-bicarbonate buffer. The plates were incubated overnight at + 40oC. Excess antigens were washed off in 2 washes with PBST using automatic microplate washer (SLT Labinstruments ® Austria).

100il of test sera diluted to 1:500 in PBST-BSA was added to duplicate wells of the paltes and incubated at 37°C for 30 minutes. The plates were rocked manually at every 10minutes interval during the incubation. Thereafter, the plates were washed 3 times with PBST and flipped to dry.

100il of conjugate was added to each well at 1:2,000 dilution in PBST-BSA and the plates were incubated at 37°C for 30 minutes, and manually shaken every 10 minutes. Excess conjugate was removed in 3 washed with PBST, the 100 il of freshly prepared substrate/chromogen was added to each well and the plates were incubated at 37°C for 15 minutes. Plates were immediately read at 450nm wavelength in microplate reader (SLT Labinstruments ® Austria).

In each plate, control wells consisting of specific pathogen free chicken serum, hyperimmune serum to ND diluted to 1:500 with PBST-BSA, as well as blank wells consisting of PBST-BSA alone were included. The positive test samples were those whose optimal density (OD) values were either equal to or above 1.5 times the OD of negative control.

RESULTS

Sampling Area/Location	No. of samples tested	No +ve with ELISA	Prevalen rate %	Mean OD of +ve (± SD)
1. University of Ibadan Campus	64	54	83.4	0.218 ± 0.060
2. Agbowo	57	43	75.4	0.198 ± 0.030
3. Oremeji	40	21	52.5	0.207 ± 0.140
Total	161	118	73.3	

Table 1: Prevalence of Antibodies to NDV in Indigenous Nigerian Chickens in Ibadan,

Using ELISA

Of the thee different areas sampled viz: University Campus, Agbowo and Oremeji prevalence rates of 83.4%, 75.4% and 52.5% were obtained respectively. The combined prevalence of NDV antibodies among 161 indigenous chickens sampled in Ibadan was found to be 73.3%.

The mean optical density readings obtained for positive reactors for the different areas were 0.128 ± 0.060 (U.I), 0.198 + 0.030 (Agbowo) and 0.207 ± 0.140 (Oremeji).

DISCUSSION

The antibodies observed in these birds is in response to exposure to field strains of Newcastle disease virus because the birds were not vaccinated against the disease.

The prevalence of NDV antibodies in this study ranged between 52.5% and 83.4%. Previous data on the prevalence of antibodies to NDV with the use of HI test showed that 22% of unvaccinated exotic chickens had demonstrable antibodies (6), while 51.4%, (18 out of 35) was found for local/indigenous chickens in Ibadan in particular and 41.04% in Nigeria (12). In this study, however, a higher prevalence of 73.3% was observed among indigenous chickens in Ibadan. This higher prevalence may be attributed to the higher sensitivity of the ELISA method compared to the HI test.

Adu et al (12), using haemagglutination test postulated that some birds may habour the virus in the absence of antibodies, or presence of very low levels of antibody. Thus the higher prevalence rate observed in our study could be as a result of this group of birds with low antibody titres which could not be detected by HA test but detectable by the ELISA.

The variation in the prevalence of NDV antibodies in the 3 areas studied could be attributed to the level of poultry production in these areas. For example the university community high poultry production especially in the Teaching and Research Farm in since samples were collected from households in this vicinity, it was possible that the indigenous chickens reared here, were constantly exposed to the virus through outbreaks in the exotic breeds, hence the higher prevalence than Oremeji area with a few commercial poultry units. Since the movement of the indigenous chickens is not controlled they can possibly constitute infected carriers as suggested by Adene et al, (15), and hence perpetuate the disease especially among susceptible exotic breeds. There is therefore the need for adequate fencing of poultry farm premises (18).

The result of this study shows that the ELISA test could be a useful tool in the assessment of NDV activity in a poultry population and would also be useful in the assessment of humoral response to vaccination. The extent of ND virus activity in apparently healthy Nigerian indigenous chicken in significantly high hence the need to take into cognizance the role of rural birds in the epidemiology of the disease.

