ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

JULY 2003 **VOLUME 4** **NUMBER 2**

Official Publication of the African Society for Clinical Microbiology

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

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002084/2313

FUNGAL INFECTIONS IN JOS: A 9-YEAR STUDY

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The prevalent mycoses and their aetiologic agents were studied in 1,287 patients in Jos and environs. 917 (71.3%) of the study population were infected. A male preponderance was observed and the age groups 11-20 and 21 –30 were predominantly infected. The most frequently isolated aetiologic agent was *Candida spp* and the commonest site of infection was the trunk. Unusual dissemination of lesions caused by *Trichosporon beigelii* infections was observed. The seasonal occurrence of mycoses and the effect on prevailing aetiologic fungi was studied.

INTRODUCTION

Fungal infections have, in the recent past, received greater recognition from various parts of the world, especially with the upsurge of several fungal pathogens. This upsurge has been attributed to factors such as immunodeficiency, the use of invasive devices or procedures, indiscriminate administration of broad-spectrum antimicrobial agents, which make the individual susceptible to even the otherwise non-pathogenic fungi (1-4). Some persons are at increased risk of susceptibility to infection because of their geographical location, level of health care delivery and ignorance (5-6).

In Nigeria, the most commonly encountered mycoses include superficial and cutaneous infections, but very few reports are available from some parts of the country on the epidemiology of these infections. Focus in the Northern Nigeria on mycoses has been on occupational groups, particularly on the Jos Plateau where mining is a major occupation (7).

The present report highlights the types and some aspects of epidemiology of mycoses in Jos, Plateau State, in the past 9 years.

MATERIALS AND METHODS

The sample population was made up of 1,287 patients, 690 males and 597 females, referred to the Mycology Laboratory of the Medical Microbiology Department of the University of Jos, from the Jos University Teaching Hospital, private clinics, the primary healthcare units within Jos and adjoining local settlements, between January 1991 to December 1999.

Personal data on sex, age, occupation, domicile, recreational activities and salient features of the lesions were recorded. Samples of

skin scrapings, hair, nail clippings, vaginal, wound and ear swabs, diarrhoeic stool and biopsy specimens were analyzed.

A portion of each specimen was examined under the light microscope, in 20% potassium hydroxide mount. Cultures of the specimens were also made on slopes of Sabouraud's Dextrose Agar supplemented with chloramphenicol (0.05mg/ml) and cycloheximide (0.5mg/ml). Cultures were incubated at room temperature (25-30^0 C) for one to six weeks, examined regularly for fungal growth. The yeast isolates were further subjected to a battery of biochemical and physiological tests to identify them to specific levels. Isolates identification was achieved by comparison with standard descriptions in books and manuals (8-10).

RESULTS

Of the 1,287 patients, 917 (71.3%) had fungal infections (Table 1). A mean yearly increase in incidence rate of 7.04% was recorded. Of the positive cases, 502 (54.7%) were males while 415 (45.3%) were females. There was a male preponderance of infection, generally. Of the 690 male subjects, 502 (72.9%) were infected while 415 (69.3%) of the 597 females were infected. The predominantly infected age groups were the 21 – 30 (24.0%) and 11 – 20 (20.0%) (Fig.1). The overall number of infected adults was higher than children.

The fungal lesions were found in various parts of the body, most commonly the trunk and multiple sites (Fig.2). Only four samples of tissue biopsy were analyzed and all yielded positive fungus cultures.

The most frequently encountered aetiologic agents over the nine year period are presented in Fig.3. Yeasts and yeast-like fungi predominated followed by dermatophytes with 444 (48.7%) and 392 (42.7%) isolates, respectively. *Candida spp* (69.0%) and *Trichophyton mentagrophytes* (41.9%) out numbered the yeast and dermatophyte isolates, respectively, in occurrence. Of the *Candida* isolates, *C. albicans* predominated.

Aspergillus fumigatus formed majority of the hyaline hyphomycetes and more than 70% was isolated from respiratory infections. *Wangiella dermatitidis* was the predominant dematiaceous fungus with 11 (31.4%) of the 35 isolates.

The mean monthly rate of infection in the population is shown in Fig. 4, the highest being in August (14.2%) and the lowest in December (4%). A striking observation here was the high occurrence of *Trichosporon beigelii* in the population, the lesions of which were frequently generalized.

Table 1: Incidence of Mycoses in the population

Year	No. sampled	No. infected		Total	%
		Male	Female		
1991	232	51	39	90	38.8
1992	98	23	27	50	51.0
1993	83	30	23	53	63.8
1994	83	32	16	47	56.6
1995	112	57	34	91	81.2
1996	123	51	51	102	82.9
1997	197	83	90	173	87.3
1998	179	90	66	157	87.1
1999	180	85	69	154	85.5
Total	1287	502	415	917	71.3

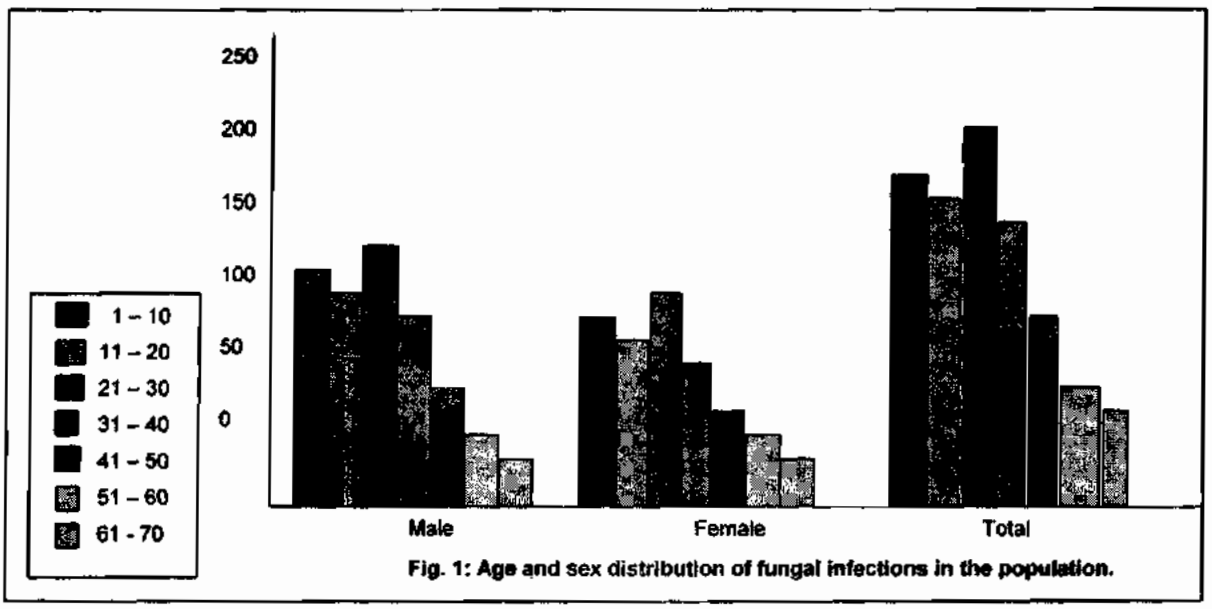

Fig. 1: Age and sex distribution of fungal infections in the population.

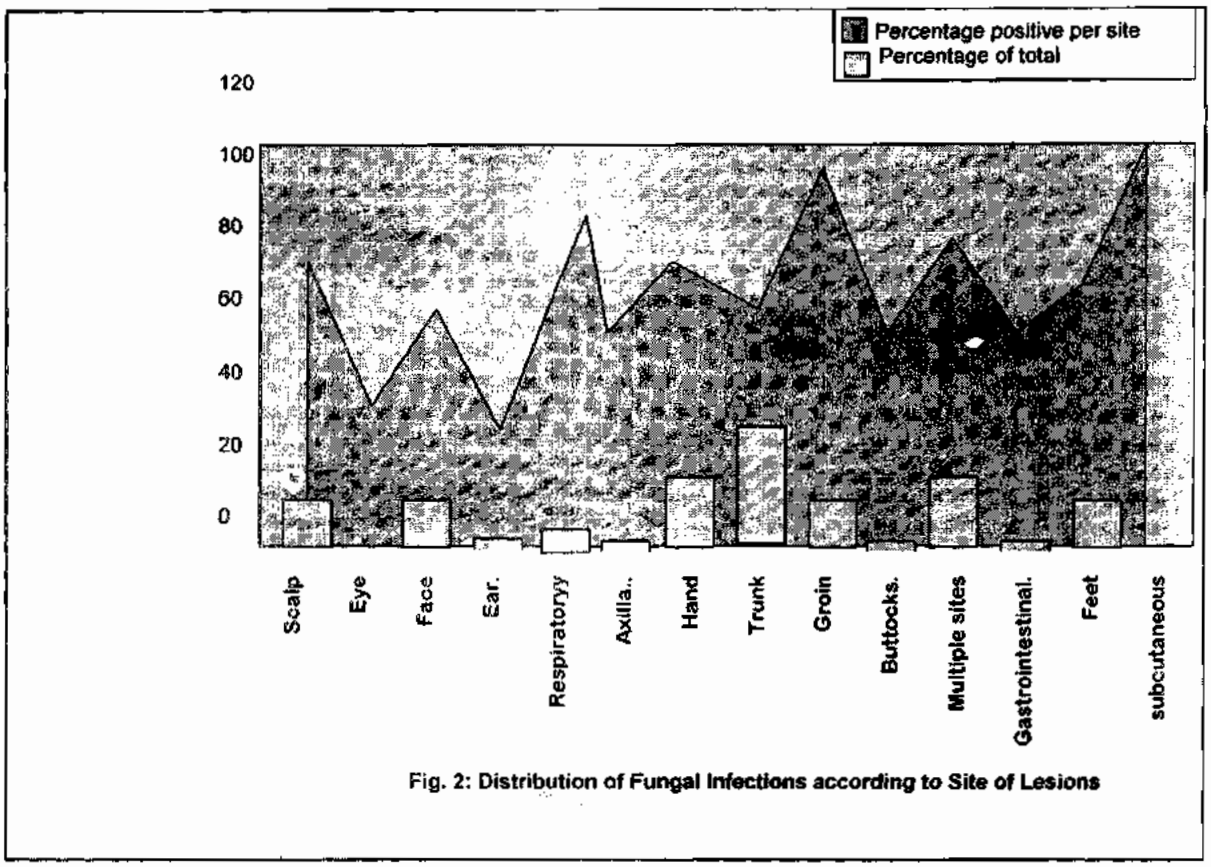

Fig. 2: Distribution of Fungal Infections according to Site of Lesions

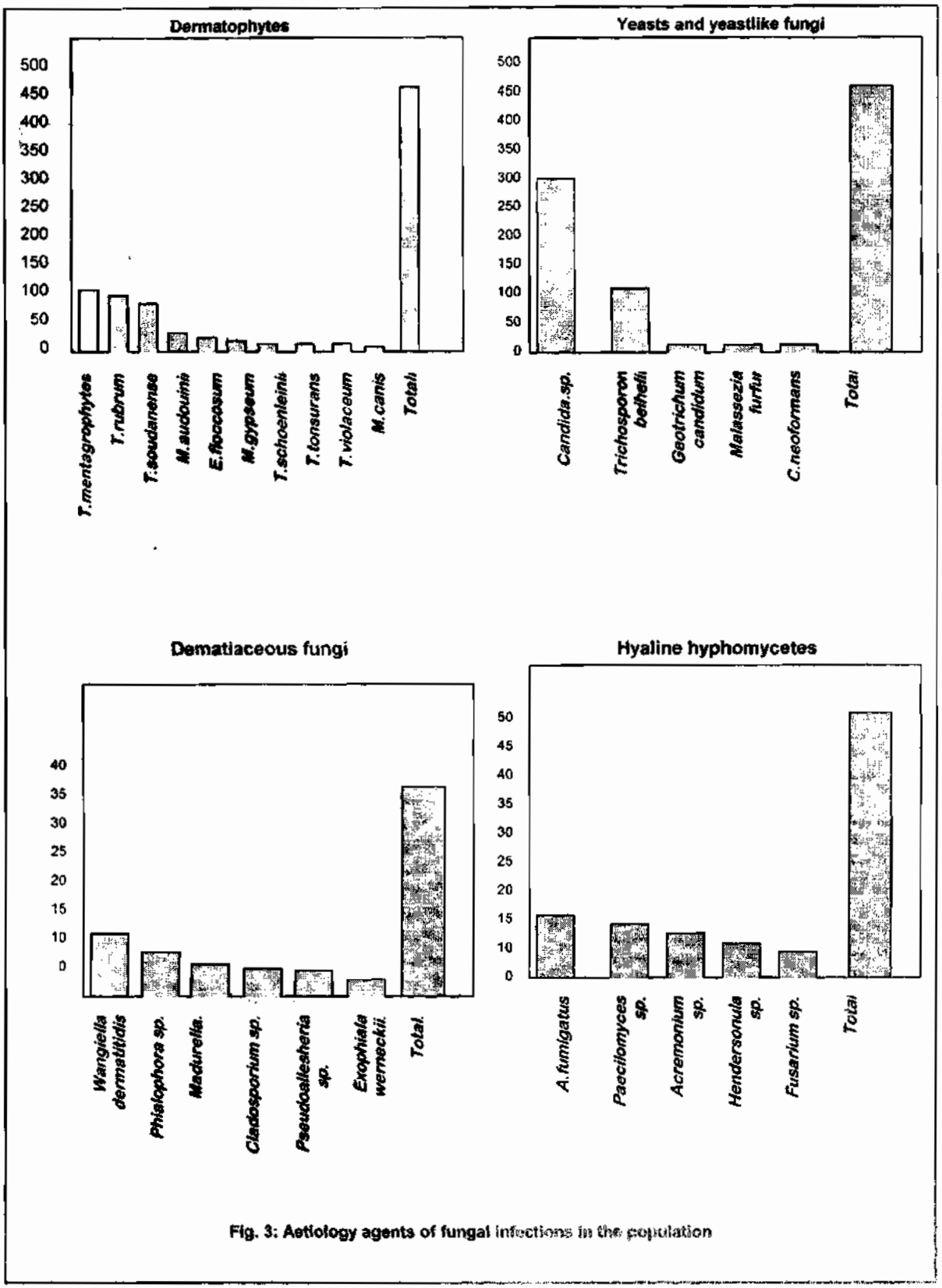

Fig. 3: Aetiology agents of fungal infections in the population

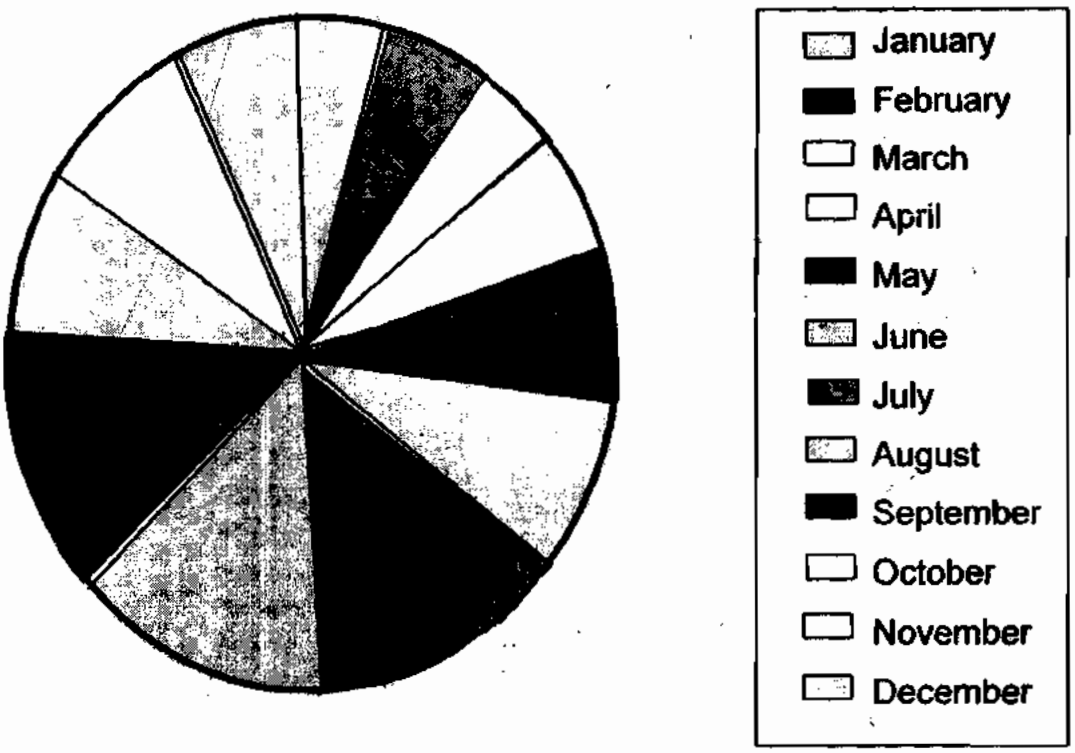

Fig. 4: Monthly Incidence of Mycoses in the Population

DISCUSSION

This study has revealed the increasing prominence of fungal infections as have been documented from other parts of the world (1-4). Over the years a significant increase was observed in the rate of occurrence of these infections. Some reports (2,3) have revealed that more stressors and factors of immunocompromise have contributed a lot to this trend. Although the present study did not investigate such factors, they may not be far from affecting the present population. More males were infected than females, similar to observations elsewhere within the country (12). Reports from parts of the world, however suggest an equal male – female ratio. This might not be unrelated to the life style of the citizens of Jos, and other parts of Nigeria where males are more exposed to outdoor sources of fungi such as recreational areas and engagement in mining activities.

The predominantly infected age groups, i.e. 21-30 and 11-20 years, include the most active in the populations both at work and play, hence their closer contact with sources of fungal pathogens. Also some these youths are completely ignorant of measures for prevention and control of fungal infections, and the living conditions in boarding schools and hostels where majority resides, favour the spread of mycotic infections. Positive lesions were obtained from various parts of he body, especially the trunk. This was most commonly ringworm infections (tinea corporis), in agreement with the findings of Al-sogar *et al* (10) in Saudi Arabia, and Egere and Gugnani, in Eastern Nigeria. Little is done in our laboratory on diagnosis of systemic mycoses because of limited resources, but the few tissue biopsies analyzed in this study yielded positive results. This is an important need for consideration by the authorities concerned.

Yeasts were predominant as causative agents of mycoses in this study. *Candida* species were the most frequently isolated, contrary to reports in Eastern Nigeria and some tropical countries. *Trichosporon beigelii,* as a single species was isolated in considerably high numbers. A number of investigators have highlighted the increasing importance of this organism, both in immuno-suppressed and immuno-competent individuals. The generalized lesions of this organism in a significant number of apparently immuno-competent subjects in this study seems to buttress the reports of other workers elsewhere (1,13).

The predominant dermatophyte species isolated was *Trichophyton mentagrophytes,* in contrast to the reports from the

southern parts of the country, and other places (10,12). Also the aetiologic agents in this study are at variance with those obtained in a number of reports. There could be climatic influence on the aetiology of mycoses, as suggested by some workers. The month of August recorded the highest number of infections, contrary to other reports (10) where the hot and dusty weather was suggested to promote spread of mycoses. In Jos the rains are relatively heavy in August and the atmosphere almost dust free. Probably because of the warmth and moisture obtained in this period, the development of fungal pathogens is favoured. The lowest infection rate was recorded in December in this study. This is one of the coldest months in the year here, exposure to outdoor activities like farming and swimming is minimal. This report seems to be the first to consider effects of seasonal variation on mycoses and their aetiologies in Northern Nigeria.

ACKNOWLEDGEMENT

We thank the Almighty God for the success of this work. Our sincere appreciation also goes to Mr. Yohanna Mato and Mrs Satya Noma, both laboratory assistants in the Mycology laboratory. Mrs. P Anele and Mr. Solomon Danladi for secretarial assistance.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002075/2314

INCIDENCE OF DERMATOPHYTE INFECTIONS AMONGST SOME OCCUPATIONAL AND SELECT GROUPS IN JOS.

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Fifty-nine Agro farm workers, 265 inmates from Jos main prison, 60 hair weavers and 40 car washers were examined in Jos for dermatophyte infections. Dermatophyte isolates included *Trichophyton* and *Microsporum species*. The highest infection rate of 75% was recorded among the farm workers with toeweb infections due to *T. mentagrophytes* and *M. canis*. 25% of the other infections were from the groin region due to *T. tonsurans* and *T. mentagrophytes*. Fifty-nine percent of infection by *T. mentagrophytes* in prison inmates was restricted to the groin region. Other species of fungi isolated from the inmates were *Trichosporon cutaneum* and *Aspergillus niger*. Among the car washers tinea manum, ringworm of the hand, andinterdigital infections due to *T.mentagrophytes* were observed. There was no visible infection among either the female hair weavers or the female prison inmates.

Keywords: Dermatophytosis, *T. mentagrophytes, T. tonsurans, M. canis, Tinea pedis, Tinea cruris.*

INTRODUCTION

Cutaneous fungal infections caused by dermatophytes and commonly known as ringworm infection have been known to be occupationally acquired, as reported by Philpot (1) and Ogbonna *et al* (2). Such infections have been found to be common among anthropophilic or human species. The characteristic among such species is that they are associated with community living and the spread of such infections require close human contact. This might occur as a result of communal use of certain facilities such as baths (3), combs, brushes and articles of clothing (4). Incidence of ringworm infections through such sources could then be found among workers who share working boots and overalls such as coal miners, factory workers, camp and prison inmates. It has even been reported in families by English (5) where overcrowding and concentrated use of common facilities result in infections. *Tinea pedis* (ringworm of the toeweb) could be spread through desquamated scales adhering to moist surfaces such as bathrooms or changing room floors (6).

Zoonosis is found in workers whose occupation lead to contact with infected animals or through contaminated working equipment. Such workers could include herdsmen (2). Hairdressers and barbers also run the risk of infection through infected hair and scalp of their customers. The combined effect of host factors, moisture, warmth, duration of exposure to pathogen and other environmental stresses have been found by Gentles and Holmes (7), to

play important role in the initiation and establishment inflection within a community.

This study was undertaken to report on the first survey of incidence of dermatophyte infection and environment effects among selected occupational groups in the Jos Plateau of Nigeria.

MATERIALS AND METHODS

Study population

The workers were agricultural farm workers from Zalaki farm about 25 km from Jos metropolis, car washers and hair weavers from within Jos. In addition, inmates of Jos main prison were also examined. The farm workers were selected due to their proximity and contact with the animals as possible source of infection. The hair weavers were examined owing to their possible handling of hairs of their infected customers, while the car washers could be exposed to particles of soil adhering to dirty cars, which could contain spores of dermatophytes. The prison inmates were selected because they are exposed to over-crowding and poor environmental sanitation conditions. All sampling was carried out between September and November 1991.

The climate of Jos Plateau is peculiar to itself in Nigeria. There is Savannah woodland vegetation and temperate climate quite different from the hot humid parts of other parts of Northern Nigeria. The geographical location confers on it Mediterranean climate conditions with dry cold weather and very low humidity.

Specimen collection and processing

Fifty-nine animal farm workers whose ages were between 25-45 years were sampled. Their bodies were thoroughly examined for the presence of cutaneous dermatophyte infections. Lesions remotely resembling fungal infections were cleaned with 70% alcohol and scrapings were then collected from affected body areas with the aid of sterile blade into sterile paper packets, which were then transported to the laboratory for further fungal analysis. The scrapings were cultured on Sabouraud Dextrose Agar supplemented with 0.05 mg/ml chloramphenicol and 0.5 mg/ml cycloheximide in order to suppress growth of bacterial contaminants. Cows on the farm were examined as possible source of human infection among the farm workers. Tufts of hair were collected from infected cows in paper wrappers and cultured for the presence of keratinophilic fungi using the "hair baiting" technique as described by Mckenzie. This involved the use of sterile petri dishes, which were half-filled with soil that have been sterilized by autoclaving at 121°C

for 30 minutes for three successive times. The infected hairs from the cows were then sprinkled on the sterilized soil after the soil has been sufficiently moistened with sterile distilled water. The petri dishes were incubated at room temperature in the dark by placing them in closed cupboard.

In Jos main prison, 265 male inmates whose ages range from 18-70 years were thoroughly examined. Scrapings were collected from the affected parts of the body after cleaning the area with 70% alcohol. The scrapings were plated out on Sabouraud Dextrose Agar containing appropriate antibiotics as in the case of the farm workers.

In the case of the 60 female weavers and 40 car washers whose ages range from 30-45 years respectively, bodily examination was only restricted to areas of the body not covered by clothing due to the outdoor nature of their jobs. Skin scrapings collected from interdigital infections of the car washers were subjected to the same treatment for the isolation of dermatophyte fungi as described for farm worker above.

The resultant culture plates from all the sampling except those cultured on sterilized soil were incubated at 37°C and examined on daily basis for the presence of dermatophytes.

The dermatophytes that appeared mainly after 10-14 days were subjected to series of subculturing until pure cultures were obtained. The isolates were then identified with the aid of the microscope and existing stock cultures. References were made to Rebel and Taplin (8); Rippon (9); Campbell and Stewart (10). Wherever necessary, slides cultures were made. Analysis of variance was used in the interpretation of the results.

RESULTS

The dermatophyte from the various occupational groups as shown Table I included *Trichophyton and Microsporum* species. *Trichophyton tonsurans* observed in toe-web infection among the farm workers was also isolated from one of the cows. Among the prison inmates, out of a total of 41 positive cultures, 9 (21.95%) were due to infection caused by *T. mentagrophytes* and 7 (17.07%) by *T. cutaneum*. All infections were recorded among the male inmates, but none in their female counterparts and female hairdressers. In the case of the car washers, only interdigital infections caused by *T. mentagrophytes* were recorded.

Table 1: Distribution of Dermatophytes and related fungi amongst occupational and select groups in the Jos, Plateau State.

SITES OF INFECTION

Organisms	GROIN		TOE-WEB		NAIL		FOLD OF BUTTOCKS		SHOULDERS		TOTAL
	P.I	F.W	P.I	F.W	P.I	F.W	P.I	F.W	P.I	F.W	
Trichophyton mentagrophytes	2(4.87%)	1(8.33%)	0	3(35.0%)	0	0	0	0	0	0	6
Trichophyton tosurans	0	2(15.66%)	0	2(16.66%)	0	0	0	0	0	0	6
Microsporon canis	0	0	0	2(16.66%)	0	0	0	0	0	0	2
Trichosporon cutaneum	7(17.07%)	0	0	0	0	0	0	0	0	0	7
Aspergillus niger	3(7.31%)	0	0	0	0	0	0	0	0	0	3
Pityosporum	0	0	0	0	0	0	0	0	5(12.19%)	0	5
Candida	2(29.26%)	0	0	0	0	0	0	0	0	0	2
Mucor sp	0	0	0	2(16.66%)	0	0	0	0	0	0	2
Dermatitis	0	0	0	0	0	0	10(24.39%)	0	0	0	10
TOTAL	14	3	0	9	0	0	10	0	5	0	41(100%)

KEY: P.I. = Prison Inmates,
F.W. = Farm Workers

Table 2: Analysis of Variance for data on distribution of dermatophytes and related fungi amongst Zalaki Farm Workers

ANOVA

Source of Variations

	SS	df	MS	F	P.Value	Fcrit
Rows	0.800	3	0.267	2.285	0.357	3.490
Columns	15.300	4	3.825	17.00	0.000	3.259
Error	2.700	12	0.225			
TOTAL	18.800	19				

Multiple Range Value = 0.129

Table 3: Analysis of Variance for data on distribution of dermatophyte and related fungi amongst Jos Prison Inmates.

ANOVA

Source of Variations

	SS	df	MS	F	P.Value	Fcrit
Rows	18.971	6	3.162	0.377	0.886	2.508
Columns	66.971	4	16.743	1.999	0.127	2.776
Error	201.029	24	8.376			
TOTAL	286.971	34				

Multiple Range Value = 3.419565

DISCUSSION

The results obtained have shown that infection with dermatophyte species is an occupational hazard. This agrees with previous findings from other investigators (2,6,11). Continous exposure of the farm workers to infected cows could result in their being infected with dermatophyte species that are zoontic. The farm workers at Zalaki had 75.0% rate of interdigital infections. The conditions of service could have enhanced this high infection rate among the workers. The workers were observed to share pithead shower facilities at the end each day's work. Dermatophytes have been isolated from floors of pithead baths, swimming pools and changing rooms (3). English (5) has also observed that fungi have been known to survive for months in shed skin scales even after serveral laundering of wool. Also the use of alkaline soap could have enhanced the spread of infection, among the community. In a survey of a naval establishment in Cranston England, Broughton (6) found that tinea pedis was particularly common among seamen experiencing much desquamation due to the use of alkaline soap. It would therefore appear that the communal use of shower facilities, the use opf common overall and soap could have all played significant roles in the spread of ringworm infections amongst those workers. Moreover, the use of heavy footwear in damp hot weather condition may also have provided suitable environment for their infection (11).

The dermatophyte species, *T. mentagrophytes* and *T. tonsurans* isolated form the workers are known anthropophilic species not restricted to any geographic region. However, the isolation of *T. tonsurans* in one of the infected cows is worthy of note since this anthropophilic species has not been listed as a zoophilic and neither has it been isolated an from an animal. Its mode of transmission though uncertain could have been from man to animal or vice versa. Infections from infected animal have been known to be acquired from building or equipment contaminated by contact with infected animals, while sources of infection of farm animals could include barns, fences and soil. Infections among the prisoners were mainly in the groin accounting for 58.53% of total infections. Tinea cruris has been considered an infection of sedentary people and to be more common among males than females. This could account for the non-isolation of species of these fungi from the Jos female prison inmates (7.8%) in view of the prevailing environmental conditions of over-crowding, poor personal hygienic and nutritional conditions. The dry low humidity of the Jos Plateau could have contributed to the low infection rate, as it reduces sweating, which reduces ringworm infection to the barest minimum.

This can be compared to situations in India where Tinea corporis and Tinea pedis are uncommon but Tinea cruris particularly of the lower covered waistline and groin assumes particular clinical significance due to the not humid climate of the region (12).

Ringworm infections were not recorded among the female hair weavers. The absence of infection among them in spite of their handling and association with people's hair could be due to the fact that the hair weavers wash their hands after attending to each customer, coupled with the age range of their customers who were found to be adults between the age range of 20-60 years. Tinea capitis is an infection that is common among children, the highest incidence occurring between the ages 4-14 years. The risk of contracting infections from other parts of the client's body is reduced, as bodily contact is minimal.

The constant exposure of car washers to water and use of different types of detergent must have been predisposing factors to their tinea pedis and tinea manum infection.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY, 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002075/2314

INHIBITION OF SWARMING BY UREA AND ITS DIAGNOSTIC IMPLICATIONS AMONG UROPATHOGENIC PROTEUS SPECIES FROM LAGOS, NIGERIA

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The anti-swarming property of urea and effects on antibiotic susceptibility among 52 uropathogenic *Proteus* strains from Lagos, Nigeria were investigated. Urea caused a reduction in swarming and number of swarmed cells at 0.5% (n = 42, DOCZ = 15.5mm), 0.75% (n= 24, DOCZ = 10.7mm), 1% (n = 17, DOCZ = 3.4mm) and 1.25% (n = 8, DOCZ = 1.7mm). Compared to DOCZ obtained at 0.5% urea, the further reduction in DOCZ at other urea concentrations was found to be significant ($p < 0.05$). Urea at less than 0.75% allowed identification of *E. coli, K. pneumoniae and S. saprophyticus* in mixed cultures containing *Proteus spp,* while colonies of *Pseudomonas aeruginosa* were distinctly identified at 1% urea with swarming restrained at 1.25% urea. At 1.25% urea, antibiotic susceptibility testing by agar diffusion method revealed significant increase and decrease in the number of *Proteus* strains that showed resistance to amoxicillin and nitrofurantoin. Compared with the control, significant increases in the MICs of gentamicin or nitrofurantoin and streptomycin were found at ≥ 0.5% and ≥ 0.75% urea respectively ($p<0.05$). The identification of extended spectrum beta lactamases (ESBL) producing strains were unaffected by urea. This study has demonstrated urea induced swarming inhibition of uropathogenic *Proteus in vitro.* However, results suggest the use of urea with great caution in diagnostic practices for optimal clinical and public health benefits in Nigeria.

Keywords: Antibiotic susceptibility, anti-swarming, urea, uropathogenic proteus, Nigeria, DOCZ (= Diameter of outermost colony zone mean value)

INTRODUCTION

Proteus spp are Gram-negative facultative anaerobic rods of tremendous clinical and public health importance particularly in developing countries (1). These bacteria are frequently implicated as aetiologic agents of urinary tract infections (UTI), which may lead to kidney damage and complicate pregnancies if untreated (2, 3). In Nigeria, several hospital and community based studies have put the isolation rates of *Proteus spp* at 3-8%, with *Proteus mirabilis* and *P. vulgaris* as predominant nosocomial pathogens among patients with indwelling catheters, benign prostatic hypertrophy, and vesicoureteral reflux (4, 5, 6). Of great concern is the mortality rate report of 17.1% in African neonates with UTIs that are untraceable by the radiologic examination of the urinary tract (7).

One of the unique characteristic features of *Proteus spp* on tolerable culture media is the ability to spread and form a thin film with distinct colonial zones on agar surfaces. This phenomenon called swarming, involves the differentiation of vegetative motile cells to hyperflagellated-elongated cells capable of coordinated and

concerted mass population migration (8). The susceptibility of catheterized patients to *Proteus* associated UTI has been demonstrated in vitro using Foley catherters (9). Swarming has also been shown to be one of the requirements for the colonization of urothelial cells *in vivo* and *in vitro* (10, 11). One of the drawbacks of *Proteus* swarming in the laboratory is the inherent difficulty in the detection of other pathogens in polymicrobial infection cases. Fons *et al* (12), reported the difficulty in distinguishing colonies of *Pseudomonas aeruginosa* among *P. mirabilis* swarm cells on agar plates.

In routine diagnostic laboratories, the use of nutrient and blood agar media for *Proteus* culture storage and antibiotic susceptibility testing, as practiced in developing countries, may compromise purity of stocks for genetic studies and many cases of polymicrobial infections involving *Proteus* may not be noticeable. This may hinder the use of drug combination to implement effective clinical cure, provide a reason for the incidence recurrent bacteruria in treated patients and promote antibiotic resistance of these 'silent' organisms. False antibiotic susceptibility outcome of pathogens *in vitro* also contributes to the spread of drug resistant strains in a community and promotes clinical failure.

Urea, P-nitrophenylglycerol (PNPG), and activated charcoal have experimentally been demonstrated to possess anti-swarming properties and recommended for routine laboratory usage (12,13,14). However the use of PNPG in culture media for antibiotic susceptibility testing has been queried (15). In Nigeria, urea is commonly used in culture media designed for the identification of pathogens of UTIs including *Proteus spp* (4, 5). However, reports have been silent on *Proteus* swarming prevention possibilities and consequences on antibiotic susceptibility outcome. This study investigated the clinical importance associated with the use of urea in *Proteus* identification media in terms of pattern of swarming inhibition, the effect on antibiotic susceptibility and extended spectrum beta lactamase (ESBL) classification.

MATERIALS AND METHODS

Bacterial strains

Fifty-two *Proteus spp* isolated from randomly selected 408 mid stream urine samples of in-patients and outpatients at different clinics and hospitals in Lagos, were used in this study. The isolates were identified on McConkey, Blood agar and composite media using criteria which included non-lactose fermentation, swarming ability,

urease and phenylalanine deaminase production (16). For mixed culture assay, pure strains of *P.aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus* and *E. coli* were obtained from the Microbiology Laboratory of the Nigerian Institute of Medical Research (NIMR), Lagos.

Swarming inhibition assay

Nutrient agar plates containing 5% sheep erythrocytes and supplemented with and without 0.5-1.25% urea (Sigma, USA) were used to grow the selected *Proteus* strains. A loopful of standardized inoculum (2×10^4 CFU/spot) of each strain was concentrically inoculated at the center of the agar and incubated at 37°C for 24 hours under aerobic conditions. Urea negative plates were used as controls. The swarming profile of *P. mirabilis* ATCC49565 was examined in parallel with those of the test organisms. The degree of swarming was measured as the diameter of the outermost colonial zone (DOCZ). DOCZs were interpreted as mean ± standard deviation, to allow statistical deductions using student's t- test and chi-square analysis. P value less than 0.05 was indicated as significant.

Mixed culture assay

Four swarmed isolates of the tested *Proteus spp* were rapidly selected and suspended in Mueller-Hinton broth containing at least any two of *P.aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus* and *E. coli*. The resulting mixed cultures were then used to inoculate Blood agar supplemented with urea (0.5-1.25%). Mixed culture plates containing a reference swarming strain of *P. mirabilis* ATCC49565 was also examined. Mixed culture plates without urea also served as control.

Antibiotic susceptibility testing

The response of untreated *Proteus* isolates and urea survivors to 13 antibiotics used in Nigeria was investigated by agar diffusion method according to Bauer *et al* (17). The antibiotics from Abtek Biological Limited, Liverpool, England mounted on inoculated plates were tetracycline 10 µg, ampicillin 25 µg, amoxicillin 25 µg, co-amoxyclav 30 µg, cotrimoxazole 25 µg, nitrofurantoin 200 µg, ceftazidime 30 µg, ceftriazone 30 µg, nalidixic acid 30 µg, streptomycin 25 µg, gentamicin 10 µg, ciprofloxacin 10 µg, and ofloxacin 5 µg. Diameters of zones of inhibition were measured to the nearest millimeters and values were interpreted as resistant and susceptible based on comparison with antibiotic susceptibility profile of *E. coli* ATCC 25922, a drug sensitive strain.

Determination of minimum inhibitory concentration (MIC) and selection of Extended Spectrum Beta Lactamase (ESBL) producing strains

The MICs of the selected antibiotics were determined by a microdilution method. *Proteus* strains from urea containing and deficient plates were first grown overnight on cysteine lactose electrolyte deficient (CLED) agar at 37°C under aerobic condition. Four distinct colonies of each strain were then subcultured in 4 ml of IsoSensitest broth (Oxoid, UK) and incubated at 37°C to attain a turbidity that was adjusted to 10^6 CFU/ml with the broth. Stock solutions of the antibiotics were prepared fresh at 128 mg/L for ampicillin, amoxycillin, co-amoxiclav, nitrofurantoin, streptomycin and tetracycline; 64 mg/L for cotrimoxazole; 32 mg/L for nalidixic, acid, gentamicin, ceftazidime and ceftriaxone; and 4 mg/L for ciprofloxacin and ofloxacin. In a 12 by 8 wells tray, 50 µL of antibiotic solution occupied the first two rows and subsequently double diluted to fill the remaining rows except the 12^{th} row, which served as a positive control. 50 µl of inoculum at 5 x 10^5 CFU/well were then added to the wells in increasing order of antibiotic concentrations. Antibiotic susceptibility of a control strain of *P. mirabilis* ATCC 49565 was examined in parallel with test organisms. All the plates were sealed and incubated at 37°C for 24 hours. Growth was assessed as turbidity observed on transillumination. The MIC of each antibiotic was defined as the lowest concentration that inhibits growth. Interpretation of MICs as resistant or susceptible was in line with NCCLS break points of the antibiotics tested (18). The significance of the mean MIC value differences between urea treated *Proteus* and untreated isolates was also evaluated statistically. Extended beta-lactamase producing strains were selected as those with ceftazidime: ceftazidime-clavulanate ratio greater than or equal to 16 according to Livermore and Yuan (19). Values of 8 were regarded as indeterminate.

RESULTS

Survival and variations in the ability of urea at 0.5- 1.25% to refrain swarming of *Proteus* isolates were highlighted in Figure 1. All the *Proteus* strains studied survived urea at 0.5-1.25% concentrations. At 0.5% urea, 42 out of the 52 isolates swarmed and produced an average diameter of outermost colony zone (DOCZ) value of 15.5 mm. At 0.75% urea, 24 isolates swarmed producing mean DOCZ value of 10.7 mm. The number of swarmed *Proteus* further decreased

from 17 to 8 following 1-1.25% urea treatments. Mean DOCZ values of 3.4 mm and 1.7 mm were produced respectively. Urea at 0.5% was observed to allow distinct identification of *E. coli* and *K. pneumoniae* in mixed culture assay. *Staphylococcus saprophyticus* colonies were identified at 0.75% urea while at 0.75 and 1%, *Pseudomonas aeruginosa* was identified, with swarming inhibited (Table 1).