Further research on the varied pathtypes of ND associated with indigenous chickens is envisaged to properly aid in the control of ND in Nigeria.

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AN ASSESSMENT OF EXISTING COMMON TRADITIONAL METHODS OF WATER PURIFICATION

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Classical water purification methods include boiling, filtration, irradiation and the use of chemicals while traditional water purification methods in use are boiling, filtration, sedimentation, long storage and solar radiation. Waterborne diseases are m ore common in the rural communities where potable water supply coverage is usually low. Therefore, this study was designed to assess and modify existing water purification methods in use in the rural communities so as to encourage their regular use.

Water samples collected from various sources serving six rural communities in Agege, Epe and Ikorodu Local Government areas of Lagos State were purified using each of the traditional methods. Viable counts were carried out on each of the water samples before and after the purification process. Water samples contamination with known pathogens were also included in the test.

The boiling method was the most efficient giving 100% decontamination after three minutes of continuous boiling. The solar method gave varying degrees of decontamination of the water samples (42-100%) depending on the turbidity of the water and the type of container used for the test. The long storage method and the cloth filtration methods decontaminated the water by (0.6-4.2%) and 41% respectively.

The solar water purification method should be encouraged. Turbid water samples should be cloth filtered prior to exposure to the sun for maximum efficiency.

INTRODUCTION

It is often said, "water has no enemy" This emphasizes the importance of water to living things. For men, access to potable water greatly affects disease burden. The focus of the water decade (1981 – 1990) activities in developing areas of the world was on changing the overall emphasis from capital intensive projects to low cost locally maintained alternative technologies (1). Therefore building on traditionally known and used water treatmen practices is expected to have the potentials for reducing morbidity and mortality rate of waterborne diseases. Water treatment is purifying water to a level safe for drinking, free of all pathogens and toxic substances, having pleasant appearance and being tasteless and odourless (2). The presence of 10 or less coliforms in 100ml of water in unchlorinated water is usually disregarded (3). Classical purification methods in use are filtration, boiling, long storage, irradiation, the use of metals like silver an copper, use of oxidants such as the halogens and halogen compounds, ozone, hydrogen peroxiden and potassium permangenate.

Traditional methods of water purification include cloth filtration, sedimentation and boiling. Coagulants of plant and soil origins have been used for water purification in developing countries are in form of such fluvial clays earth from termite hills, seed of *Moringa oliferia* (2), potash alum (trona) (4,5). Trona, a naturally occurring grey or yellowish white deposit used locally as tenderizer, oil-emulsifier, preservative and a food condiment that is alkaline (pH 9.0) and water soluble is made up of hydrated acid sodium carbonate $Na_2Co_3NaHCo_32H_2O$. Trona has also been reported to contain potassium, chloride and sulphite ions (5).

Storage of water reduces the number of bacteria by 90% in five to ten days (6). Pioneering studies in Beirut reported that the near ultra-violent region (A) of the sun in tropical and sub-tropical regions destroyed 99.9% of Coliforms in water contained in transparent plastic or glass bottles in 90 minutes provided the volume of the water was three litres or less (7,8,9). Pathogens such as *Salmonella typhi, Shigella spp*, and *Vibrio Cholerae* were repoted to be more sensitive to the sun rays than coliforms (10,11). The minimum dosage of solar intensity recommended to inactivate vegetative bacteria is 0.44KWh/m^2, (12) and in Nigeria an average solar intensity of 3.7KWh/m^2 per day in the semi-arid areas of the country.

The fruits of Xylopea aethiopica are sometimes put into jars of water to purify the water (13). The leaves of *Ocimum gatissium, Psiditum guagjava (guava)* and *Anacardium occidentale* (cashew) are used in the management of diarrhoea in the eastern part of Nigeria (14). *Terminalia avicennoides,* was reported to possess vibriocidal properties (15) and Lennea *welwischii* and *Phyllantus discoideus* were reported to show anti-bacteria activities against the *Enterobacteraceae* (16). The need to purify water in our rural communities and other developing countries is of utmost importance in the reduction of morbidity and mortality due to waterborne diseases, this study was therefore designed to search for and validate simple, cheap and practicable methods of water purification using locally available materials.