However, the number of cells that displayed resistance to amoxicillin, gentamicin, nitrofurantoin and ofloxacin by disk diffusion method at 1-1.25% urea differed (Table 2) and those of amoxicillin and nitrofurantoin were statistically significant ($p<0.05$). Table 3 summarized data for MICs of the 13 antibiotics tested. Significant increases in the MICs of nitrofurantoin or gentamicin, and streptomycin were obtained at ≥ 0.5 and ≥ 0.75% urea respectively ($p<0.05$). Furthermore, two *Proteus* strains were identified as extended beta lactamases producers in plates devoid of urea and those containing 0.5 - 1.25% urea. However, no isolate was identified as indeterminate for ESBL production among the strains cultured without urea, whereas, between one and two indeterminate identifications were recorded among the urea treated strains (Table 4).

Table 1: Isolates identification from mixed cultures containing swarmed *Proteus* strains.

Mixed culture assay	Isolate identification scheme
1.	*Proteus* strains
2.	*Proteus* strains, *E. coli, K. pneumoniae,*
3.	*Proteus* strains, *E. coli, K. pneumoniae, S. saprophyticus, P. aeruginosa*[a]
4.	*Proteus* strains, *E. coli, K. pneumoniae, S. saprophyticus, P. aeruginosa*[b]
5.	*Proteus* strains, *E. coli, K. pneumoniae, S. saprophyticus, P. aeruginosa*[c].

Keys: 1 = Assay without urea; 2 = Assay containing 0.5% urea; 3 = Assay containing 0.75% urea; 4 = Assay containing 1% urea; 5 = Assay containing 1.25% urea. *a, P. aeruginosa* not distinctly identified; *b, P. aeruginosa* swarmed cells identified distinctly; *c, P. aeruginosa identified without swarming* .

Table 2: Resistance to antibiotics in the presence and absence of urea by the 52 Proteus strains using disk diffusion method.

	Proteus spp.				
	E	a	b	c	d
Antibiotics	n (%)	n (%)	n (%)	n (%)	n (%)
Ampicillin	32 (61.5)	32 (61.5)	32 (61.5)	32 (61.5)	32 (61.5)
Amoxicillin	31 (59.6)	31 (59.6)	31 (59.6)	31 (59.6)	36 (69.2)*
Co-amoxiclav	29 (55.8)	29 (55.8)	28 (53.8)	28 (53.8)	28 (53.8)
Cotrimoxazole	48 (92.3)	48 (92.3)	48 (92.3)	48 (92.3)	46 (88.6)
Ceftazidime	3 (5.8)	3 (5.8)	3 (5.8)	3 (5.8)	3 (5.8)
Ceftrixone	3 (5.8)	3 (5.8)	3 (5.8)	3 (5.8)	3 (5.8)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	2 (3.8)	2 (3.8)	5 (9.6)	5 (9.6)	7 (13.5)
Nalidixic acid	2 (3.8)	2 (3.8)	2 (3.8)	2 (3.8)	2 (3.8)
Nitrofurantoin	7 (13.5)	7 (13.5)	7 (13.5)	6 (11.5)	4 (7.7)*
Ofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Streptomycin	34 (65.4)	34 (65.4)	34 (65.4)	32 (61.5)	32 (61.5)
Tetracycline	50 (96.2)	50 (96.2)	50 (96.2)	50 (96.2)	50 (96.2)

Keywords:Urea supplementation: (E, without urea; a, 0.5% urea; b, 0.75% urea; c, 1.0% urea; d, 1.25% urea), n (%), number and percentage of antibiotic resistant strains.
* = significant at 95% confidence limit by chi square analysis.

Table 3: Minimum inhibitory concentrations of antibiotic resistant Proteus strains by microbroth dilution method.

	Proteus spp. MIC (mg/L)				
	E	a	b	c	d
Antibiotics	n ( )	n ( )	n ( )	n ( )	n ( )
Ampicillin	32 (75+33.1)	32 (74+33.9)	32 (76+32.3)	32 (76+32.3)	32 (77+34.3)
Amoxicillin	33 (81+33.5)	33 (81+33.5)	33 (82+32.4)	33 (83+34.3)	33 (80+31.5)
Co-amoxiclav	29 (77.2+33.7)	29 (77.2+33.7)	29 (79.4+34.9)	31(79.5+35.9)	31 (80.5+31.9)
Cotrimoxazole	48 (82.7+33.6)	48 (83.3+32.8)	48 (84+34.1)	48 (84.7+33.3)	48 (84+34.1)
Ceftazidime	3 (18.7+12.2)	3 (21.3+9.2)	3 (18.7+12.2)	3 (21.3+9.2)	3 (26.7+9.2)
Ceftrixone	3 (21.3+9.2)	3 (26.7+9.2)	3 (26.7+9.2)	3 (21.3+9.2)	3 (21.3+9.2)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	2 (8.2+1.8)	2 (12.0+5.7)*	5 (20.8+10.7)*	5 (22.4+8.6)*	7 (21.7+10.0)*
Nalidixic acid	2 (12.0+5.7)	2 (12.0+5.7)	2 (16.0+0.0)	2 (12.0+5.7)	2 (12.0+5.7)
Nitrofurantoin	7 (59.4+34.2)	7 (73.1+40.1)*	7 (82.3+31.2)*	7 (73.1+40.1)*	7 (86.9+40.1)*
Ofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Streptomycin	35 (85.9+33.6)	35 (85.9+33.6)	35 (90.5+33.3)*	35 (91.4+32.1)*	35 (92.3+33.6)*
Tetracycline	50 (90.9+33.8)	50 (90.9+33.8)	50 (92.2+34.0)	50 (92.2+34.0)	50 (92.2+34.0)

Keywords:Urea supplementation: (E, without urea; a, 0.5% urea; b, 0.75% urea; c, 1.0% urea; d, 1.25% urea), n ( ), number of antibiotic resistant strains, numbers in parentheses are mean ± SD of MIC values, * = significant at 95% confidence limit by Student's t-test.

Table 4: **Identification of the extended beta lactamases producing strains among the *Proteus spp.***

	Proteus spp.				
	E	a	b	c	d
ESBLs n (%)	2 (3.8)	2 (3.8)	2 (3.8)	2 (3.8)	2 (3.8)
Inderminate	0 (0)	1 (1.9)	1 (1.9)	1 (1.9)	2 (3.8)

Keywords: Urea supplementation: (E, without urea; a, 0.5% urea; b, 0.75% urea; c, 1.0% urea; d, 1.25% urea), n (%), number and percentage of ESBL producing strains or inderterminate result.

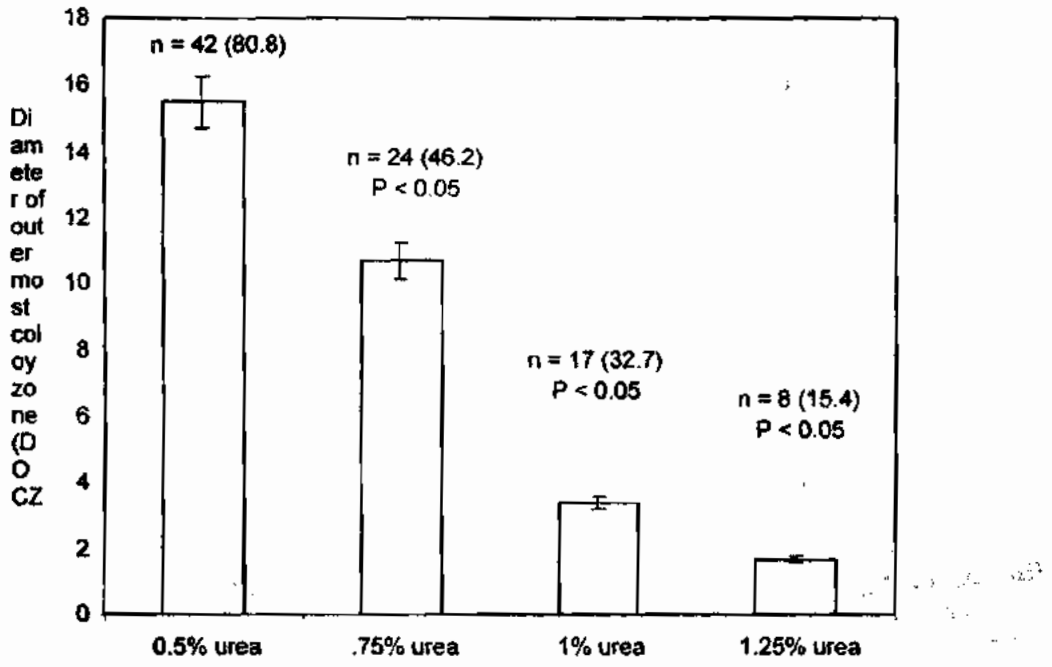

Figure 1: Effect of urea on swarming among *Proteus* isolates from Lagos, Nigeria.
Bars represent mean DOCZ valuesand projections on bars indicate deviations from mean.
n = number of swarmed *Proteus* strains, figures in parentheses indicate percentages.

DISCUSSION

Urea is primarily used in selective and composite media to identify urease-producing microorganisms (20). In recent times, the possibilities of exploiting the anti-swarming property of urea to aid isolation and identification of single colonies on solid media are being tested (12). In the present study, we found urea useful in refraining swarming of *Proteus spp* causing urinary tract infections in Lagos. *In vitro*, urea was also observed to allow isolation and identification of *K. pneumoniae, Pseudomonas aeruginosa; S. saprophyticus* and *E. coli. Pseudomonas aeruginosa* identified at 1% urea is higher than the 0.5% urea reported by Fons *et al* (12). The disparity in urea concentration could be attributed to strain variation and difference in swarming ability. Antigenic differences and varying capsular polysaccharide composition have been found among swarming *Proteus spp* (21). All the *Proteus* isolates examined survived urea at 1.25% and this suggests that urea is not inhibitory to cell growth at this concentration. This may not be unexpected since many media formulations for identification and speciation of bacteria contain up to 2% urea (22).

In this study, we found that the responses of our isolates to amoxicillin, nitrofurantoin, gentamicin and streptomycin were compromised following exposure to urea. This became more evident when the minimum inhibitory concentrations of these antibiotics were determined. In a study conducted by Ward *et al* (15), P-nitrophenylglycerol was found to increase the MICs of aminoglycosides; gentamicin and tobramycin and decrease the MICs of ticarcillin, ciprofloxacin and colistin against *Pseudomonas aeruginosa.* In Nigeria, several prospective studies have condemned the use of streptomycin and amoxicillin for empirical treatment of bacterial infections (23, 24). Recently, high amoxicillin resistant *Helicobacter pylori* strains were found in the biopsy samples of patients with gastritis and peptic ulcer in Western Nigeria (25). Although MICs of gentamicin and nitrofurantoin among the urea treated and untreated *Proteus* strains were above their respective break points (17), it is very important to adopt a cautionary use of urea when investigating susceptibility of *Proteus* to these antibiotics. The prevalence of bacterial pathogens that show resistance to these antibiotics in human infections is generally low in Nigeria (23). Therefore, discrepancies associated with the assessment of pathogens to these antibiotics may jeopardize control

measures and heighten the risk of multidrug resistant infections. The identification of pathogens as ESBL further provides an insight into their mechanisms of resistance to beta-lactam drugs. ESBL pathogens have been experimentally demonstrated to express stably de-repressed, constitutive chromosomal class 1β-lactamases, which hydrolyze most β-lactam antibiotics except carbapenems (26). This study has demonstrated the inability of urea at ≤ 1.25% to cause no discrepancies in ESBL classification and thus provides an additional credit to its diagnostic usefulness in clinical medicine and public health.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002083/2316

THE EFFECT OF SOME NIGERIAN LOCAL HERBS ON HELICOBACTER PYLORI

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Four Nigerian medicinal plants commonly used in the treatment of bacterial infections were tested for antimicrobial activity against twenty local strains of *Helicobacter pylori* recovered from patients with gastro-duodenal ulcers and gastritis. In vitro agar diffusion assay revealed anti-*Helicobacter pylori* activity of ethanolic extracts of *C. papaya* and *M. lucida* to 80% (16/20) of the isolates tested. While the ethanolic extracts of *O. gratissimum* and *P. amarus* inhibited the colonial growth of 35% (7/20) of these strains. The zones of inhibition ranged from 5 – 20 mm in diameter. Contrastingly, the aqueous extracts of these plants appeared to lack anti-Helicobacter pylori activity except in *M. lucida* and *O. gratissimum* where inhibition of a total of three isolates was observed. The present results suggest the presence of anti-*Helicobacter pylori* principles in ethanolic extracts of *C. papaya* and *M. lucida* and support their future use in the treatment of ulcers and gastritis in Nigeria.

INTRODUCTION

It is over a decade that *Helicobacter pylori* infections were known as a major cause of gastro-duodenal ulcers, gastritis and stomach cancer with greater burden of cases documented in developing countries (1, 2). Most effective therapies employ a synergic action between a gastric acid release inhibitor and one or more antibiotics to eradicate *Helicobacter pylori*, its urease and associated diseases (3, 4). However, the implementation of these therapies in communities where the poor bears the greater brunt of the disease is cost ineffective as concerned drugs are poorly patronized (5).

In Nigeria, *Helicobacter pylori* is fastly replacing non streroidal anti-inflammatory drugs (NSAIDs) as causal agent of gastro-duodenal ulcers and duodenal perforation cases are also of significant increase (6). More worrisome is the increased resistance trend of *Helicobacter pylori* isolates to metronidazole, amoxycillin and tetracycline *in vitro* (7) in a manner that discourage their future clinical use against *Helicobacter pylori* infection in the country. In Nigeria, a triple therapy involving omeperazole, metronidazole and amoxycillin are widely used in severe cases and treatment failures with this combination have been reported (8). Alternative triple

therapies that could be used are expensive, have undesirable side effects (9) and a long list of contraindications (10). The discoveries that Allium vegetables habour anti-*Helicobacter pylori* substances (11,12) have further heightened the global search for similar compounds in other medicinal plants and this to some extent has yielded encouraging results that have the therapeutic applications in areas of discovery as a barrier (13). *Morinda lucida* (*Rubiaceae*), *Ocimum gratissimum (Lamiaceae), Carica papaya* (*Caricaceae*) and *Phyllanthus amarus* (*Euphorbiaceae*) extracts are among the folkloric remedies that have been confirmed scientifically to possess clinical values against protozoal and bacterial infections in Nigeria (14, 15, 16, 17). These plants grow abundantly in Nigerian soils and are not ethnically or age group biased in use. This further explains why their potentials in the future treatment of gastritis and ulcers in Nigeria are investigated.

MATERIALS AND METHOD

Plant materials

The plants, were collected from various local markets in Lagos, identified and confirmed by Mrs. B. Opere of the Department of Botany, Lagos State University. Voucher samples of these plants were subsequently deposited in the Department. The plants used were listed in Table 1.

Extraction

A simple extraction procedure of Olukoya *et al* (18) was adopted to prepare aqueous and organic extracts of the plants tested. To prepare aqueous extracts, 1.1 g of plants' leaves (previously dried at 50°C and ground into fine powders) were steeped in 10 ml of sterile-distilled water at 30 – 32°C for five days. The organic extracts were prepared by steeping 1.2 g of plant materials in 5 ml of 40% ethanol. Extracts were then passed through Hemmings filters (BTI UK) and the resulting sterile filtrates were aseptically transferred to sterile bottles and labeled as crude extracts of individual plants. The organic extracts were subsequently reconstituted with phosphate buffered saline solution (pH 7.2) to nullify the effect of ethanol on the tested organisms. A mixture of 0.1 ml of sterile water and 5 ml of 40% ethanol was prepared as a control.

Microbial cultures

Twenty strains of *Helicobacter pylori* recovered from the biopsy samples of patients with gastritis and gastro-duodenal ulcers from Western Nigeria were used as test organisms. *Helicobacter pylori* ATCC 49503 was used as control. All organisms were cultured on Columbia agar base (Oxoid, CM331) containing 7% sheep blood.

Sensitivity Testing

Antimicrobial susceptibility testing was carried out using the agar diffusion technique. In brief, Isosensitest agar (Oxoid, UK) plates were holed (6 mm in diameter) with the aid of a sterile cork-borer and seeded with 10 µL of *H. pylori* suspension (McFarland 3). The plates were dried in the air and 100 µL of plant extract was introduced into the wells. The plates were incubated microaerophilically (5% O_2, 10% CO_2) at 37°C for 4 days. Holes containing bacterial suspension (10 µL of 9 x 10^8 CFU/ml) and sterile water or ethanol (100 µL) were used as controls. Diameters of zones of inhibition of both the tested organisms and standard strain (*H. pylori* ATCC 45903) were measured in millimeters (mm) and recorded.

RESULTS.

Table 2 gave the summary of the antimicrobial activity of aqueous and ethanolic extracts of the plants against the twenty *H. pylori* isolates and the standard strain. The ethanolic extracts of *C. papaya, M. lucida, O. gratissimum* and *P. amarus* provided evidence of anti-Helicobacter pylori activity in 80% (16/20) and 30% (7/20) of the isolates tested. *Helicobacter pylori* ATCC 49503 was observed to be susceptible to the organic extracts of *C. papaya* and *M. lucida* only. The zones of growth inhibition were 5 – 20 mm in diameter. Apart from the water extracts of *M. lucida* and *O. gratissimum*, which inhibited the growth of 10% (2/20, 5 – 15 mm) and 5% (1/20, 10 – 15 mm) of the isolates tested, aqueous extracts of other plants were found susceptible to these isolates. The standard strain was resistant to all aqueous preparations. Unlike sterile water wells, no colonial growth was found in 40% ethanol control wells.

Table 1: The local herbs selected for testing

Botanical name	Local name*	Plant part tested
Carica papaya	Ibepe	Leaf
Morinda lucida	Ejirin	Leaf
Octimum gratissimum	Efirin	Leaf
Phylllanthus amarus	Ehinolubinsowo	Leaf

* Nigerian (Yoruba) names.

Table 2: Antimicrobial activity of ethanol and water extracts of the four local herbs

Strain code no	*C. papaya*		*M. lucida*		*O. gratissimum*		*P. amarus*		Sterile water	40% Ethanol
	E	W	E	W	E	W	E	W		
Hp 1	0	0	2+	0	0	0	0	0	0	2+
Hp 2	2+	0	2+	0	1+	0	0	0	0	3+
Hp 3	1+	0	2+	0	0	0	0	0	0	1+
Hp 4	2+	0	2+	2+	0	0	0	0	0	2+
Hp 5	1+	0	3+	0	2+	0	2+	0	0	3+
Hp 6	3+	0	1+	0	0	0	0	0	0	1+
Hp 7	2+	0	2+	0	2+	0	0	0	0	2+
Hp 8	0	0	0	0	0	0	0	0	0	1+
Hp 9	2+	0	0	0	0	0	0	0	0	2+
Hp 10	2+	0	2+	0	0	0	1+	0	0	2+
Hp 11	2+	0	2+	0	2+	0	2+	0	0	3+
Hp 12	1+	0	3+	0	2+	0	2+	0	0	2+
Hp 13	2+	0	1+	1+	0	0	0	0	0	3+
Hp 14	0	0	0	0	0	0	0	0	0	2+
Hp 15	2+	0	2+	0	0	2+	1+	0	0	3+
Hp 16	1+	0	2+	0	1+	0	1+	0	0	2+
Hp 17	2+	0	3+	0	0	0	0	0	0	2+
Hp 18	0	0	0	0	0	0	0	0	0	2+
Hp 19	2+	0	2+	0	1+	0	1+	0	0	3+
Hp 20	3+	0	3+	0	0	0	0	0	0	2+
Hp 49305	2+	0	2+	0	0	0	0	0	0	3+

Keys: W = water extract; E = Ethanolic extract; 0 = No inhibition; 1+ = 5 – 9 mm diameter zone of inhibition; 2+ = 10 – 15mm; 3+ = 16 – 20mm; Hp = *Helicobacter pylori* strains.