MATERIALS AND METHODS

Water samples: water samples from wells, river, stream, and pond sources serving six rural communities in Lagos State which in an earlier study were found to be contaminated were coded S1-S10 a potable water sample coded SC served as control. All the samples were purified by each of the purification methods as described. Water samples were contaminated in the laboratory with 1.5 x 10^4/ml E. coli (ATCC 25922).

Boiling: one hundred millilitres of each water sample were heated to 100°C and 1ml each was withdrawn at the start of boiling, after 1,2,3,4 and 5 min-

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utes. Viable counts of all the samples were performed as described by Miles and Misra (17) using nutrient agar, blood and Maconkey agar (Oxoid) and incubated at 37°C for 24 hrs.

Filtration: one hundred millilitres of each water sample were filtered through sterile white cotton material and viable counts performed on the filtrates.

Long storage: two and a half litres of each eater sample were stored in sterile clay pots and plastic containers with fitted lids at room temperature. Viable counts were then performed on the water samples withdrawn from each container after 2, 5 10, 15, 21 days of storage.

Addition of local materials: Plants parts used (bark, leaves, or seeds, table 1) were weighed, washed in distilled water, rinsed in methylated spirit and dried in the oven at 60oC for 30 minutes and then macerated in a clean sterile mortar. The plant parts were then put the water samples.

Samples to give a final concentration of 1% w/v and left for 4hrs before viable counts were performed. Viable count were repeated after 24hrs. For Trona (potash alum), various concentration, 0.05, 0.1,0.25 and 1.0% and for aluminium sulphate (alum) 50mg/lit concentrations were tested likewise

Use of sunlight: water samples S1 – S10 put in plastic and glass bottles, (1.5L) were kept in the sunlight for 4hrs. A duplicate set of bottles was left in a cupboard in the laboratory away from sunlight for the same period of time as controls. Viable counts were performed on all samples and repeated after 24hrs.

Large volume of water (8L) put in different wide shallow containers (enamel, aluminium and plastic), and covered with thin clean transparent polythene sheets, knotted firmly at the sides were also exposed to sunlight. Some known water pathogens like *Salmonella typhi, Shigella dysenteriae, Vibrios cholerae*, (local strains), *Escherichia coli* (ATCC 25922) were introduced into the water samples and the solar decontamination process repeated. On cloudly days, the containers were half filled and exposed to the low intensity of sunlight for solar decontamination. The containers were aerated by shaking them at intervals.

Solar and cloth filtration: samples S1 – S10 were passed through a clean white cloth for filtration. The filtrates were then put in the enamel container and purified by the solar decontamination method.

Botanical Name (Family)	Voucher Sample	Local Name	Plants part
Lennea welwitschii (Hievn) Engl. (Anacardiae)	LUTH 020	Orira (Y)	Bark
Phyplianlia dixoideus Mueli-Arg (Euphorbiaceae)	LUTH 2021	Ashasha(Y)	Bark
Terminalia avicennoides (Combretaceae)	LUTH 376	Idi (Y)	Bark
Moringa oilefiera	IDIKA 1	Ewe igbale (Y)	Seed
Xylopea aethiopica (Annonaeceae)	IDIKA 2	Uda(I)	Fruit
Momordica foetida	IDIKA 3	Ejirin (Y)	Leaves
Ocumum gratissimum	IDIKA 4	Efirin (Y) Nchanwu(I)	Leaves
Ocumum gratissimum			
Parinari spp	IDIKA 5	Abere (Y)	Leaves
Agerantum conyzoide	IDIKA 6	Imisu (Y)	Leaves
Psidium guagjava	IDIKA 7	Guava (E)	Leaves & stem

Table 1: Parts of Local herbs selected for testign water purification ability
KEY: *Y = Yoruba* *I = Igbo* *E = English*

RESULTS

Boiling method: A 1005 decontamination of all the water samples tested was obtained after three minutes of boiling (Table 2).

Solar decontamination method: water samples S1 – S10 were decontaminated by 40-94% while laboratory water samples contaminated with known pathogens were decontaminated by 95.4-100% (Table and 3).