DISCUSSION

In this study, ethanolic extracts of *C. papaya* and *M. lucida* were observed to prevent the growth of 80% of *Helicobacter pylori* strains tested in vitro. Similar extracts of *O. gratissimum* and *P. amarus* also demonstrated anti-*Helicobacter pylori* activity but in only 35% (7/20) of the isolates. The lack of inhibition observed in water wells confirmed the viability of all the isolates tested. While the inhibitory effect of 40% ethanol attested to the appropriateness of the reconstitution procedure, the poor antibacterial activity of the water extracts of these plants implies that water has inadequate power to extract anti-*Helicobacter pylori* principles from these plants. However, With respect to organic extraction, this finding has provided scientific evidence for antibacterial activity of *Morinda lucida* in vitro as most scientific findings described the plant as an anti-malaria herb (14). The study of Agomo *et al* (19), which demonstrated a complex array of cellular responses to *M. lucida*

administered to mice infected with *Plasmodium yoeli nigeriensis* seemed to provide an indication that numerous biological properties are inherent in this plant. *Ocimum gratissimum* has been extensively demonstrated to inhibit aetiologic agents of diarrhoeal *in vitro* and *in vivo* (15, 20). However, the result obtained from this study should be interpreted with caution as strains of *O. gratissimum* are characterized by varying chemical composition (21). The present study has also extended the biological functions of *Carica papaya* whose seeds have demonstratable evidence of having antifertility effects in rats (22). Although, antimalaria activity of *P. amarus* in mice and rats has been observed in the laboratory (*Personnal communication*), this is first time anti-*Helicobacter pylori* activity will be ascribed to this plant in Nigeria. Based on our findings, there is no doubt that these plants hold tremendous clinical promise especially in rural communities, which provide the greater number of patients and severe cases of gastro-duodenal ulcer. The present study is still in its infancy and therefore invite more research studies to elucidate the active anti-*Helicobacter pylori* substances in these plants, investigate synergy associated with extract combination leading to ultimate suggestion of whether these plants can be combined with orthodox drugs to met the criteria of gastric acid suppression, *H. pylori* eradication and stomach protection in the treatment of ulcers and gastritis. In conclusion, the present study has revealed, the tremendous potentials inherent in ethanolic preparations of *C. papaya* and *M. lucida* if adopted for future treatment of ulcers and gastritis in Nigeria.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002076/2317

BACTERIOLOGICAL QUALITY OF SOBO DRINKS RETAILED WITHIN ILORIN METROPOLIS

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Sobo drinks retailed within Ilorin metropolis were investigated for their bacteriological quality, in terms of the total bacterial count and types of bacterial species present. The sobo drinks were found to have an average pH of 3.2. The bacterial counts were generally high ranging from $5.0x10^4$ to $24x10^4$ CFU/ml. Six bacterial species; *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Klebsiella spp, Enterobacter spp,* and *Escherichia coli* were isolated. Consideration of the distribution pattern showed that samples from Unilorin Main Campus and General Post Office contained all the isolates, while three of the isolates; *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* were common to all samples. Possible sources of the contaminants and their public health implications are discussed and suggestions offered on ways of ensuring safety of the sobo drinks.

INTRODUCTION

Sobo is a drink produced from carefully selected bright red sorrels of Rossell hemp (*Hibiscus sabdariffa*). It is a hot water extract of sorrel of the Rossell hemp: a shrub with distinct bright red coloured sorrels that is fibrous, and is native to West Africa (1). The drink is commonly used as refreshment and in the entertainment of guest at social occasions. The sorrels are sorted and cleaned before soaking in water. It is then heated to boiling after which the plant materials are removed by sieving. Sugar and other additives (flavours) are then added as desired. The hot extract is allowed to cool after which it is dispensed into nylons or plastic containers. It is the cooled extract, which is usually refrigerated before retailing that is taken as 'sobo'. The consumption of sobo has continued to spread across Nigeria been helped mainly by the worsening economic fortune of the majority of people and the escalating price of soft drinks and beverages that are commonly patronized.

Foods frequently serve as vehicle for spread of several organisms some of which are pathogenic (2). Many picnic, suppers and banquets have come to a disastrous end when home-prepared foods and drink serve not only as food and drinks for guest but also as the vehicle for transmitting Staphylococcal food poisoning (3). In view of the fact that sobo is never subjected to any form of post-production treatment that can eliminate, or at least reduce the bacterial load in the drink, it could be a potential source

of health hazard. Also the activities involved in the cooling, and subsequent dispensing of the drink into containers also represent potential sources of health hazards. Some researchers (4) have reported that some gastrointestinal illness characterized by diarrhoea, abdominal cramps, and vomiting which may be assumed as been of unknown aetiology may arise from drinking drinks contaminated with microorganisms. In this study retailed sobo drinks were purchased from various location within Ilorin. They were analyzed for their bacteriological quality as indicated by the number (counts) and kinds of bacteria they harbour.

MATERIALS AND METHODS

Samples of sobo drinks were purchased from retailers from five locations within the metropolis; these were Unilorin Secondary School, Unilorin Main Campus, Ipata Market, Oja-Oba, and General Post Office. These were immediately taken to the laboratory for analysis. The pH of all the samples was determined using Pye Unican pH meter (Model 292 MK2). The bacterial populations were determined by preparing tenfold serial dilution of the samples, and then plating 1 ml of desired dilution on nutrient agar using pour plate count method (5). Representative colonies of bacterial isolates were selected and purified by subculturing on nutrient agar using the streaking method. Pure cultures were then characterized and subsequently identified according to Bergy's Manual of Determinative Bacteriology (6).

RESULTS

The pH of the samples ranged from 2.6 to 4.1 (Table 1), the mean pH was 3.04. The bacterial counts were generally high; it ranged from 5 x 10^4 to 24 x 10^4 CFU/ml (Table 2). A sample from Unilorin Secondary School had the highest count (24 x 10^4 CFU/ml) while a sample from Ipata Market had the lowest count (5 x 10^4 CFU/ ml). A total of six bacterial species were identified. These were *Bacillus subtilus, Staphylococcus aureus, Streptococcus faecalis, Klebsiella spp, Enterobacter spp* and *Escherichia coli.* Their distribution among the collection points is shown in Table 3. Samples from Unilorin Main Campus and General Post Office were observed to contain all the isolates and three of the isolates; *Escherichia coli, Bacillus subtilus* and *Staphylococcus aureus* were common to all samples. *Klebsiella* was the least encountered; been found only in some samples from Unilorin Main Campus and General Post Office.

Table 1: pH of Sobo Samples Examined

Collection Point.	pH				
	Collection Time				
	1	2	3	4	5
Unilorin Secondary School.	2.6	3.0	3.2	3.3	3.0
Unilorin Main Campus.	3.2	3.2	3.3	3.2	3.1
Ipata Market.	3.2	3.1	4.1	3.1	3.2
Oja-Oba	3.0	3.2	3.2	3.2	3.2
General Post Office.	3.2	3.2	3.0	3.2	3.2

Table 2: Population of Bacteria in Sobo Samples Examined

Collection Point.	Mean Total Bacterial Count (cfu/ml) x 10^4				
	Collection Time				
	1	2	3	4	5
Unilorin Secondary School.	9.0	16.0	24.0	14.0	13.0
Unilorin Main Campus.	21.0	12.0	6.0	13.0	11.0
Ipata Market.	5.0	16.0	8.0	11.0	7.0
Oja-Oba	16.0	9.0	11.0	16.0	8.0
General Post Office.	13.0	7.0	8.0	11.0	14.0

Table 3: Distribution of Bacterial Isolates Among Collection Points.

Bacterial species	Source of Sample				
	USS	UMC	IMT	OJA	GPO
Staphylococcus aureus	+	+	+	+	+
Streptococcus faecalis	+	+	+	-	+
Bacillus subtilis	+	+	+	+	+
Klebsiella sp	-	+	-	-	+
Enterobacter sp	-	+	-	+	+
Escherichia coli	+	+	+	+	+

Key
Uss: Unilorin Secondary School.
IMT: Ipata Market
GPO: General Post Office.
UMC: Unilorin Main Campus.
OJA: Oja Oba

DISCUSSION

The pH values obtained indicate that the sobo drinks were acidic in nature and hence normally not favour the proliferation of bacteria. However pH alone is not a sufficient parameter to predict the chances of survival and proliferation of bacteria in the sobo drinks. The high bacterial counts obtained are indicative of poor production conditions. In the light of the amount of heating that goes into sobo production, the presence of bacteria can only be as a result of post heating contamination. Simlar

post treatment contamination of water has been reported (7). This can occur during cooling of the hot extract, addition of flavours and sweetener, or dispensing of the extract into nylons. Utensils and water used during the post heating stages can also serve as sources of contamination. Water used in processing has been identified as the major source of contamination of locally made drinks (8).

The presence of *Staphylococcus aureus* in all samples is indicative of human contamination after heating. This could be from direct human contact such as fingers dipped into the extract to taste the sweetness, or indirectly through additives or utensils. The organism is associated with enterotoxin characterized by short incubation period (1-8 hours), violent nausea, vomiting and diarrhoea. There is no fever (9). The presence of *Bacillus subtilis* is indicative of environmental contamination, which could have resulted from exposure of the extract to air or contact of utensils used with the soil or from the water used in the post heating stages. The presence of *E. coli, Klebsiella* and *Enterobacter spp* suggests faecal contamination, while the presence of *Streptococcus faecalis* particularly indicates a fairly recent faecal contamination (10). Some strains of *E. coli* are associated with production of heat stable enterotoxins, hence, their presence constitute a health risk (11). Although there are no specific guidelines as at present to give a standard for assessing sobo quality, the WHO standard for drinking water could be used since sobo serves as a drink. The standard requires that drinking water should not contain pathogenic organisms and should be free of bacteria indicative of polution with excreta (11). Hence it can be adjudged that the sobo drinks retailed in most location within Ilorin, as obtained in this study, are not bacteriologically fit for consumption.

Generally the drinks appear to have been contaminated due to poor saniatary conditions, unhygienic production practices and prolonged exposure to the environment. Therefore to ensure the safety of the sobo drinks, producers must maintain a clean environment, minimize contact with the extract and exposure to the environment after heating and also maintain a high personal hygiene level. Also utensils should be properly washed before use and clean water should be used at all stages of production. On a larger note further studies examining the chemistry of the extract and its effect(s) on visceral organs and

body system are desirable before an overall acceptability can be placed on the sobo drink.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002069/2316

BACTERIOLOGICAL EXAMINATION OF CHRONIC OSTEOMYELITIS CASES IN ILE-IFE, SOUTHWESTERN NIGERIA

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The bacteriological examination of chronic osteomyelitis cases in Ile-Ife, revealed *Proteus mirabilis* as the predominant isolate encountered (23.1%). Staphylococci constituted 30.7% of the total bacterial isolates with *Staphylococcus aureus* accounting for only 10.2%. Other Gram-positive cocci cultured include, *Staphylococcus spp* (20.5%), coagulase negative staphylococci (CONS) (12.8%) and *Streptococcus spp* (1.2%). Gram-negative rods constituted 55.1% of the total isolates with *Pseudomonas aeruginosa* being 8.5%, *Eschericia coli* 5.1% *Citrobacter freundii, Salmonella spp* 2.5% each. Antibiotic sensitivity test revealed all isolates to be multi-resistant to traditional antimicrobials, which is of epidemiologcal importance in treating cases of chronic osteomyelitis in this environment. The study suggests institution of aggressive therapeutic interventions to avert possible sequalae.

INTRODUCTION

Osteomyelitis is an inflammation of the bone caused mainly by microorganisms and can be categorized into acute and chronic forms. According to Ofiaeli (1), chronic osteomyelitis is perhaps one of the commonest orthopaedic diseases in the tropics among children and adolescents under the age of twenty years. Multiple factors predispose individuals to chronic osteomyelitis among which are mismanagement of acute osteomyelitis (2), haemoglobinopathies such as sickle cell diseases (3,4,5). Haematogenous bone infections, which persist and defy antibiotic therapy, environmental and personal hygiene (6). Prompt bacteriological investigations and aggressive management of patients with chronic ostomyelitis are essential for effective resolution and prognosis of this condition. While treatment modalities of chronic osteomyelitis involve various surgical interventions (7), there is no consensus as to the best surgical option although appropriate, effective and prompt intervention of antimicrobials is desirable when the microbial agents are cultured from patients. Reports from the literature indicate paucity of information on the incidence of chronic osteomyelitis cases in Ile-Ife and its environs except for a study undertaken by Ogunjumo in 1981(6). The present prospective study reports on the current status

of this condition in Ile-Ife and its environs with regard to the patients profile, predisposing factors and the treatment modalities in an effort to assist clinicians in the effective management of this condition to reduce cost and period of hospitalization of these patients.

MATERIALS AND METHOD

Study population

Criteria for inclusion in study were; establishment of osteomyelitis by radiography and only patients who presented at the hospital and were willing to submit themselves for treatment were included. All patients described above in all age groups were considered. 82 consecutive in-patients who satisfied these criteria were admitted into the study. Their ages ranged from 6 months to 90 years, of which 58 were males and 24 were females. The study was conducted between April 1995 to April 1997 at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife, Osun State, SouthWestern Nigeria. The complex is a referral centre for more than half a million people in Ile-Ife and its environs.

Collection of samples

Samples were collected by clinicians from each patient either during routine visit to the hospitals or at the operating table with sterile cotton-tipped applicators or sterile needles and syringes. At least 2-5 ml of venous blood was similarly obtained from each patient. All samples were collected with care to prevent any contamination during transit. Some of these specimens were introduced into transport medium containing Brain Heart Infusion (BHI) broth and Thioglycolate broth (TB) (Difco, Detriot, Michigan, USA).

Processing of samples

Each sample was collected with sterile cotton-tipped applicators and applied unto freshly prepared Mannitol Salt Agar (MSA), Blood Agar (BA) plate and Eosin Methylene Blue (EMB) agar. Each sample was also introduced into Cooked Meat medium and Thioglycolate broth (TB). All inoculated plates were thereafter incubated at 37°C for growth. Approximately 2 ml of venous blood was obtained from each subject and introduced into Thioglycolate broth (TB), which was subsequently incubated at 37°C for growth. The bacterial colonies that grew on the plates were picked and classified into different groups based on their Gram reaction. Those colonies resembling staphylococci were inoculated onto Mannitol Salt Agar and those isolates that fermented mannitol were picked. These were tested for coagulase production using both the slide and tube agglutination test employing pooled

human plasma. Gram-negative isolates that grew on EMB agar were further tested on Triple Sugar Iron (TSI agar), Koser's Citrate and Sulphide Indole Motility (SIM) agar and further characterized on other conventional media.

Antibiotic susceptibility test

Antibiotic susceptibility of the isolated organisms was determined by the standard agar dilution method of Ericsson and Sherris (8). The antibiotic discs employed included Ampicillin (Amp) 10 µg, Chloramphenicol (Chl) 10 µg, Cloxacillin (Cxc) 5 µg, Erythromycin (Ery) 5 µg, Nalidixic acid (Nal) 10 µg, Gentamicin (Gen) 10 µg, Penicillin (Pen), 1 unit, Ofloxacin (Ofx) 10 µg, Streptomycin (Str) 10 µg and Tetracycline (Tet) 10 µg, all obtained from Abtek Biological Ltd, Liverpool, U.K. Lot No. 5B02/P. The test medium was Mueller-Hinton agar (Oxoid, UK). *S aureus* ATCC 25923 and *Enterobacter aerogenes* ATCC 13042 were run as control organisms.

RESULTS

A total of 82 patients were seen. 58(70.7%) were males and 24(29.2%) were females. The incidence of chronic osteomyelitis was lowest among subjects aged fifteen years and below with 11(13.4%) patients, of whom 8(72.7 %) were males. Overall the incidence of the condition was more than double in males as against the females (See Table 1).

Table 2 shows the types of bone commonly involved in cases of chronic osteomyelitis during the study. Forty-six (56.1%) of these involved the tibia followed by femur (13.4%), humerus (8.5%), ulna (6.1%) and radius (4.8%).

The type of surgical interventions carried out on the patients is shown in Table 3. Wound debridement constituted a large proportion of surgery occurring in 32 cases followed by sequestrectomy, saucerization and curettage in 22 cases. Local muscle flaps accounted for 10; bone grafting, skin grafting and antibiotic impregnation constituted 2 cases each, while plating was done for non-union in only one case. The sources of infections in the chronic osteomyelitis cases varied. Post-traumatic sources were seen in 50 cases and accounted for a considerable proportion of infections. Decubitus ulcers contributed 5 cases. This was followed by diabetic foot with 4 cases. Leg ulcer, pin-tract infection, and crush injury accounted for 3 cases each. Gunshot injuries occurred in 2 cases and acute osteomyelitis occurred in only one case.

Bacterial isolates cultured from chronic osteomyelitis specimens

Seventy-eight bacterial isolates were cultured from 82 cases of chronic osteomyelitis averaging 1.05 microbe per subject. Gram-positive cocci constituted 44.9% of the entire isolates. Staphylococci accounted for 97.1% of these isolates. Among the staphylococci, *Staphylococcus spp* accounted for 47.1%, coagulase negative staphylococci (CONS) 29.4% and *Staphylococcus aureus* 23.5%. Gram-negative rods accounted for 55.1% of bacterial isolates with *Proteus mirabilis* predominating (41.9%) followed by *Pseudomonas aeruginosa* (25.9%), *Escherichia coli* (13.9%), *Klebsiella aerogenes* (9.3%) and *Citrobacter freundii* and *Salmonella spp* constituting 4.6% each of the Gram-negative short rods. None of the blood culture showed any growth.

Antibiotic susceptibility profile of some of the bacterial isolates

The antibiotic susceptibility pattern of the bacterial isolates is shown in Table 6. The staphylococci were all virtually resistant to Benzylpenicillin and Ampicillin. All the *Staphylococcus aureus* and CONS isolates were resistant to these two penicillins but sensitive to Cloxacillin and Ofloxacin both of which are beta-lactamase resistant drugs. *Pseudomonas aeruginosa* isolates were resistant to all drugs used except Gentamicin which 45% of *P. aeruginosa* isolates were sensitive. A similar trend of resistance was seen with *Proteus mirabilis* and *E. coli* isolates (Table 6).

TABLE 1: PROILE OF CHRONIC OSTEOMYELITIS CASES SEEN AT OAUTHC BETWEEN 1995-1997

Age	Number (%) Male	Number (%) Female
0-15	8(13.8)	3-(12.5)
16-30	20(34.5)	10-(41.7)
31-45	17(29.3)	5-(20.8)
>46	13(22.4)	6 – (25.0)
Total	58 (70.7)	24 (29.2)

TABLE 2: TYPES OF BONE INVOLVED IN CASES OF CHRONIC OSTEOMYELITIS AT OAUTHC, ILE-IFE

BONE TYPE	FREQUENCY (%)
Tibia	46(56.1)
Femur	11(13.4)
Humerus	7(8.5)
Ulna	5(6.1)
Radius	4(4.8)
Phalanges	2(2.4)
Sacral Bone	2(2.4)
Tarsal	1(1.2)
Clavicle	1(1.2)
Knee	1(1.2)
Elbow	1(1.2)
Ribs	1(1.2)

TABLE 3: TYPES OF SURGICAL INTERVENTIONS EMPLOYED AT THE OAUTHC, ILE-IFE

PROCEDURE	FREQUENCY
Sequestrectomy, saucerization and curettage	22
Wound debridement	32
Local Muscle flaps	10
Amputation	7
Drainage of Pus	5
Bone grafting	2
Skin grafting	2
Antibiotic impregnation	2
Plating (after control of infection)	1

TABLE 4: SOURCES OF INFECTION IN CHRONIC OSTEOMYELITIS AT OAUTHC, ILE-IFE

ROUTE OF INFECTION	FREQUENCY
Post traumatic	50
Decubitus ulcers	5
Diabetic foot	4
Leg ulcer	3
Pintract infection	3
Sacral pressure (bed sore)	3
Crush injury	2
Gunshot injury	2
Acute osteomyelitis	1

TABLE 5: BACTERIAL SPECIES ISOLATED FROM CHRONIC OSTEOMYELITIS PATIENTS AT THE OAUTHC, ILE-IFE

BACTERIAL ISOLATES	FREQUENCY (%)
Gram Positive	
Staphylococcus spp.	16(20.5)
Coagulase nagative Staphylococci (CONS)	10(12.8)
Staphylococcus aureus	8(10.0)
Streptococcus spp.	1(1.2)
Gram Negative rods	
Proteus mirabilis	18(23.1)
Pseudomonas aeruginosa	11(14.1)
E.coli	6(8.5)
Klebsiella aerogenes	4(5.1)
Citrobacter freundii	2(2.5)
Salmonella spp.	2(2.5)
Total	78

TABLE 6: ANTIBIOTIC RESISTANT PROFILE OF BACTERIAL ISOLATES

Organism	Total No. Tested	% Resistant								
		PEN	AMP	ERY	CHL	STR	TET	GEN	CXC	OFX
Staphylococcus aureus	8	100	100	450	25	25	100	25	0	0
Staphylococcus spp	16	75	44	13	44	100	100	13	0	0
Coagulase Negative Staphylococci (CONS)	10	100*	100	20	20	20	100	0	0	0
Streptococcus spp	1	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	11	100	100	100	100	100	100	45	0	0
Proteus mirabilis	18	100	100	100	100	100	100	33	0	0
Klebsiella aerogenes	4	100	100	100	100	100	100	25	0	0
Escherichia coli	6	100	100	100	100	100	100	33	0	0
Salmonella spp	2	100	0	0	0	0	0	0	0	0

*** Only major organisms were tested**

DISCUSSION

This work was prompted because of the paucity of information regarding chronic osteomyeliitis in Ile-Ife and its environs. Except for the study carried out by Ogunjumo in 1981 (6), there is no other report of chronic osteomyelistis in this environment. Unlike acute osteomyelitis, a substantial proportion of the patients with chronic osteomyelitis were adults (78%) and a number of these were males (67%) indicating the predominance of this condition amongst males (2.41:1). Our results show that the tibia was the predominant bone affected followed by the femur and humerus. The majority of the chronic osteomyelitis cases and others were as a result of post-traumatic infections due to compound fracture, crush injury and pin tract infection. Although other organisms have been implicated in chronic osteomyelitis, bacterial species are the most common. The bacterial aetiology of chronic osteomyelitis varies but according to most studies (9,16), *Staphylococcus aureus* seems to be the predominant organism encountered. In a study carried out at this Centre by Ogunjumo (5), *S. aureus* was the predominant organism isolated from patients. While staphylococci still remain the significant organisms cultured from chronic osteomylitis patients, our study reveals that *Staphylococcus spp* (20.5%) has become the predominant Gram positive cocci recovered, followed by coagulase negative staphylococci (CONS) (12.8%). *S. aureus* came a distant third (10.3%). This observation underscores the changing pattern of bacterial aetiology of this condition even within the same environment.