Method	Duration	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	SC
Boiling	At boiling	0	8	7	24	3	3	6	4	5	20	0
	1 min	0	3	4	10	3	0	2	0	1	10	0
	2 min	0	2	0	6	1	0	0	0	0	3	0
	3 min	0	0	0	0	0	0	0	0	0	0	0
Cloth filtration	2 days	97	272	280	480	364	386	156	280	398	268	6
Long storage	5 days	70	210	260	350	270	300	120	200	190	350	0
(clay pot)	10 days	50	150	180	320	200	280	100	180	170	300	0
	15 days	40	150	206	320	202	280	80	150	170	280	2
	21 days	80	170	200	350	200	350	60	150	200	200	8
Long storage	1 days	95	180	200	400	200	350	60	150	200	200	8
(Plastic can)	5 days	82	210	230	380	300	320	120	300	200	380	0
	10 days	80	190	200	380	260	300	100	250	200	320	7
	15 days	100	186	200	400	280	250	100	250	210	320	4
	21 days	150	180	200	450	250	250	120	250	200	400	2
Solar	4hrs	6	10	60	300	200	30	15	10	12	12	0
0.5% Potash alum	4hrs	220	220	50	70	100	60	60	120	20	50	0
	24hrs	200	200	40	50	50	60	50	100	20	40	0
viable count prior to purification		100	280	290	500	500	380	400	500	350	300	500	0

Viable Counts cfu/ml of water

TABLE 2
Comparison of the effects of the different purification methods employed on the water samples from the six communities in Lagos.

Purification method	Duration	HEC	HST	HSD	HVO	HVI
		Viable counts cfu/ml of water				
Boiling	At boiling	0	95	4	2	2
	1 min	0	10	0	1	1
	2min	0	3	0	0	0
	3 min	450	0	0	0	0
Cloth filtration	2 days	400	350	300	370	380
Long storage in clay pot	5 days	150	300	300	350	350
	10 days	220	250	280	290	300
	15 days	250	250	280	250	280
	21 days	250	200	280	250	260
Long storage in Plastic containers	2 days	380	350	300	400	380
	5 days	200	300	280	370	320
	10 days	250	280	280	300	300
	15 days	250	250	300	300	300
	21 days	300	250	320	300	300
Solar energy	4 hrs	25	10	10	0	0
Use of 0.5% Potash alum	4 hrs	80	100	300	200	150
	24hrs	20	95	60	50	40
Viable count prior to purification		470	510	512	500	508

TABLE 3
The effect of the difference purification methods on water pathogens introduced into sterilized water from a well.
Key:
HEC – Water contaminated with E. coli (150 x 10^4/ml)
HSD - Water contaminated with Shigella dysenteriae (150 x 10^4/ml)
HST - Water contaminated with Salmonella typhi (150 x 10^4/ml)

HVO - Water contaminated with V. cholerae (Ogawa) (150 x 10^4/ml)
HVI - Water contaminated with V. cholerae (Inaba) (150 x 10^4/ml)

After filtration of S1-S10 with cotton cloth, 80-96% solar decontamination was obtained Water samples in aluminium and enamel containers were deconminated by 93-100% (Table 4) solar and air combination used by cloudly days gave 98.2-100% decontamination (Table 5) of the water pathogens.

Type of container	Viable counts cfu/1ml of water			
	Day temp.	HEC	HST	HSD
1.5L Plastic Bottle	36°C	0(100)	0(100)	0(100)
1.5L glass jar	36°C	10(98)	8(98.4)	10(98)
1.5L Plastic Bottle	32°C	0(100)	4(99.2)	0(100)
1.5L glass jar	32°C	18(98)	20(96)	18(98)
1.5L Plastic Bottle	30°C	10(98)	10(98)	8(98.4)
1.5L glass jar	30°C	22(95)	12(97.6)	2(99.6)
viable counts of samples prior to purification		480	500	500

TABLE 4:
Effect of four hours exposure of Laboratory contaminated water samples to sunlight
Key:
HEC – Water contaminated with 150 x 10^4 E.coli
HST - Water contaminated with 150 x 10^4 S. Typhi.
HSD - Water contaminated with 150 x 10^4 S. dysnteriae
( ) = % Bacterial Reduction.

Addition of local plant and natural compounds: Local plant parts and soil materials like limestone used in this study failed to exhibit anti-bacterial activity. Aluminimum sulphate (alum) at 50mg/L, the concentration used in water treatment, did not destroy the bacteria in water. However trona at 0.5% w/v concentration was found to be inhibitory to the bacteria in the water samples by 50-80% and by 78.7-96% after 4 and 24hrs respectively (Table 2). Water pathogens showed 40-80% decontamination (Table 3).

	Viable counts cfu/1ml of water				
Plastic Bottle	0(490)	100	25(490)	94.9	0.781
	0(500)	100	20(500)	96.0	0.775
	0(220)	100	17(220)	92.3	2.694
	2(400)	99.5	100(400)	75.0	3.076
Enamel Basin	0(490)	100	20(400)	95.8	0.781
	0(500)	100	14(500)	97.2	2.775
	0(220)	100	10(220)	95.5	2.694
	0(300)	100	40(400)	90.0	3.076

TABLE 5:
The effect of the combination of Solar Irradiation and Oxygen (Aeration) on the Decontamination of Water.
Values in bracket are viable counts of water samples prior to exposure to sunlight

Key:
½ FC = Half filled container
FC = Completely filled container
1 = Irradiation

Long storage method: the water samples in clay pots and plastic containers showed in average bacterial reduction of 41% after 5 days of storage (Table 2). The counts remained constant or increase in some cases by the 21st day of storage.

Cloth filtration method: the bacterial count in the water samples tested was reduced by 0.6 –4.2% using this method (Table 2).

DISCUSSION:

This study has shown boiling as the most efficient of the five methods tested. Though a very effective methods of destroying bacteria, viruses, spores, cercaria, amoeba cyst, worms and parasitic eggs (2) it alters the taste of water and consumes a large amount of fuel, and leads to deforestation where wood is used (2). The fumes can be injurious to health by causing damage to the lungs and eyes (2). It is pertinent to note that *S. typhi and S. dysenteriae* survived after 1 min and 2min of boiling respectively suggesting that water should be allowed to boil for at least five minutes for effective water purification. It is also expensive as a report from India stated that boiling drinking water required about 33% of the income of most of the inhabitants(18).

The efficiency of the solar purification method in this study agrees with the views of Odeyemi that peasants living in cholera endemic areas may achieve considerable reduction in the incidence, prevalence, morbidity and morality of waterborne diseases by merely exposing their domestic water supplies to solar radiation for about 5 hours(19). In this study, the effect of the soal radiation on turbid water samples was very much lower than its effects on the laboratory contaminated water samples. This is probably due to the exerted attenuating effects on the transmission of the sun rays by the particles present in the water which tend to shield and protect the bacteria as was earlier explained by Odeyemi (20). This was confirmed by our finding where a combination of the cloth filtration and solar decontamination methods yielded better results than either method when used alone. This study also validated the solar and air combination for water purification on cloudy days.

The local herbs used in this study failed to exhibit anti-bacteria property. It is possible that their active ingredients are not water soluble. On the other hand, trona which decontaminated the water samples was found to increase the blood pressure of rats in separate study (21).

CONCLUSION

Boiling and solar methods were found to be suitable for purifying domestic water in the rural areas. However solar method being simple, practicable and cheap is therefore recommended for use in the rural communities. The use of potash alum (trona) which is cheap and effective would require further studies on its subsequent toxicological effect invivo using animal models such as rats. The other methods were not found suitable in this study.

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EFFECT OF ACUTE CAPRINE TRYPANOSOMIASIS ON HAEMOGLOBIN, UREA AND SERUM ELECTROLYTES

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The effect of acute caprine trypanosomiasis on haemoglobin (Hb) concentration, urea and serum electrolytes was studied in Red Sokoto goats infected with Trypanosoma vivax. The course of infection lasted only two weeks when the infected goats died of fulminating parasitaemia and high fever. Haemoglobin concentation of the infected goats was only slightly decreased. However, the serum urea level was significantly increased (P <0.05) while Cl, K+ and HCO_3^- levels were slightly increased above pre-infection values by week two post infection (PI). Serum Na+ increased only in the first week PI but returned to pre-infection values by the second week.