However, Gram negative short rods constituted 55.1% of the total bacterial isolates cultured from various specimens with *Proteus mirabilis* accounting for 41.9%) of the Gram negative rods. This was followed by *Pseudomonas aeruginosa* (25.9%). Overall, *Proteus mirabilis* was the predominant bacterial isolates in chronic osteomyelities at this centre. While Ogunjumo (6) previously reported *S. aureus* as the predominant organism in chronic osteomyelitis at this centre, our study however, corroborates Oguachuba (10) who reported *Proteus spp* predominance in Jos, Northern Nigeria. The increasing prominence of *Pseudomonas aeruginosa* in chronic osteomyelitis is of concern because of this organism resistance to various antimicrobial agents. However, none of the subjects from whom pseudomonas were cultured had sickle cell disease (11). An average of two types of organisms

each was cultured from 24 patients that participated in the study. Seven of the clinical specimens cultured did not grow on all the media employed. The occurrence of multiple organisms in some specimens cultured, suggests polymicrobial nature of chronic osteomyelitis which appears to be a feature of this condition compared with acute osteomyelitis cases, where over 50% of clinical specimens cultured contained a single organism (12).

Many factors other than the age affect the development of chronic osteomyelitis (1,5,13). Osteomyelitis resulting from direct extension of a decubitus ulcer, wound infection or open fracture may be difficult to detect clinically before the infection progresses to chronic osteomyelitis. Therefore early recognition of acute osteomyelitis is highly desirable for favourable prognosis. Even when roentgenographic abnormalities of the bone may be helpful in confirming the presence of infection, if roentgenographically diagnostic changes are delayed, the process may often progress to chronic form. In patients with chronic recurrent osteomyelitis, antimicrobial therapy is usually dictated by the microflora present within the infected bone. Even when cultures of sinus tract drainage yield bacteria, which are not present in the bone or fail to yield organisms, such cultures of sinus tract are only useful as guidelines for initial therapy.

The selection of antimicrobial agents for the treatment of chronic osteomyelitis caused by Gram-negative rods may be difficult. The *in vitro* sensitivity testings carried out with these isolates underscores this point. Virtually all the gram-negative rods recovered from the clinical source were multiply resistant to the various antibiotics used at least *in vitro*. Except for four isolates of *Staphylococcus spp*, all the other staphylococcal isolates were resistant to Benzylpenicillin and Ampicillin, suggesting these were beta-lactamase producers. Such observation with *S. aureus* isolates had been reported by Mollan and Piggot (12), in which seventy-four percent of these strains were resistant to Benzylpenicillin. Gentamicin was marginally effective in *vitro* with only five isolates of *Pseudomonas aeruginosa* sensitive. Similarly the *Proteus mirabilis* recovered from the patients were resistant to most antibiotics used in treating Gram-negative infections except to Gentamicin. The ineffectiveness of many of these antibiotics *in vitro* against the majority of our isolates corroborates reports of some investigators of the existence of multiply resistant enteric rods even amongst apparently healthy

individuals in this environment and elsewhere (14,15).

We recommend that clinicians practicing in this environment should be mindful of this phenomenon and therefore employ in their treatment modalities as combination of effective drugs and also follow-up patients' treatment for considerable length of time in order to resolve the condition. According to Jensen and Lassen (17), Fusidic acid given in combination with other antibiotics were effective in preventing emergence of resistant strains of *S.aureus* when administered as first line treatment. It is interesting that the bacterial isolates cultured in our study responded very well to Cloxacillin and Ofloxacin both of which are known to be effective against beta-lactamase producers.

While antimicrobial therapy is desirable in the control of chronic osteomyelitis, surgery remains the therapeutic and diagnostic procedure, which should be carried out early to resolve the condition (1,18). Our study therefore recommends prompt detection of acute osteomyelitis cases so that these do not progress to chronic form. Aggressive chemotherapeutic management of acute osteomyelitis is suggested. Early bacteriological investigations to determine bacterial aetiology are highly desirable so that first line antimicrobials are rigorously administered. Since the majority of chronic osteomyelitis cases are posttraumatic, orthopaedic surgeons should be more conscious of the risk of such injuries progressing to chronic forms and educate patients of prompt and proper management at initial stages to avert subsequent complications. The prevalence of multiply antibiotic resistant organisms in this study should be brought to the notice of Health Administrators in the Ministry of Health who should educate the public and dissuade communities in engaging in self-medication.

ACKNOWLEDGEMENT

This study was supported by funds received from Obafemi Awolowo University Research Committee. The authors acknowledge the assistance of Mrs. M.O. Disu in the preparation of this manuscript.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002059/2319

STUDIES ON DIABETIC FOOT ULCERS IN PATIENTS AT JOS UNIVERSITY TEACHING HOSPITAL, NIGERIA

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An epidemioloigcal and microbiological studies of diabetic foot ulcers were carried out in our hospital, with a view to reducing the amputation and mortality rate associated with the disease. Wound swabs from 38 Diabetes Mellitus (DM) foot ulcer patients were investigated using culture methods for both strict aerobes and anaerobes. The bacterial isolates were subjected to antibiotic susceptibility tests using the disc diffusion method. Baseline biochemical and haematological analysis were also carried out. The prevalence of the disease was stratified in relation to some clinical and laboratory parameters, gender, age, educational and occupational status of the patients. The prevalence of the diasese was 24.7%, with an amputation and mortality rates of 18.4% and 15.8% respectively. Only 13% had DM for less than 1 year, while 53% for more than 10 years. 28.9% have regular shoe-wearing habits. Duration of healing ranged from 2 weeks to 24 weeks (mean = 2.7months). 31% of the patients with marked periosteal reaction had lower extremity amputation or died before amputation could be done. *Staphylococus aureus* (31%), *Proteus spp* (16%), *Pseudomonas aeruginosa* (10%), *Klebsiella spp* (6%), *Peptococcus spp* (6%), *Bacteroides fragilis* (3%), *Streptococcus pyogenes* (3%), *Escherichia coli* (3%), *Candida albicans* (3%), *Streptococcus viridans* (1%), *Flavobacterium spp* (1.5%) and *Bacteroides melaninogenicus* (1%) were isolated. Most of the bacteria isolates were sensitive to pefloxacin. Our results demonstrate a very high rate of diabetic foot ulcer with the corresponding high rate of amputation and mortality. A multi-disciplinary approach to the management of DM foot ulcers is advocated.
Efforts should be made to carry out cultures of samples from refractory ulcers to rule out yeast colonization which if not treated will delay wound healing.

Key words: Diabetic foot ulcers, Microbial and antimicrobial surveillance, Refractory ulcers.

INTRODUCTION

Diabetes Mellitus is increasingly being recognized as a major medical problem in our environment, and the threat of lower extremity amputation (LEA) casts an ominous shadow over the lives of diabetic patients. Diabetic ulcers are the most common foot lesions leading to lower extremity amputation (1). Management of the diabetic foot requires a thorough knowledge of the major risk factors for amputation, frequent routine evaluation and meticulous preventive maintenance (2). The etiology of lower extremity diabetic ulcer includes injury complicated by underlying neuropathy, ischaema or both. The use of recombinant growth factor is becoming more widespread as more is learnt about their essential role in wound healing (3).

According to a study by Bild (4), more than 50,000 amputations were performed on diabetic patients each year in the United States of America. Reported prevalence rates of diabetic foot ulcers in different

groups of Nigeria diabetics range from 0.9% to 8.3% (5-8). This complication led to limb amputation in 40% of cases (8). Foot infections caused a third of all skin infections in 1,050 Nigeria diabetics (9), and accounted for 25% of deaths in ketocidosis (8). In a study by Walters *et al* (10) in the United Kingdom, the prevalence of diabetic foot ulceration was 7.4%.

Psychosocial problems from limb amputation persistently retard rapid rehabilitation. In Nigeria with inadequate health care facilities and uniform lack of comprehensive rehabilitation progammes, it is important to recognize foot lesions early and thereby offer early and effective treatment. Amputation is expensive (about $500 per case, not including the costs of rehabilitation) and there is difficulty in psychological readjustment.

When managing diabetic foot ulcers, one is faced with the dilemma of whether to opt for radical surgery or to continue with conservative measures. We therefore, decided to carry out a prospective study to see how we can effectively reduce the amputation rate and the mortality associaed with diabetic ulcers in our environment.

PATIENTS AND METHODS

Patients

One hundred and fifty four diabetics were admitted from January 1998 to December 1999 for various reasons ranging from poor control of diabetes to septicaemia. All were prospectively studied. Each of the patients with diabetic foot ulcer(s) was interviewed using the close ended questionnaire and then examined clinically. For example, with respcet to shoe wearing habit, patients were asked whether it is regular or none. In this study, diabetic is defined according to WHO guidelines of glucose more than 7.0 mmol/L (>126 mg/dl) for fasting blood sugar or more than 11.1 mmol/L (>200 mg/dl) 2 hours postprandial (11).

Bacteriology

The wound swabs were collected as much as possible before any antibiotic therapy and inoculated immediately onto freshly prepared Blood Agar (BA), Chocolate Agar (Choc.) and MaConkey (McC) Agar plates. Pre-heated Robertson's Cooked Meat medium (RCM) was also inoculated and incubated aerobically at 35°C. One set of BA, Choc and McC agar plates was incubated aerobically at 35°C for 18-24 hours. The other set was incubated anaerobically at 35°C for 48 to 72 hours. The RCM broth cultures were subcultured after 48 hours and 72 hours onto the solid media and incubated

appropriately. The identifications of the isolates were done using the standard methods (12). Antibiotic susceptibility tests were carried out on the pathogenic isolates using the NCCLS techniques (13).

Haematology

Full blood count and Erythrocyte Sedimentation Rate (ESR) values were carried out on the patients' blood samples collected on admission (14).

Chemical pathology

Short-term glycaemic control was assessed by fasting glycosuria, acetonuria and fasting blood glucose levels. Urinalysis of the patients' samples were carried out using the MediTest strips and proteinuria was defined as the presence of greater than 50 mg/dl of protein.

Plain foot radiographs

Serial plain radiographs on the affected limbs were carried out on 32 of the 38 patients with diabetic foot ulcers. Two of the six patients that did not do X-ray could not afford the cost and their ulcers healed within six weeks, while the remaining four had above knee amputation immediately they were brought to the hospital so as to save their lives.

Surgery

Prophyhlactic surgery was done when necessary by serial debridement and large lesions were skin-grafted. In a few cases, where the life of the patients was in danger, amputation (especially above the knee) was inevitably carried out.

Antibotic therapy

The patients were treated based on the *in vitro* susceptibility pattern of the isolates, in addition to Metronidazole, Vitamin C, Vitamin B-complex and Insulin therapy. This was in addition to the scrupulous twice-daily dressing of the ulcers with hydrogen peroxide and eusol after warm hypertonic saline immersion of the affected whole foot for 30 minutes

RESULTS

Table 1 shows the duration of diabetes mellitus at the time of presentation to the hospital with diabetic foot ulcers. Only 5 (13%) of the 38 patients studied had duration of diabetes mellitus for less than one year. All the ulcers were located on the hind limbs and 7 (18%) had bilateral ulcers on both hind limbs while 31 (82%) had unilateral ulcers. The duration of healing ranged from 0.5 month to 6 months with a mean of 2.73 months. None of the patients was hospitalised for the entire duration of the healing. As soon as the blood glucose level is stabilised, they were discharged and then followed up in an ambulatory setting. The fasting blood glucose at the time of admission ranged from 6.0 mmol/L to 28 mmol/L. The packed cell volume (PCV) ranged from 18% to 45%; white blood cell (WBC) count ranged from 3,500/mm^3 to 15,700/mm^3; differential neutrophil

counts ranged from 48% to 78%; lymhocyte counts from 19% to 50% and the erythrocyle sedimentation rate (ESR) ranged from 6 mm/hour to 150 mm/hour. All these were the baseline data at the time of admission.

The plain foot x-rays showed periosteal reaction, osteomyelitis and soft tissue swelling. A few of the x-rays were normal while 31% of the patients who had marked periosteal reaction had their limbs amputated or died before amputation could be done

The sex distribution of the diabetes patients that attended the hospital for the 2 year period is shown in Table 2. There is no significant difference in the prevalence of diabetic foot ulcers in relation to gender ($p>0.05$). For the 2-year period, the prevalence of the diabetic foot ulcers in our hospital was 24.7%. Table 3 shows the prevalence of some important clinical and laboratory parameters in the patients with diabetes foot ulcers while Table 4 shows the prevalence in relation to the ages of the patients. No ulcer was seen in patients of less than 20 years and more than 80 years of age while the 51-60 years group had the highest with 44.7%.

The prevalence of diabetes foot ulcers in relation to educational status of the patients is shown onTable 5. Patients with no formal education recorded the highest with 36.8% ($P<0.05$). Table 6 shows the prevalence of the diseases in rotation to the occupational status of the patients. Civil servants recorded the highest with 39.2%, but there is no statistical difference ($P>0.05$).

The microorganisms isolated from the diabetic foot ulcers foot ulcers are shown on Table 7. Twenty-two (58%) of the patients had poly-microbial flora, while 4(6%) showed no growth and strict anaerobes, aerobes and yeasts were encountered. Table 8 summarizes the percentage susceptibility patterns of the bacterial isolates to some of the commonly prescribed antibiotics in our hospital. Most of the isolates were sensitive to pefloxaclin, except for *Klebsiella spp* that recorded only 25% sensitivity.

The summary of the laboratory data comparing patients whose ulcers healed and those who went home for amputation or died is shown in Table 9. There were no significant differences in the haematocrit values, white blood cell counts, erythrocyte sedimentation rates, differential neutrophil counts, fasting blood sugar and fasting glycosuria on first visits. Only 8(21.1%) of the patients had acetonuria on their first visit; out of which 7(87.5%) under went amputation or died.

Table 1: Duration of Diabetes Mellitus at the time of presentation with ulcer.

Duration (years)	No of Patients	% of Total
<1	5	13
1-5	7	18
6-10	6	16
>10	20	53

Table 2: Sex Distribution of Diabetic Foot Ulcers in JUTH (January 1998-December 1999).

Admission	Males	Females	Total
Total DM	96	58	154
Total DM Foot	24	14	38
Relative frequency of DM Foot	25%	24.1%	24.7%

Table 3: Prevalence of some Important Clinical and Laboratory Parameters in the Patients.

Parameter	Number Examined	Number positive	%Positive
Positive knowledge of foot care.	38	11	28.9
Regular shoe wearing habit	38	30	78.9
Presence of previous foot ulcer	38	12	31.6
Presence of unilateral lesion	38	31	81.6
Spontaneous initiating factor	38	30	78'9
Presence of foot pain	38	26	68.4
Presence of shooting or lancinating pain	38	27	71.1
Presence of pain at rest	38	24	63.2
Presence of intermittent claudication	37	11	28.9
Presence or nocturnal diarrhoea	38	14	36.8
Presence of postural dizziness	24	15	39.5
Presence of impotence	38	21	87.5
Presence of fever	38	22	57.9
Presence of acetonuria	38	8	21.1
Presence of albuminuria	38	24	63.2
Amputation	38	7	18.4
Mortality		6	15.8

Table 4: Prevalence of Diabetes Foot ulcers in relation to Age at JUTH, Nigeria

Age Group (years)	Number with Ulcer	% with Ulcer
21-30	2	5.3
31-40	4	10.5
41-50	9	23.7
51-60	17	44.7
61-70	4	10.5
71-80	2	5.3

Tables 5: Prevalence of Diabetes Foot in Relation to Educational Status

Educational Level	No. of Ulcer	% with Ulcer
None	14	36.8
Primary School	10	26.3
Secondary school	6	15.8
Tertiary Institution	8	21.1

Table 6: Prevalence of Diabetes Foot Ulcers in Relation to Occupational Status of the Patients

Occupational Status	No with Ulcer	% with Ulcer
Farmers	5	13.2
Traders	8	21.1
Civil servants	13	39.2
House-wives	7	18.4
Others (like Butchers, motor mechanics, applicants)	5	13.2

Table 7: Microbial Isolates from Diabetic Foot Ulcers at JUTH, Nigeria

Microorganism	No of Isolates	% of Isolates
S. aureus	21	31
S. pyogenes	2	3
S. viridans	1	1
Cl. perfringens	4	6
Peptococcus spp	4	6
Klebsiella spp	4	6
Proteus spp	11	16
B. fragilis	2	3
B. metaninogenicus	1	1
Flavobacterium spp	1	1
P. aeruginosa	7	10
Candida albican	2	3
Acinetobacter spp	1	1
Esherichia coli	2	3
No growth	4	6

Table 8: Percentage Susceptibility Patterns of the Bacterial Isolates to Clindamycin (CLN), Augumentin (AMC), Ampicillin (PN), Cotrimoxazole (SXT), Tetracycline (Te) and Pefloxacine (Pef).

	Antibiotics					
	CLN	**AMC**	**PN**	**SXT**	**Te**	**Pef**
Bacteria						
S. aureus	29*	48	0	10	48	90
S. pyogenes	100	100	100	0	0	100
S.viridans	100	100	100	0	0	100
CL. Perfringens	75	100	50	0	75	100
Peptococcus *sp.*	100	75	25	0	25	100
Klebsiella *sp.*	NA+	25	0	25	0	25
Proteus *sp*	NA	36	0	0	9	73
B. *fragilis*	50	50	50	50	50	100
B. *melaninogenicus*	0	0	0	0	0	100
Flavobacterium *sp.*	0	0	0	0	100	100
P. *aeruginosa*	NA	NA	NA	NA	NA	86
Acinetobacter *sp.*	NA	0	0	0	0	100
E. *coli*	NA	100	0	0	0	100

* % susceptible
\+ Not applicable.

Table 9: Laboratory Parameters of Patients with healed Diabeti Ulcers Amputated/Dead Patients.

	PCV (Mean + SE)	WBC (Mean + SE)	ESR (Mean + SE)	Neutrophils (Mean + SE)	FBS (Mean + SE)	Aceton uria Num-ber	FG Number
Healed Ulcers	37.88±1.165	6816±558.62	41.167±6.574	57.12±1.365	12.748±0.832	01	21
Limb/death	30.538±1.96	1111±1086.623	81.154±10.837	67.846±2.412	19.269±1.45	07	13
P-Value (using kruskal-Wallis ANOVA	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P>0.05	P > 0.05

DISCUSSION

This study has shown a relatively high prevalence rate of diabetic foot ulcers (24.7%) among the DM patients in our hospital with 18.4% and 15.8% amputation and mortality rates. The population was limited to patients who attended the hospital during the study period either as in patients or ambulatory patients. It is very likely that the burden of diabetic foot ulcers may be more in the general population (15years and above) within the metropolis with a DM prevalence of 3.1% (16). We believe that a combination of conservative approach and aggressive prophylactic surgery in high-risk patients, if started very early in the course of the disease will reduce the amputation and mortality rates in our environment, which hitherto was high. Previously many limbs that should have been

saved were amputated without trying the combination of conservative approach and aggressive prophylactic surgery. Also some DM foot ulcers patients shy away from attending the hospital for fear of amputation and eventually some of them die due to infections. We believe that with adequate health education, which should be given to the DM patients particularly on their clinic days, they will be willing to report any ulcers to the physician no matter how trivial. Proper management of the ulcers includes the use of proper footwear, non-weight-bearing limb support, use of appropriate antibiotics, debridement, aggressive revascularization, control of serum glucose levels and careful monitoring of the ulcers. Empirical antibiotic selection was replaced by culture-guided definitive therapy as soon as possible and this greatly improved the condition of the patients. It was discovered in the study, that the prevalence of diabetic ulcers was low in those with positive knowledge of foot care and this confirms the advantage of regular health education for the diabetics. The prevalence of the disease in relation to age is in agreement with the known knowledge that diabetes mellitus is highest in the 51-60 year age group. A few diabetic with no formal education had the highest prevalence of ulcers due mostly to ignorance.