INTRODUCTION

African animal trypanosomiasis is a debilitating parasitic disease of livestock in sub-humid Africa resulting to huge economic losses annually (1,2). The disease has bee described as probably the single mot devatating disease in Africa in terms of poverty and lost agricultural production (2). The disease causes not less that 3 million livestock deaths each year and reduces calving rate, livestock numbers, milk yield, meat supply, work efficiency of draft animals and mixed farming (1). Despite the impact of the disease on man and his domestic animals, the exact factors involved in the pathogenesis or trypanosomiasis is not yet fully understood. The severity of pathology is dependent on the specie of infecting trypanosome and the host (3,4).

Trypanosoma vivax is highly pathogenic and a major threat to runminants in West Africa (3,5). The disease in small ruminants hitherto, was believed to be of less economic importance. However, reports on both natural and experimental infections in sheep and goats (1,6) show that the impact of trypanosomiasis in small ruminants is substantial. Anaemia and other haematological changes and serum biochemical changes associated with the disease has been described (4,7). In this investigation we report haemoglobin levels, urea and serum electrolytes associated with acute T. vivax infection in Red Sokoto goats.

MATERIALS AND METHODS

Six adult female Red Sokoto goats weighing 12.3 to 20.7kg body weight (BW) were used for the study. All the animals were purchased from local markets around Kaduna and screened for haemoarasites before use; all the animals were negative for trypanosomes by either haematocrit centrigugation technique (HCT) or by Buffy Coat Method (BCM) (8). During the acclimatization period which lasted three weeks, each animals was treated with Ivomec®, MSD-AGVET, U.S.A. at the does of 1.0ml/50kg B.W. subcutanously for internal and ecto parasite control. Oxytetracycline Long Acting (Pfizer, Ikeja, Nigeria) was also administered at the does of 1.0ml/10kg BW through the intramuscular (IM) route. Each goat was treated with diaminazene acetuavate (Berenil ®), vetimex, Bladel-Hollan, at 7.0mg/kg BW, IM. The animals were fed fresh grass, maize brain mixed with concentrates and water ad libitum. Four of the goats served as experimental (infected) group while the remaining two goats, served as control group. T. vivax (NITR/Federe) isolated from cattle and cryopreserved once, into a donor female Red Sokoto goat from where they were harvested from the jugular blood for inoculation 2 $x10^6$ parasites were inoculated into the goats intravenously. Blood samples collected weekly by jugular venipuncutres were put into ethylene diamine tetracetic acid (EDTA) bottles and sterile Universal bottles for Hb determination and serum separation respectively. Sera samples were stored at 20°C till analysed. Haemoglobin estimation was done using the cynomethaemoglobin method (9) to determine the Hb concentration. Sodium (Na+) and Potassium (K+) concentrations were determined using the flame photmeter (Corning Model 400, Corning Scientific Limited, England). Chloride (Cl-) and bicarbonate (HCO_3^-) were measured according to Toro and Ackerman (10) while serum urea level was determined as described by Harrison (11).

RESULTS

The infected goats became parasiteamic 3 to 4 days post infectio (PI). The infection in the Red Sokoto goats was virulent, characterized by fulminating parasitaemia and high fever. By week 2 PI, all the infected goats had died. The mean Hb level dropped slightly from 10.44g/dl + 1.34 before infection to 8.97g/dl by week 2 PI (P>0.05, fig.1).

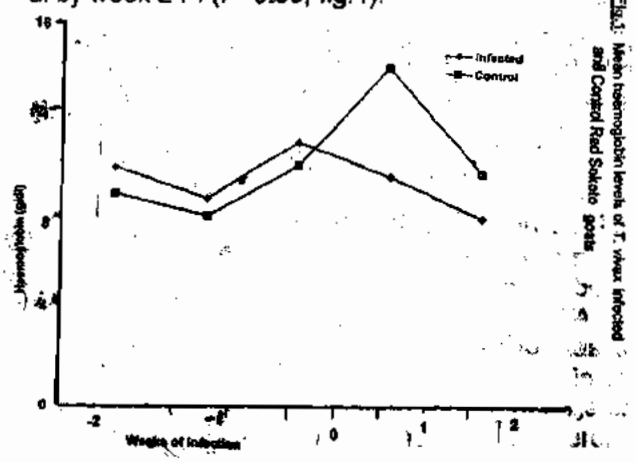

Fig.1: Mean haemoglobin levels of T. vivax infected and Control Red Sokoto goats.