Some of the important parameters which are relevant to development of foot ulcers in diabetic patients such as peripheral neuropathy, peripheral pulses, presence of calluses, blisters, corns and trophic changes are being prepared for publication. Although new therapies, such as the use of exogenous recombinant growth factors are being developed for refractory ulcers (17), care must be taken to isolate and identify all the microbial flora of the ulcers.

The bacterial isolates showed a high rate of mulitiple resistance to commonly used antibiotics. This is mainly due to the ready availability and the easy access access to those antibiotic by the general population without medical prescription. It is most likely that majority of the *Staphylococcus aureus* strains isolated in this study are MRSA as earlier reported by Ikeh (18), who got a prevalence of 43% out of the 180 consecutive isolate of *S. aureus* in our hospital. In this study, we encountered few isolates of *Candida albicans* as part of the mixed microbial flora and the patients responded very well on the inclusion of ketoconazole to the treatment regimen. Diabetic ulcers shoud be thoroughly evaluated and appropriately treated through established protocols utilizing all members of the team. This certainly

reduces the duration of hospital admission, morbidity and loss of limbs. Although foot problems in diabetics cannot be eradicated completely, it is always better to diagnose and manage diabetic ulcers effectively, educate and motivate patients to care for their feet so as to minimize complications and decrease health care costs. In the absence of facilities for culture and antibiotic susceptibility tests, pefloxacine may be useful as a monotherapy in diabetic foot ulcers due to the marked clinical and laboratory reponse in our study.

Except for acetonuria, none of the conducted laboratory parameters determined the outcome of the diabetic foot ulcers disease. This shows that with proper managemnet of the patients, the morbidity, amputation and the mortality rate can be drastically reduced if the management is started early. The study population was limited to patients who attented the hospital either as inpatients or ambulatory patients, and it is very likely that the burden of diabetic foot ulcers is more in the general population.

ACKNOWLEDGEMENTS

The authors wish to thank the nursing staff of the affected wards for their cooperation. This study was supported in part by the senate Research grant (RGC/1997-98/010) of University of Jos, Nigeria.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002075/2320

AETIOLOGIC AGENTS OF DIARRHOEA IN CHILDREN UNDER FIVE YEARS OF AGE IN OSOGBO, OSUN STATE.

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A survey of the aetiological agents of diarrhoea in children under 5 years of age was carried out in Osogbo, Osun State. A total of 135 patients visiting the outpatient and children emergency units of LAUTECH Teaching Hospital were examined. Strains of *Shigella* isolated were tested for antibiotic sensitivity. Consideration of the distribution showed that *Escherichia coli* was more prevalent in children aged between 3 to 5 years (57%) and was the most encountered of the organisms isolated (present in 77.8% of all samples) while *Shigella spp* was found in 16.3% of samples, *Vibro cholerae* 0.7% and other coliforms 5.2%. Statistical analysis showed that *E. coli* was significantly associated with diarrhoea in the patients aged 3-5 years (P< 0.05). *Shigella spp* was shown to show some resistance to tetracycline and high sensitivity to ofloxacin.

INTRODUCTION

Diarrhoea is a leading cause of morbidity and mortality among children in developing countries (1). It has been found to be associated with bacteria, viruses and protozoa. However the pathogens most commonly associated with children diarrhoea werefound to include *E. coli, Vibrio spp,* Salmonella, *Shigella* spp, and parasitic agent, *Giardia lamblia* (3). Rotavirus has been implicated in recent study, being more pronounced during the dry season (4). Some factors such as poor food hygiene, water and sanitation combine to facilitate the spread of enteropathogens and epidemics in such areas (5). *E. coli* has been implicated as the prevalent aetiological agent of Infantile diarrhoea in Nigeria; exhibiting numerous strains(6). The epidemiological significance of each *E. coli* strain in childhood diarrhoea varies with geographical area (7). Although extensive investigation of diarrhoea in Nigeria have not been reported, the diarrhoea-specific mortality in children less than 5 years of age in Africa has been estimated at about 10 6 per 1000. Although oral rehydration has been shown to reduce early child mortality (8), it is a general belief that malnutrition may increase the duration or severity of diarrhoea in young children, while diarrhoea may lead to symptoms of malnutrition in them (8). This study investigates the aetiological agents of childhood diarrhoea in Osogbo

and their susceptiblity to some antibacterial drug.

MATERIALS AND METHODS

Study area/ sample source

The study was carried out at the children and emergency and out patient departments of the Teaching Hospital, LAUTECH CHS Osogbo. Stool samples were collected from patients presenting with diarrhoea into sterile sample bottle. One hundred and five stool samples were collected over a period 10 months (from middle of July 2001 to April 2002) and analyzed.

Microbial Analysis

The samples were examined for signs of diarrhoea (9). Tenfold serial dilution was carried out on those samples found consistent with the description for diarrhoea using sterile normal saline as diluent. 1.0ml of appropriate dilutions were inoculated onto Thiosulfate-Citrate-Bile Salt-Sucrose agar medium, Salmonella-Shigella agar medium and MacConkey agar, according to described method (6). Rectal swab specimens were directly inoculated onto each agar plate by routine techniques. The colonies that grew on each medium were examined and the organisms characterized. The *Shigella* strains isolated were tested for susceptibility to some antibiotics (ampicillin, tetracycline, cefdinir, ofloxacin and erythromycin.) and the minimum inhibitory concentration (MIC) of each drug was determined by plate dilution technique.

RESULTS

All the samples were positively identified as diarrheal stool. Bacteriological analysis showed that of a total of 135 isolates recovered from the stool samples; 77 were strains of *Escherichia coli,* others are *Shigella 22, Vibrio · cholerae* 1, and other coliforms 7 (Table 1). A consideration of the age distribution shows that *E. coli* was more prevalent in children aged between 3 to 5 years (57%) and was the most encountered of the organisms isolated. A strain of *Vibrio cholerae* was also observed in a sample from children within the ages of 3-5yrs. Seasonal variations in incidence of bacterial diarrhoea is shown on Table 2. It was found to be prevalent during wet season ($P>0.05$). A total of 93 (68.9%) isolates were recovered during the wet season compared to 42 (31.1%) recovered in the dry season. The frequency of isolation of *E. coli* was also higher in the wet season than dry season (52.6% and 25.2% respectively).

Table 3 shows the susceptibility pattern of the 22 strains of *Shigella spp* examined. All the shigella isolates were

resistant to tetracycline and erythromycin but were susceptible to ofloxacin. Two peaks of MICs were found for ampicillin 3.1 and 100 mg/ml or greater. Almost all the MICs of cefdinir were distributed in a narrow range, from 0.1 to 0.4 mg/ml.

Table 1: Incidence and age distribution of enteropathogens isolated

Pathogen	Yr < 1yr	Yr 1-2%	Yr 3-5%	(Total n = 135)
E.coli	16(11.9%)	12(8.9)%	77(57%)	
Shigella	2(1.5) %	12(8.9)%	8(59%)	
Other coliforms	2(1.5) %	3(2.2) %	2(1.5) %	
Vibro cholerae	-(-)	(-)	1(0.7%)	
	20	**27**	**88**	**135**

Table 2: Incidence and Seasonal Variation of Enteropathogens Isolated From Patients presenting at Hospital in Osogbo

Organism	Nos of Isolates.		
	Dry	Rainy	Total%
Shigella	6(4.4)%	16(11.9)%	22(16.3)%
E.coli	34(25.2)%	71(52.6)%	105(77.8)%
Other coliforms	2(1.5)%	5(3.7)%	7(5.2)%
Vibro cholerae	0(-)	1(0.7)%	1(0.7)%

Table 3: Drug susceptibility pattern of shigella spp isolated from patients Presenting at hospital in Osogbo.

Minimum Inhibitory Concentration (MIC) mg/ml					
	ABPC	AT	EM	CFDN	OFLX
< 0.0.25	0	0	0	0	0
0.05	0	0	0	0	8
0.1	0	0	0	1	11
0.4	0	0	0	12	2
0.8	0	0	0	9	1
1.6	1	1	0	0	0
3.1	5	0	0	0	0
6.3	3	0	2	0	0
12.5	1	0	1	0	0
25	0	0	3	0	0
50	0	6	6	0	0
> 100	12	13	10	0	0

ABPC: Ampicilin, AT: Tetracyclin CFDN: Cefdinir

EM: Erythromycin OFLX: Ofloxacin

DISCUSSION

Diarrhoeal diseases are part of the main social problems in Osogbo, as in other developing countries in tropics (1,11). Clarification of the enteropathogens involved in diarrhoeal diseases in the country is an essential step toward the implementation of effective primary health care activities against the diseases. This study provides information on the prevalence of enteropathogens in a distinctive area, Osogbo, Osun State. Although it may not give a complete picture of the genuine spectrum of diarrhoeal diseases in the community, the survey is still significant as it is, based on information available to us, the first intensive survey of the etiological agents of diarrhoeal disease in Osogbo.

Esherichia coli and *Shigella spp* were identified in this study as the major causes of diarrhoeal diseases in Osogbo. They are bacteria that have been consistently identified as aetiological agent of diarrhoea. Statistical analysis showed that *E. coli* was significantly associated with diarrhoea in patients aged 3-5 years ($P<0.05$). It was found to be more prevalent than the other enteropathogens, which is consistent with the result of other researchers (11, 12). While the isolation of *Shigella spp* is also similar to results obtained by other workers, the frequency of isolation of *Shigella spp* (16.3%) in this area was higher than those reported in other tropical countries (1, 9). This could be indicative of the important role *Shigella spp* plays in the outbreak of diarrhoea in children aged less than 5 years. The seasonal variation in incidence of the pathogen is similar to results obtained elsewhere (13, 14).

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY, 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002095/2321

PHAGE AMPLIFICATION TECHNOLOGY AND ANTI-TUBERCULOUS DRUG SUSCEPTIBILITY TESTING IN NIGERIA

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The emergence of multi-drug resistant tuberculosis (MDR-TB) defined as combined resistance to the two most effective anti-tuberculosis drugs, rifampicin and isoniazid, threatens to create a public health hazard of unprecedented proportion. The fact that MDR-TR is more difficult and expensive to cure creates the need for prompt diagnosis. Conventionally, the proportion method on Lowenstein Jensen (L J) medium is used in most developing countries as the 'gold standard' in the drug susceptibility testing of *Mycobacterium tuberculosis* (MTB) and it takes 3-4 weeks to give results from an MTB culture.

The use of phage as a diagnostic is fast gaining ground today. It involves targeting viable MTB cells from culture with a specific mycobacteriophage. After a one hour incubation, it is treated with an antivirus to destroy the phage that are not protected with the bacilli. Upon addition of cells of growing, non-pathogenic *Mycobacterium smegmatis* (sensor cells), progeny phage from the MTB cells infect the sensor cells, thus amplifying the effect of the phage. When plated in an agar medium overnight, plaques occur in the cell lawn indicating the presence of viable MTB in an original sample. A comparison is made between the number of plaques produced in a drug-free control and a sample incubated in the presence of the drug. While the presence of plaques beyond a cut-of point indicates drug resistance, the absence of plaques indicates that the drug destroyed MTB cells. Overall accuracy from several trials so far conducted is put at 97-98% compared with the 'gold standard'. With the phage amplification method, antituberculosis drug susceptibility results are obtained from MTB culture within 48 hours as opposed to the L J proportion method, which gives resulted in 3 to 4 weeks. Also, phage, as a diagnostic, is much more applicable in Nigeria laboratories than newer, rapid methods which requires specially dedicated instrumentation and are therefore very expensive. Phage amplification technology requires no special equipment and the results can be read visually.

Key words: Tuberculosis, drug susceptibility, phage, treatment, FASTPlaque-TB, rifampicin

INTRODUCTION

The World Health Organization (WHO) in 1993 declared tuberculosis (TB) a global emergency (1). Coupled with it deadly alliance with HIV/AIDS (2), TB has emerged as a public health hazard of unprecedented proportion especially for the developing world which bears 95% of its burden (3). Eight million new cases and 2-3 million deaths from TB are recorded annually (4). Nigeria is one of the 22 countries, which bears 80% of all incident cases of TB worldwide. She is also one of the 16 countries in which the progress of TB control is of greatest concern (5). Average annual case notification is put at 25,000 in the year 2000 (6), however, 26,641 cases were recorded (7). In an epidemiological study of TB in Lagos, Nigeria, a significant increase from an incident rate of 21% in 1982 to 42% in 1992 was reported (8). Also, a study by Wokoma in 1999 showed a 7.74% increase in the incidence

rate of TB in Port Harcourt, between 1993 and 1999 (9). These were hospital-based studies. It is important to note that disease surveillance in Nigeria is neither intensive nor extensive and so cannot provide exact numbers of new cases or deaths occurring from TB in a year. Due to the stigma attached to TB in the Nigerian society, several cases go unreported. Of the cases reported, up to half or more are not diagnosed and therefor not treated. Smear microscopy has a sensitivity of 30-70% depending on whether direct or processed (concentrated) smears are examined (10).

TB is a curable disease, which ravaged the globe for over two centuries after its emergence. The discovery of streptomycin in 1946 offered a respite to TB sufferers because prior to its discovery, TB patients were subjected to mutilating surgical procedures such as thoracoplasty in which large sections of the chest wall were excised in order to close cavities. TB came to be known as "captain of all men of death" as it continued to kill several famous people because there was no cure (12, 13). Several other anti-TB drugs were later discovered and they included rifampicin, pyrazinamide, ethambutol, ethionamide, thioacetazone, kanamycin, viomycin, capreomycin and cycloserine. Of these, rifampicin and pyrazinamide are bactericidal in vivo while the others are bacteriostatic (13). For the treatment of TB, the WHO has listed five essential drugs, which include isoniazid (H), rifampicin (R), pyrazinamide (Z), streptomycin (S) and ethambutol (E). These are the most effective drugs against TB and are referred to as first line drugs. The second group consists of capreomycin, cycloserine, ethionamide, kanamycin, and a host of others. They are referred to as 'second line' drugs and are only used in cases of relapse, treatment failure or chronic cases (14,15). Usually a treatment regimen is made up of two phases namely, the initial phase during which the first-line drugs are used in combination to ensure rapid smear conversion from positive to negative, and a continuation phase (15). In Nigeria, the full regimen is rifampicin (R)/isoniazid (H) (combined tablet), pyrazinamide (Z) and ethambutol (E) daily for two months followed by thiazina (isoniazid plus thiacetazone) or ethambutol daily for 6 months. This is standardized according to the patient's age and body weight as well as the type of TB (16).

DRUG RESISTANCE IN TUBERCULOSIS

The greatest threat to chemotherapy today is the

frequency and rapidity with which bacteria develop resistance to drugs (17). Several workers have demonstrated drug resistance. In a study by Fattorini and co-workers, the activity of sixteen antimicrobial agents was tested against drug resistant strains of *Mycobacterium tuberculosis* (MTB). Of the first line drugs, isoniazid was ineffective against all strains while resistance to streptomycin; rifampicin, pyrazinamide and ethambutol were 80.4%, 71.7%, 39.1% and 8.7% respectively. Amongst second line anti-TB drugs, resistance to ciprofloxacin, ofloxacin, sparfloxacin, amikacin and kanamycin was 20%. About 10% of strains were resistant to capreomycin and cycloserine and 4.3% were resistant to ethionamide (18). In another study by Karamat and co-workers, four first line anti-TB drugs were tested on 300 isolates form clinical samples at the Armed Forces Institution of Pathology, Rawalpindi. Of these, 52.66% were resistant to one drug at least. Of the resistant isolates, 26.33% were resistant to isoniazid, 24.0% to rifampicin, 28.0% to streptomycin and 23.33% to ethambutol with or without resistance to other drugs.

Besides MTB resistant to single drugs, there have been dramatic outbreaks of multidrug resistant tuberculosis (MDR-TB). It is described as a man-made disease because it is caused by improper treatment, inadequate drug supplies as well as erratic and indiscriminate use of drugs. The availability of drugs over the shelf in most developing countries including Nigeria has led to uncontrolled self-medication (20). Multi-drug resistance in TB has been described as combined resistance to rifampicin and isoniazid. Patients with MDR-TB face chronic disabilioty and death and they represent an infectious hazard for society (21). In the study by karamat and co-workers (19), multi-drug resistance was found in 41 isolates (13.66%). Studies have shown that rifampicin resistance is a good predictor of MDR-TB. This conclusion was drawn after studies in several parts of the world pointed to the fact that MTB strains resistant to rifampicin are often invariably resistant to isoniazid at least and are thus considered MDR-TB (22). Some of such studies include those conducted in Estonia, Ethiopia, India (Delhi) and Latvia in which of the rifampicin resistant strains, 100%, 100%, 95% and 96% respectively were MDR-TB (22,23).

ANTI-TUBERCULOSIS DRUG SUSCEPTIBILITY TESTING

The management of MDR-TB requires a quick diagnosis as well as rapid and accurate susceptibility results to ensure early administration of a new regimen,

usually based on a quinolone, for retreatment of the patient. This is a major problem in developing countries including Nigeria.

In Nigeria, TB culture is limited to the National Reference Laboratory, which so far does not have a good liaison with peripheral laboratories where TB diagnosis by direct smear microscopy (16) is done under the National TB Control Programme. Even when such samples are sent to the reference laboratory, it takes 3-4 weeks after the availability of culture (which itself takes 6-8 weeks) for drug susceptibility results to be available. This is because under currently available infrastructure, it is only feasible to carry out drug susceptibility testing using the conventional proportion method on Lowenstein Jensen (LJ) medium.

The proportion method, which can also be done on the agar based Middlebrook 7H10 and 7H11 media is regarded as the 'gold standard' in drug susceptibility testing of TB (24) and is recommended by the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) (22). Though susceptibility testing can be performed directly on sputum samples through this method to give results within 3-4 weeks, there is often the problem of inoculum standardization. Besides the proportion method, there exist other methods, which include the resistance ratio and absolute concentration method, which are not commonly used.

The need for prompt treatment of MDR-TB has led to a search for more rapid method of susceptibility testing. There exists today, semi-automated methods like the Bactec 460 system (Becton Dickinson) which gives results within 7-21 days, the fully automated MB/Bac T (Organon Teknika) and the Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson) which also has manual use. While the Bactec 460 system detects growth by the evolution of radiolabeled $^{14}CO_2$, the other two use non-radioactive materials. The results obtained by testing sputum samples directly can be quite confusing and misleading because of insufficient growth of the control as well as contamination (22). Special skills and experience are required to read the results correctly. It will also be tantamount to a gross waste of reagents to perform susceptibility tests on organisms, which may turn out to be something other than MTB. It is easier and more economical to perform susceptibility tests after culture results are available. In most developing countries including Nigeria, the cost of these machines, their servicing, cost of reagents and

personnel expertise prohibit their use.

The WHO Global Tuberculosis Programme (GTB) recognizes the need for new, rapid and affordable diagnostic in developing countries to replace the slower conventional methods. The GTB is committed to the promotion of new diagnostic of proven value in TB control programmes (26).

PHAGE AMPLIFICATION TECHNOLOGY

The use of mycobacteriophage in the disagnosis of TB is fast gaining ground today. Research into the use of phage in diagnosis dates back to 1947, when Gardner and Weiser identified phages that are specific for the mycobacteria (27). In 1960, Redmond and Cater isolated phages that specifically infect *M. tuberculosis* and *M. bovis* (28). The fact that phages are specific in their infection of hosts is the backbone of this technology. In the procedure, a specific phage that infects members of the MTB complex is used to target viable MTB in a specimen. If it is a positive sample, the phages infect the MTB cells (27). Any phage not incorporated within MTB cells are destroyed on treatment with a specific virucide. To confirm the presence of phage in the MTB cells, rapid growing *Mycobacterium smegmatis*, a non-pathogenic mycobacterium, which is also susceptible to infection by the phage is added. Using the pour plate method, this mixture is inoculated into molten agar, which is allowed to solidify, and the plate is incubated overnight. The phage particles within the MTB cells replicate and lyse the cells to release progeny phage. These then infect the cells of *M. smegmatis*, thereby amplifying the presence of the phage. Since *M. smegmatis* is a rapid grower, growth is seen in the medium overnight and plaques are seen where lysis of the cells occurred. A comparison of the number of plaques observed is made between culture suspensions incubated in both the presence and absence of the antibiotic. The number of plaques visualized from a given sample is related to the number of viable tubercle bacilli in the original sample (22,29,30-33).