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Serum urea levels and electrolyte changes in Red Sokoto goats are shown on table 1. The Urea level of infected animals increased significantly ($P<0.05$) from preinfection value of 4.9 + 2.50 (mmol/L) to 10.8 + 1.7 (mmol/L) by week 2 PI. The serum Cl-, K+ and HCO3 levels increased slightly above pre-infection values ($P>0.05$) by week 2. However Na+ increase only on week 1 PI but returned to pre-infection values by the second week.

PARAMETER	PRE-INFECTION	POST - INFECTION	
		WEEK 1	WEEK 2
Urea (mmol/L)	4.9 ± 2.5	7.16 ± 0.3	10.8 ± 1.7
Na^+ (mmol/L)	143.08 ± 5.8	160.0 ± 10.5	143.66± 15.6
Cl(mmol/L)	101. ± 1.7	92.66 ± 8.5	98.00 ± 5.3
K^+(mmol/L)	3.60 ± 0.6	4.4 ± 1.0	5.2 ± 0.9
HCO_3(mmol/L)	23.00 ± 0.5	25.0 ± 0.8	24.01 ± 1.2

Table 1 : UREA AND SERUM ELECTROLYTES IN T. VIVAX INFECTED RED SOKOTO GOATS

DISCUSSION

The acute nature of T. vivax infection in goats resulting, to death without significant fall in the haemoglobin levels in infected animals suggest that Red Sokoto goats are highly susceptible to T.vivax and death may result from other pathogenic factors beside anaemia. The course of T. vivax infection observed in this study differs from the observations of Kalu et al (7) and Akinwale et al (12) in the same breed of goats. This is probably as a result of high virulence of the strain of parasite used. High urea levels recorded in this study has previously been observed in acute and sub acute trypanosomiasis in cattle caused by T. vivax (13) and T. rhodesiense (14) and T. gambiense infected monkeys (15). Urea levels are elevated during periods of hgh parasitaemia and fever which occur in acute infection (4). The causes of elevated Blood Urea Nitrogen (BUN) include kidney disease such as glomerulonephritis, urinary tract obstruction and excessive protein catabolism associated with severe toxic and febrile conditions (4). Fever and glomerulonepritis, are consistent features of trypanosomiasis and acute disease course in Red Sokoto goats which was characterized by fulminating parasitaemia and high fever. These factors therefore may have acted together to precipiate very high increase in blood urea level, and perhaps with accompanying early renal damage. The slight increase in serum bicarbonate level is in agreement with previous observations in T. brucei – infected bicarbonate ions by the kidney. The observed increase in sodium ions are also attributable to renal dysfunction (18). Slight decrease in serum chloride recorded in this study doest not agree with previous repot on T. vivax infected goats (7). This might have arisen from the acute nature of the disease in the Red Sokoto goats. Kadima et al (17) however reported fluctuating levels of serum sodium and chloride ions which was associated with fluctuating parasiteamia in T. vivax – infected cattle. Serum potassium cations also increased in the T. vivax infected Red Sokoto goats. A similar increase was reported in T. brucei and T. equiperdum infections of rats(4) and T. gambiense – infected Monkeys (15). Anosa, (4) reported that increases in the serum K+ levels of trypanosome infected animals correlated with decreased in Red blood cell (RBC) values. He attributed it to release of K+ from RBC and damaged tissue coupled with the effects of kidney damage. The mild anaemia observed in this study may therefore have been responsible for the mild increase in the serum K+ level in the infected goats.

The findings of this study suggest that kidney damage occur probably very early in T. vivax infected goats and may be one of the factors in the pathogenesis of trypanosomiasis-in animals resulting to early death.

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