FASTPLAQUETB-MDRI™

The FASTPlaque TB-MDRI™ (FPTB-MDRi) is a commercially available kit (Biotec Laboratories, Ipswich, UK) for the rapid detection of MTB resistance to rifampicin, a proven indicator of MDR-TB (22,23). Results of the trials conducted so far have been very impressive with 100% sensitivity and overall accuracy of 97%-98% compared with the LJ proportion method, which is used in most developing countries including Nigeria.

PHAGE AMPLIFICATION TECHNOLOGY VERSUS CONVENTIONAL METHODS

In comparison with the LJ proportion method, phage amplification technology is rapid; giving conclusive results within 48 hours after culture is available. With the FASTPlaque TB-MDRi, further studies have indicated that it may be possible to achieve a 24-hour test by the use of a shorter rifampicin incubation period (31). Availability of susceptibility results in a shorter time will reduce morbidity and mortality from MDR-TB and treatment can be promptly initiated. It will thus check the spread of MDR-TB in the community. Phage amplification technology does not involve complicated procedures requiring extra-ordinary expertise. It involves simple microbiological skills like centrifugation, pipetting and pour plate culture method. The results are read visually and it requires no specially dedicated instrumentation hence it is less expensive than the new automated methods and it is applicable under currently available infrastructure in most laboratories in developing countries. One of the attractions of the FASTPlaque TB-MDRi™ Kit (Biotec Laboratories, Ipswich, UK) is that it contains all materials-reagents, freeze-dried virus, *M. smegmatis*, virucide, and the drug, medium and reagent bottles needed for the test. It is thus very convenient. The FASTPlaque TB-MDRi™ assay has been evaluated and compared with conventional methods in several studies. In a study conducted at the TB Reference Laboratories of the South African Institute of Medical Research (SAIMR) in Johannesbourg (LAB I) and Cape Town (LAB II), the ability of the FPTB-MDRi kit to correctly identify rifampicin susceptibility on strains of *Mycobacterium tuberculosis* culture on solid media was evaluated and compared with conventional methods. By conventional methods, 81 out of 191 strains were found to be rifampicin resistant and 110 strains out of 191 were rifampicin susceptible. Respectively, sensitivity, specificity and overall accuracy of the FASTPlaque TB-MDRi for LAB I were, 100%, 97% and 98% and for LAB II they were 100%, 94% and 97% (22). In another study at the TB Unit of SAIMR, Johannesbourg, the ability of FPTB-MDRi to detect rifampicin resistance within 48 hours was evaluated using samples with known Bactec 460 automated culture results. In this study, the use of FPTB-MDRi was compared to the Bactec 460 radiometric method in determining TB drug susceptibility patterns to rifampicin. Also, the possibility of reducing the time for reporting

results from 48 hours to 24 hours was explored. The study showed that FPTB results are available within 48 hours (sooner than the automated Bactec 460 method). With a reduced rifampicin incubation time, the overall time for reporting results could be reduced to 24 hours. Overall accuracy was 90% (31).

COST-EFFECTIVENESS OF PHAGE AMPLIFICATION TECHNOLOGY

TB is a disease symptomatic of poverty. MDR-TB is particularly common among those who have had previous anti-TB chemotherapy, ethnic minority groups, migrants, refugees, substance abusers, HIV positive patients and the homeless (34). Cost is therefore a major consideration that determines the applicability of diagnostics in the developing world including Nigeria. This is exemplified by the fact that despite the availability of rapid sophisticated methods for the diagnosis of TB and the detection of MDR-TB, none of these are being used in National TB control programmes in most third world countries. Any new test must therefore compare favourably with the conventional methods otherwise their high cost would prohibit their use.

The cost of full diagnosis of TB (AFBX3, x-ray, culture and sensitivity) is about N2000 (approximately $15). This is highly variable depending on where the tests are done and the method used. The FASTPlaqueTB-MDRi kit costs $400 per determination of 50 tests, if one buys in small quantities from Biotec Laboratories, Ipswich, UK (35). It is difficult to work out what the cost per test would eventually be in Nigeria with the availability of discount on bulk purchases but it is unlikely to go beyond WHO recommendations of less than $10 for a drug susceptibility test. It places very little demand on the scarce resources of Nigerian laboratories because it is manual, requiring no specially dedicated instrumentation.

It has been suggested that it may be possible to reduce the cost of this technology by propagating the phage and *M. smegmatis* in a local laboratory. Studies have however shown that a particular gene in *M. smegmatis* when over expressed induces resistance to certain phages (36). Coupled with the problem of standardization, it may be better to use the commercially available kit which passes through a quality control test to ensure high performance standards before it is plunged into the market.

The ability of phage amplification technology promptly detect MDR-TB will reduce morbidity and mortality

from the disease. Also, the number of contacts that the patient has a chance to infect will be greatly reduced. Thus the number of people requiring anti-TB drug susceptibility testing as well as treatment with the more expensive second line drugs in the future will be reduced. These future savings could be used to pay for the diagnostic (26). With increased budgetary allocation to the National Tuberculosis and Leprosy Control Programme (NTLCP) and donor support, the cost of diagnosis and treatment on the individual patient would be greatly reduced.

CONCLUSIONS AND RECOMMENDATION

The emergence of MDR-TB is indeed a major threat to the treatment of TB and an important impediment to National TB Control Programmes. In order to avoid its fatal effects on individual patients and its spread within communities, there is need for prompt diagnosis. FPTB-MDRi gives susceptibility results from MTB cultures within 48 hours and is therefore recommended as a substitute for the conventional LJ proportion method, which takes about one month to provide susceptibility results. This would ensure that appropriate treatment is initiated in good time.

Cost has emerged as a major consideration and an important factor determining the applicability of new diagnostics in the National TB Control Programmes of developing counties including Nigeria. There is an urgent need for all tiers of government to prioritize TB control because of its chronic and debilitating effects on the workforce and the general population as well as its deadly alliance with HIV/AIDS, which has its highest prevalence in developing countries. Prioritization of TB control goes beyond an expression of the political will to do so. It involves actual dedication and disbursement of funds to the National TB Control Programme. This has become necessary because majority of TB patients are poor and so cannot afford exorbitant testing and treatment. Ensuring that such patients undergo and receive free or highly subsidized testing and treatment would be an indirect investment into the workforce of nations. The right to quality/affordable healthcare is entrenched in several international human rights documents as well as the Nigerian constitution.

There is the need for the NTLCP in Nigeria to embark on laboratiories inspection and to identify and equip strategically located centers where TB testing can be done nationally. This would do little to reduce the spread of MDR-TB as the onus of culture and

drug susceptibility testing lies with the National TB Reference Laboratory in Lagos which so far has no strong liaison with peripheral laboratories. It is therefore recommended that a network of laboratories be established so that samples from cases of treatment failure can be promptly submitted to the Reference Laboratory for further diagnosis. Amongst other things, provision of vehicles and biohazard containers/ice chests for the transportation of samples from peripheral centers to the National Reference center is a top priority.

The World Health Organisation as the regulatory body for National TB Control Programmes has a great role to play in promoting the evaluation of new diagnostics as well as recommending their use. It is thus recommended that the WHO assists National Reference Laboratories in developing countries where TB prevalence is highest to evaluate new kits in other to ascertain their adaptability to particular situations. The WHO also has a role to play in supporting laboratory personnel in charge of TB diagnosis to attend training courses to improve their skills especially in new technologies.

Though considered rapid compared with the L.J proportion method, FPTB-MDRi relies on culture. There is the therefore need for the manufacturers to engage in continuing research in order to produce kits that could detect MDR-TB rapidly and directly form sputum and other samples.

ACKNOWLEDGEMENT

I am most grateful to the Management and Staff of Biotec Laboratories, Ipswich, UK especially Messrs Rowland King, Andre Trollip and Dr Heidi Albert for giving me my first hands-on training in Phage Amplification Technology in Cape Town, South Africa. Special thanks to the Director General of NIMR, Lagos, Dr. E. Oni Idigbe, for giving me the opportunity to attend the training.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY, 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002087/2322

METHICILLIN RESISTANCE IN STAPHYLOCOCCAL ISOLATES FROM CLINICAL AND ASYMPTOMATIC BACTERIURIA SPECIMENS: IMPLICATIONS FOR INFECTION CONTROL

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The study assessed the importance of *Staphylococcus aureus* as a urinary pathogen and the incidence of multidrug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 86 staphylococcal isolates made up of 50 clinical isolates from urine samples submitted to the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital and 36 asymptomatic bacteriuria isolates from urine samples of 'healthy' volunteers within the university community were tested for their susceptibility to various antibiotics and production of -lactamase enzyme. A total of 27 isolates (31.4%) were methicillin resistant, with 12(44.4%) being methicillin resistant coagulase-negative staphylococci (MRCNS). Majority of the isolates tested were resistant to the cheap, readily available brand-spectrum antibiotics; ampicllin, amoxicillin, chloramphenicol, tetracycline and penicillin G. All the isolates were resistant to three or more of the antimicrobial agents tested. A total of 14/50 (28%) of the clinical isolates and 17/36 (47.2%) of the 'community' isolates from healthy volunteers were resistant to 7 or more of the antimicrobial agents tested. Analysis of the multiple antibiotic resistance (MAR) index of isolates and the production of -lactamase enzyme showed that 56 isolates representing 65.1% of the total number tested had an MAR index of 0.5 and above indicating that they probably originated from an environment where antibiotics are frequently used. The implication of these findings for instituting effective control measures aimed at reducing the pool of antibiotic-resistant organisms is discussed.

Key words: Methicillin-resistant, staphylococcus aureus, asymptomatic bacteriuria, infection control

INTRODUCTION

Antimicrobial resistance is a well recongnised problem worldwide (1, 2). The resistance organisms have however been associated primarily with hospitals; especially in intensive care units (1). The ubiquitous occurrence of staphylococci ensures that man is constantly exposed to this group of microorganisms, thus infection of various parts of the body caused by staphylococci are very common (3,4).

Staphylococcus aureus continues to be a major cause of community acquired and health-care related infections around the world (5,6). The emergences of high level of penicillin resistance followed by the development and spread of strains resistance to the semi-synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclines and aminoglycoside have made the theraphy of staphylococcal disease a global challenge (6,7,8).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a virulent organism that causes significant mortality and morbidility, especially to patients in critical care areas (9). MRSA can (and does in some cases) also contribute to an increased length of hospital stay and healthcare costs. Infections with methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci (MRCNS), have been widely reported. Those infections were initially confined to hospitals and nursing homes especially in intensive care units where a combination of debilitated patients, invasive technology, and high antimicrobial use facilitates infections by multi-drugs resistant staphylococci, enterobacteria resistant to third-generation cephalosporins and imipenem resistant non-fermentative bacteria (10). However, cases of community-acquired MRSA have been reported, primarily in persons with history of injection drug use and other high-risk patients (11).

Recently, community-acquired MRSA have been described in both adults and children who did not have extensive exposure to hospitals or apparent risk factors (12,13,14,15,16). Antimicrobial resistance often leads to therapeutic failure of empirical therapy; therefore, knowledge of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians in their routine work (17). Effective antibiotic therapy in developing countries is severely limited by the large reservoir of antibiotic resistant bacteria that exist within their population. The healthy members of any community represent its largest reservoir of bacteria resistant to antimicrobial agents (18).

The increase in antimicrobial resistance is creating a lot of problems. These have focused attention upon measures for fighting resistance, foremost of which is susceptibility surveillance (19). The rapidity of emergence of multiple antibiotics resistant organisms is not being reflected by the same rate of development of new antimicrobial agents. It is therefore conceivable that patients with serious infections will soon no longer be treatable with currently available antimicrobial agents (20).

Before instituting control measures that will be appreciated by all healthcare professionals, there must be scientific data to ascertain the extent of the problem posed by multi-drug resistant organisms like the MRSA to the outcome of antimicrobial chemotherapy in the hospital and the immediate community. Unfortunately, surveillance studies

on the epidemiology of MRSA and their antimicrobial susceptibility patterns are lacking in this environment.

This study aimed at assessing the importance of *Staphylococcus aureus* as a urinary pathogen determine the incidence of multi-drug resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical isolates from urine in a University Teaching Hospital and compare these with isolates from 'healthy' individuals within the university community. The implications for instituting effective control measures that can reduce the pool of antibiotic-resistant organisms within healthy members of the community and in the hospital setting are discussed.

MATERIALS AND METHODS

Bacteriology

Staphylococcal isolates obtained from urine sample submitted to the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, and 'healthy' student volunteers from Ahmadu Bello University were analysed.

The isolates were characterized using established methods, which included colonial morphology, Gram stain characteristics, ability to produce the enzyme peroxidase, coagulase and the presence of heat stable DNAse activity to separate the *Staphylococcus aureus* strains from the coagulase-negative staphylococci (CNS). A standard *Staphylococcus aureus* strain ATCC 13709 was obtained from the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

Chemicals and media

The media used were Nutrient Broth (NB), Nutrient (NA) and Mannitol Salt Agar (MSA) all from Oxoid. The chemicals include hydrogen peroxide (3%), deoxyribonucleic acid, sodium chloride, starch and iodine solution.

Antimicrobial sensitivity testing

The susceptibility pattern of the isolates to the following antibiotics was determined; Ampicillin 25 µg, Chloramphenicol 20 µg, Cloxacillin 10 µg, Erythromycin 10 µg, Gentamcin µg, Penicillin G 1.5 iu, Streptomycin 10 µg, Amoxycillin 25 µg, Ciprofloxacin 5 µg and Methicillin 5 µg, using the modified Kirby Bauer diffusion technique (21). The isolates were grown overnight in nutrient broth and the inocula spread on the surface of the previously prepared sterile nutrient agar plates by flooding with 2mls of the standardized suspension. Excess were drained off and allowed to dry in a warm incubator for about 15-20 minutes. Using sterile forceps, multiantibiotic discs were placed on

the dried nutrient agar plate and left at room temperature for about 25minutes to allow the antibiotics to diffuse in the agar medium. Similar treatment was extended to the standard *Staphylococcus aureus* ATCC 13709. All the plates were incubated at 37° C for 24 hours in inverted position. Thereafter, the diameter of the zones of inhibition of the isolates and the standard *Staphylococcus aureus* were measured to the nearest millimeter.

Determination of Methicillin sensitivity

Nutrient agar medium containing 5% w/v sodium chloride (22) was prepared, distributed into 20ml aliquots and sterilized at 121° C for 15 minutes. Overnight cultures of the isolates were used to flood the surfaces of the prepared agar media, drained and allowed to dry. Methicillin discs (containing 5µg of methicillin) were placed on the dried agar plate and treated as previously described above, but incubation was at 35° C. The diameter of the zones of inhibition was similarly determined.

Test for β-lactamase production

Suspensions of the isolates were prepared in triplicates by emulsifying bacterial colonies (from an overnight nutrient agar culture) with sterile loops in 0.5 ml of phosphate buffer solution containing 0.06 mg/ml (10,000 units/ml) of Penicillin G. As control, cell suspension of the standard typed culture of *Staphylococcus aureus* (ATCC 13709) was similarly set-up. They were incubated at room temperature for at least 1 hour. Thereafter, 2 drops of freshly prepared 1% aqueous starch solution were added to each bacterial suspension and shaken. To this was added 1 drop of iodine solution and allowed to stand for 10 minutes at room temperature. β-lactamase producing organisms changed the colour of the reaction mixture from blue-black to colourless within the 10 minutes.

Determination of multiple antibiotic resistance (MAR) index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolates is resistant by the total number of antibiotics tested (23,24).

$$\text{MAR index} = \frac{\text{Number to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

RESULTS

Of the staphylococcal isolates, 50 were clinical isolates from urine samples submitted to the Department of Medical Microbiology, Ahmadu Bello Teaching Hospital, while 36 were "community" isolates form urine samples of 'healthy' volunteers within the university community. A total of 27 isolates (31.4%) were

methicillin resistant, with 12 (44.4%) being MRSA, while 15 (55.6%) were MRCNS. Out of the 59 methicillin-sensitive staphylococcal isolates, 40 (67.8%) were MSSA while 19 (32.2%) were MSCNS. Figure 1 shows the proportion of the staphylococcal isolates resistant to various antibiotics.

The MAR index of isolates by the proportion, that are β-lactamase positive and methicillin-resistant is shown on Table 1. A breakdown of the analysis of the MAR index of the 'community' and clinical isolates is shown in Table 2.

All the isolates were resistant to three or more antibiotics, while 17/36 (47.2%) 'community' isolates and 14/50 (28%) clinical isolates showed multi-drug resistance to seven or more of the antibiotics tested. Figure 1 shows the susceptibility patterns of staphylococcal to different antibiotics. Table 1 shows multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* isolates, with the proportions that are β-lactamase positive and methicillin-resistant. Table 2 shows the analysis of MAR index of the clinical and asymptomatic bacteruria (community) isolates.

Figure 1: Susceptibility patterns of Staphylococcal isolates to different antibiotics

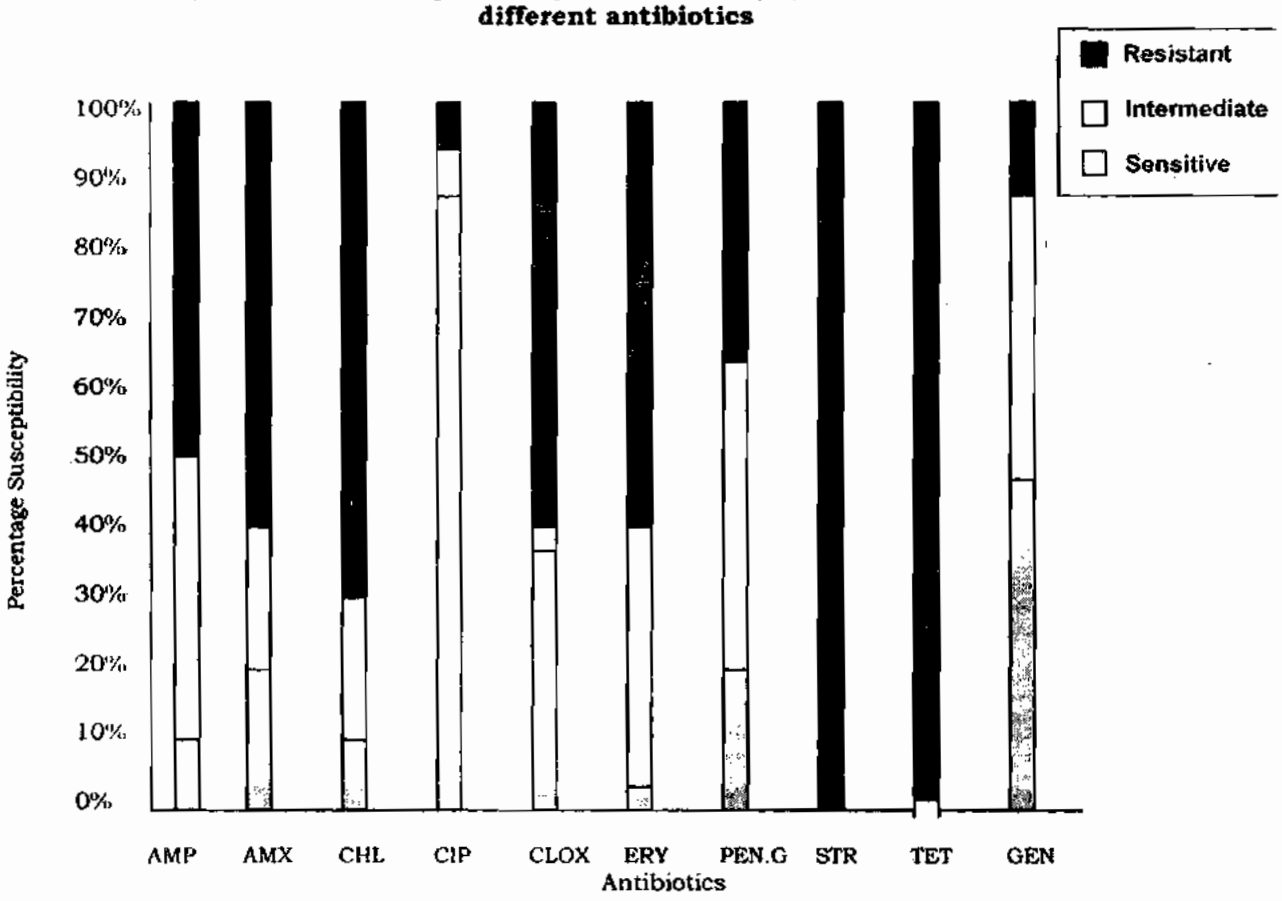

Table 1: Multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* isolates, with the proportions that are β-lactamase positive and methicillin-resistant.

MAR index	No. of isolates (%)	β-lactamase +ve (%)	MR (%)
0.3	19(22.1)	3(15.8)	2(10.5)
0.4	10(11.6)	0(0.0)	1(10.0)
0.5	12(14.0)	3(25.0)	1(8.3)
0.6	12(14.0)	7(58.3)	3(25.0)
0.7	11(12.7)	7(43.6)	6(54.5)
0.8	15(17.4)	10(66.7)	8(53.3)
0.9	6(7.0)	3(50.0)	6(100.0)
1.0	1(1.2)	1(100.0)	0(0.0)

Table 2: Analysis of MAR index of the clinical and asymptomatic bacteruria (community) isolates number of

MAR index	Community isolates	Clinical isolates
0.3	3	17
0.4	3	7
0.5	4	8
0.6	8	4
0.7	6	5
0.8	4	7
0.9	4	2
1.0	1	0
TOTAL	**36**	**50**

DISCUSSION

Nosocomial infection caused by multi-resistant organisms in developing countries respresent a major public health problem that is not universally recognized (25). Results from various studies in the past did not identify *Staphylococcus aureus* as an important urinary pathogen (3,26,27), rather there has been a focus on the CNS identified as a mjor cause of infections associated with prosthetic implants and medical devices (28) and urinary tract infection, particularly in young sexually active women (29,30). The 72.4% prevalence of *Staphylococcus aureus* in this study points to the increasing important of this organism as a urinary

pathogen and a common isolate in asymptomatic bacteriuia in this environment.

Majority of the isolates tested were resistant to the cheap, readily available broad- spectrum antibiotics; Ampicillin (89.3%), Amoxycillin (83.3%), Chloramphenicol (89.3%), Tetracycline (98.8%) and Penicillin G (83.3%). This result is consistent with the observation that clinical staphylococcal isolates are resistant to a large number of commonly prescribed antimicrobial agents (30).

The level of multi-drug resistance exhibited by the staphylococcal isolates in this study is alarming. All the isolates were resistant to three or more of the antimicrobial agent tested. The MDR strains came from both clinical and 'community' isolates. A total of 14/50 (28%) of the clinical isolates and 17/36 (47.2%) of 'community' isolates form healthy volunteers wec resistant to seven or more of the antimicrobial agents tested. The high percentage of isolates form 'healthy' individual showing high MDR goes to confirm the assertion that the healthy members of the community represent its largest reservoir of bacterial resistant to antimicrobial agents (18,31). MAR index higher that 0.2 has been said to be an indication of isolates originating from an environment where antibiotic were often used (23,24).

Since all the isolates were not from the hospital environment where antibiotic are often used but also from urine of asymptomatic 'healthy' volunteers in the university community, this observation goes to confirm the widespread abuse/misuse of antibiotics in this community. Administration of antibiotics often permits the selection and overgrowth of multiply resistant organisms (32). The selective pressures favouring resistant strains are known to arise form misuse and overuse of antimicrobials (notably extended spectrum cephalosporins) increased numbers of immunocompromised hosts, lapses in infection control (where they exist), increased use of invasive procedures and devices, and widespread use of antibiotic in agriculture and animal husbandary (33).

It has been documented that resistance properties are easily transferred between organisms of the same or different genera through the agency of plasmid. Evidence of transfer of high-level resistance to gentamicin, tobramycin and kanamycin between staphylococi of the same and different species by filter mating also exists (34). Restriction endonuclease analysis of the plasmids from five isolates of

Staphylococcus epidermidis has also supported the hypothesis that plasmid transfers tween the two species occur in nature (35). Transfer of such resistance determinants may have been responsible for the high level of MDR encountered in this study.

A breakdown of the number of isolates with a particular MAR index and proportion that are β-lactamase-positive showed that 56 isolates representing 65.1% of the total number tested, had an MAR index of 0.5 and above, while 29 isolates (33.7%) had MAR index less than 0.5. This is a relative indication of the susceptibility of the isolates to the test antibiotics. One isolate that was resistant to all the 10 antibiotics tested was isolated from the urine of a 'healthy' volunteer in the community. The isolate produced β-lactamase, was coagulase-positive but sensitive to methicillin.

Multi-drug resistant *Staphylococcus aureus* have been known to produce β-lactamase in greater amounts than strains that are fully sensitive to antibiotic or resistant to only to penicillin (22). Contrary to the findings from other studies (4,36,37), bacterial resistance to ciprofloxacin (a fluoroquinolone) as high as 13.1% was encountered in this study. The increasing resistance to such a new and expensive, reserve drug is probably an indication of the increasing level of availability, misuse and overuse.

The tremendous therapeutic advantage afforded by introduction of new antibiotic is always threatened by the emergence of increasingly resistance strains of microbes (33). Introduction of new antibiotic are essential, but their useful life will be enhanced only if used wisely and sparingly (38). The ever-present danger of individuals contracting infections in this community makes it imperative that measures aimed at reducing the pool of antibiotic resistant organisms existing within the healthy members of the community be instituted within delay.

In concert with improved prescribing habits, efforts to identify and isolate resistant organisms that can be introduced into healthcare setting from outside institutions are essential. Evidence from various studies have shown that, surveillance when used to guide polices on antibiotic use and infection control, can be helpful to control the development and spread of antimicrobial resistance within the hospital setting and the community at large (19,39,40).

We agree that persistence would be required in influencing the behaviour of healthcare professionals and to maintain optimal infection control policies and procedures within the hospital

and the community at large. In the meantime, it is highly desirable to continuously monitor the antibiotic resistance situation so as to maximize the possibility of administering an effective antimicrobial agent whenever there is need to do so.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002070/2323

BACTERIOLOGY OF CHRONIC SINUSITIS IN ILORIN, NIGERIA

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A prospective study of the bacteriology of 120 patients with chronic sinusitis and 55 control subjects seen between January 1995 and December 1998 in the Ear, Nose and Throat (ENT) Diseases Clinic of University of Ilorin Teaching Hospital, Ilorin, Nigeria was made. Whereas all cultures from the control group yielded only Staphylococcus (63.6% Coagulase positive and 36.4% Coagulase negative), cultures of patients with chronic sinusitis yielded *Staphylococcus aureus* (48.1%), *Escherichia coli* (20.4%), *Klebsiella spp.* (20.4%), Streptococci (7.4%) and *Streptococcus pneumoniae* (3.7%). The isolates were 100% sensitive to Ofloxacin, while penicillin was the least effective antimicrobial agent across board.

It was concluded that because of the difficulty in differentiating pathogenic organisms from commensals, the result of nasal swabs should be interpreted with caution. However, non-otolaryngologists involved in the management of the vast majority of patients with chronic sinusitis should request a carefully obtained posterior nasal mucosal swab.

INTRODUCTION

Chronic sinusitis is a common otolaryngologic disease worldwide, and particularly so in developing countries (1-7). The aetiology of sinusitis is often multifactorial with considerable overlap of clinical manifestations (8). Purulent nasal discharge may not necessarily signify infection. Nasal discharge with very high eosinophil count associated with allergic rhinitis may appear yellow or green (8). Also, clear nasal discharge may not always be of allergic origin.

There are multiple problems associated with management of chronic sinusitis. The difficulty in interpreting nasal mucosal swab cultures as opposed to sinus cavity samples is the main reason for preferring the latter in the management of infective chronic sinusitis as they yield mainly pathogens. Review of several studies show that the normal commensal flora of the nasal vestibule include *Staphylococcus epidermidis*, Micrococcus, *Staphylococcus aureus*, Diphtheroids and Gram negative bacteria, while that of the posterior nasal fossa include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Neisseria meningitidis* and Gram negative bacteria (8). These results raise doubts on the usefulness of nasal swab bacteriology in the treatment of infective rhinosinusitis (8-11). Apart from sampling technique, inappropriate transport method

could also interfere with optimal conditions thereby inhibiting the growth of fastidious organisms, while promoting the overgrowth of commensal flora.

As a result of failure to establish causative pathogens before starting treatment, there is a tendency to over prescribe antibiotics and hence the development of drug resistant organisms (12,13). Inspite of awareness of these potential problems, most cases of chronic sinusitis are being managed by general practitioners, with no access to obtaining specimens of sinus cavity. Therefore, this study was undertaken to establish the reliability of results of nasal culture in the management of chronic sinusitis and to recommend precautions which when taken, will maximize the effectiveness of treatment of chronic sinusitis.

MATERIALS AND METHODS

A prospective study of 120 consecutive patients with chronic sinusitis seen between January 1995 and December 1998 in the Ear, Nose and Throat (ENT) diseases clinic of the University of Ilorin Teaching Hospital, Ilorin Nigeria was made.

Fifty-five control subjects, not known to be chronic sinusitis patients, and who had no symptoms of sinusitis over the preceding month before sampling, were enrolled into the study. A provisional diagnosis of chronic sinusitis was made if there were at least two of the following signs and symptoms for duration of at least two months (14); nasal blockage, nasal discharge (mucoid, mucopurulent or purulent), postnasal drip, excessive sneezing and halitosis. Allergic, vasomotor and infective processes were not differentiated since these may co-exist or one may lead to the other.

Nasal swab specimens were carefully obtained from patients by ENT surgeons on first clinic attendance after thorough physical examination with the documentation of their relevant biodata, (e.g. age, sex) and drug history. Very slender nasal swabs were used to collect the exudate from the posterior nasal mucosa. In cases where the nasal cavity was filled with pus, this was initially suctioned to allow access to the posterior nasal mucosa. Patients on antimicrobial therapy within 72 hours of presentation were excluded from the study. The same procedure was followed for the control group.

Specimens so collected were immediately sent to the University of Ilorin Teaching Hospital laboratory (within a short distance) where streaking on Chocolate, MacConkey and Saborauds Dextrose Agar plates, were carried

out and incubated aerobically at 37°C for 18-24 hours. Isolates were identified by standard methods (15).

The antimicrobial susceptibility patterns of isolates were determined by standard disc diffusion methods (15), on Mueller-Hinton agar employing Habdisc multidiscs with the following antibiotics: penicillin 1 mu, gentamycin 10µg, colistin 25µg, cloxacillin 5µg, clotrimazole 25µg, tetracycline 10µg, erythromycin 5µg and chloramphenicol 10µg. Single disc of ofloxacin 10µg and cefuroxime 30µg were added where applicable. Growth inhibition zone diameter was measured in millimeters with a calibrated ruler after 18-24 hours incubation at 37°C. Results were interpreted as susceptible or resistant, while *Staphylococcus aureus* (NCTC 6571) and Escherichia coli (NCTC 10413), served as control organisms. Facilities for retrieving and culturing anaerobic bacteria were lacking in the centre during the period of the study.

RESULTS

A total of 120 posterior nasal swabs from 58 males and 62 females were examined. The age range was 5-70 years. Less than 5% of patients were in the paediatric age group.

Fifty-four patients (45%) had pathogenic isolate. *Staphylococcus aureus* (48.1%), *Escherichia coli* (20.4%), *Klebsiella spp.* (20.4%), Streptococci (7.8%); and Streptococcus pneumoniae (3.7%) (Table I). Of the 55 control subjects studied (16 females and 39 males between ages of 18 and 55years), 22 (40%) were culture positive. Their isolates were exclusively Staphylococci (63.6% coagulase positive and 36.4% coagulase negative).

As shown in Table II, all the isolates were susceptible to ofloxacin, while penicillin was the least effective antimicrobial agent across board.

Table 1: Significant isolates from patients with Chronic Sinusitis in Ilorin, Nigeria.

Organism	Frequency (%) (N=54)
Staph. aureus	26(48.1)
E.coli	11(20.4)
Kebsiella spp	11(20.4)
Streptococci	4(7.4)
Strept. pneumoniae	2(3.7)
Total	54(100)

Table II: Antimicrobial Susceptibility Patterns of bacterial isolate

Antimicrobial Agent (Disk Concentration)	S.aureus n=26	S.pyogenes n=4	S.pneumoniae n=2	E.coli n=11	K.pneumoniae n=11
Ampicillin (25ug)	3(11.5)	2(50.0)	1(50.0)	2(18.2)	0(0)
Penicillin 1ug	0(0)	3(75.0)	1(50.0)	0(0)	0(0)
Erythromycin (5ug)	21(80.8)	4(100.0)	2(100)	-	-
Tetracycline 10ug	5(19.2)	3(75.0)	2(100)	8(72.3)	6(54.5)
Cloxacillin (10ug)	23(88.5)	4(100)	2(100)	-	-
Gentamycin (10ug)	16(61.5)	2(50.0)	1(50.0)	10(90.1)	8(72.8)
Cotrimoxazole (25ug)	6(23.1)	3(75.0)	1(50.0)	8(72.8)	8(72.8)
Chloramphenicol (10ug)	12(46.2)	3(75.0)	1(50.0)	-	-
Colistin (25ug)	-	-		11(100)	11(100)
Ofloxacin (10ug)	26(100)	4(100)	2(100)	11(100)	11(100)
Cefuroxime (30ug)	20(77.0)	4(100)	2(100)	10(90.1)	10(90.1)

Note:- - - Not tested.

DISCUSSION

Although a few studies suggest that paranasal sinuses are normally sterile, both aerobic and anaerobic bacteria have been recovered from sinus aspirates of presumably normal human subjects (10,16,17). It is however, generally agreed that the knowledge

of the bacteriology of secretions obtained directly from maxillary sinus by needle aspiration (with careful avoidance of contamination from mucosa surfaces), provides a more reliable information essential to the planning of anti microbial therapy (8,10,11). Thus in decreasing order of reliability, sample sites can be arranged in the following sequence; sinus cavity, sinus ostium, posterior nasal mucosa and anterior nasal mucosa (8-11). For the majority of non-otolaryngologists involved in the management of chronic sinusitis the posterior nasal fossa is recommended for obtaining specimens but contamination should be avoided and the limitations in interpreting reports of such culture recognized.

As reported else where (18-21), *Staphylococcus aureus* was the commonest pathogen associated with chronic sinusitis in this study. However, there are recent reports of increasing prevalence of enteric Gram-negative bacilli in this condition (17). Although, due to lack of facilities, anaerooic organisms were not cultured in this study. It has been documented that anaerobic organisms play a major role in chronic sinusitis (8,9,19,22). Fungal sinusitis is rare, and its presence should raise the suspicion of an immune deficiency disorder (12).

All isolates in this study were 100% susceptible to ofloxacin (cefuroxime was second) while penicillin was the least effective anti microbial agent across board. Although, the gram-negative organisms were susceptible to colistin, it is no longer in common use because it is ototoxic. This study suggests that ofloxacin is the drug of choice for the management of chronic sinusitis in our environment. But where it is contraindicated as in children, cefuroxime may be employed, as it is the second most effective antibiotic in this study. Although, some earlier studies recommended gentamicin and colistin, which are ototoxic, most recent studies also recommend newer drugs such as ofloxacin and amoxicillin-clavulanate as the drugs of first choice (13,16,22). Due to the high frequency, of anaerobic organism as recorded in literature, an empirical trial of metronidazole is warranted by clinical suspicion, in cases not responding to the recommended regimen (8,22).

This study confirms that one must be careful in interpreting the result of nasal swabs because of the difficulty in differentiating pathogens from commensals. The establishment of Ear, Nose, Throat Disease and Laboratory Centers with full compliment and facilities

for reliable diagnosis and treatment of chronic sinusitis is recommended.

ACKNOWLEDGEMENT

We gratefully acknowledge the input of Prof. L.D. Edungbola who reviewed the manuscript.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY, 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2007/0/0/2324

CONTROL OF HEPATITIS B INFECTION

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Hepatitis B infection remains a public health problem word-wide because of its high endemicity in most countries of the world and the presence of the virus in many body fluids/secretions, which is responsible for several alterative modes of transmissions. Also, the fact that most of the hepatitis B virus (HBV) infections occur in infancy or early childhood where over 70% of the infections is asymptomatic and nearly over half of the primary infection results in chronic infection/ carrier is another reason. Most of the HBV related chronic liver diseases are associated with the chronic infection, which manifestations occur in adulthood. This article examined the various measures that can be used in the control of the disease taking into consideration the epidemiological factors responsible for the occurrence and distribution of the disease.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem world wide. About 2 billion people have serological evidence of infection with HBV (1), and it is estimated that 350 million of them have chronic HBV infection, while about a million die each year from chronic liver diseases, including cirrhosis and cancer of the liver (2). Man is the only reservoir of the infection, and infected person can have both short-term and long-term outcomes. When infected, a person can have either symptomatic disease (acute hepatitis B) or symptomatic infection with no signs or symptoms of the disease (2,3). In either case, the individual may either recover from the infection and develop life long immunity or develop a chronic infection that usually last throughout life. In persons with acute hepatitis B the incubation period after becoming infected is usually 3-4 months, with a range of 6 weeks to 6 months. About 1-2% of those with acute infection die from fulminating hepatitis (2), while about 5% will become chronic carriers (3). Most of the disease burden associated with HBV infection is related to the chronic condition of the disease (2).

THE EPIDEMIOLOGY OF HEPATITIS B INFECTION

Although most HBV related chronic liver disease manifest themselves in adulthood, the infection usually begins in infancy or early childhood in hyper endemic areas. Several factors including

initial age of infection, immunization status of the host and viral factors are known to affect the natural course of the disease (4). The age at which infection occurs is an important determinant factor of the outcome of the disease (2,4,5). Only about 10% of infections occurring among children under the age of 5 years are symptomatic, while 80-90% of infants infected during the first year of life and 30-50% of children infected between 1-4 years of age develop the chronic condition (5,6). However, 30-50% of adults are symptomatic when first infected but only 2-5% become chronically infected (2).

About 45% of the world's population live in areas where chronic HBV infection is highly endemic (i.e. 8% or more of the population are HBsAg positive), 43% are in areas of intermediate endemicity (2-7% are HBsAg-postive) and 12% live in areas of low endemicity (<2% are HBsAg positive) (2). The infection is transmitted by either skin puncture or mucous membrane contact with infected blood or other body fluids. The virus is found in most body fluids / secretions such as blood, wound secretion, vaginal fluid, semen, saliva and urine in varying concentrations. The highest concentration of the virus occurs in blood and wound secretions. Moderate concentrations of HBV are found in semen and vaginal fluid and lower concentration in saliva and urine (7). The presence of the virus in several body fluid / secretion justify many alternative modes of spread of the disease like perinatal transmission, spread through close personal contact, unsafe injection or use of sharps, sexual contact and blood transfusion.

Perinatal transmission from mothers infected with HBV (i.e. positive for hepatitis B Surface Antigen HBsAg) to their newborn infants is a major source of HBV infection in many countries (4,6) and this usually occurs at the time of birth. Transmission in utero is very low, less than 2% (2) and there is no evidence to support claims of transmission through breast milk (8). Any mother who is a carrier for HBV (i.e. HBsAg positive) can transmit the infection to the newborn; however, the risk of perinatal transmission is higher if such mother has hepatitis B e antigen (HBeAg) in her blood. The risk of transmission is in the range of 70-90% in mothers who are HBeAg positive and about 5-20% among those who are HBeAg negative (7).

The spread from child to child account for most HBV infection occurring in household settings (9,10), but may also occur in child day care centers and pre-nursery

school (11). The most probable mechanisms of child-to-child transmission involve contact of skin sores, breaks in skin or mucous membranes with blood or skin sore secretions (7). The disease may also spread because of contact with saliva through bites or other breaks in the skin (12,13). Also, the virus may spread from inanimate objects such as shared towels or tooth brushes and other formites since the virus can survive for at least 7 days out side the body and can be found in high titres on objects, even in the absence of visible blood (14).

Transmission associated with unsafe injection practices is a major mode of transmission of the infection in adults in many countries (15,16), while transmission through blood transfusion is a major source of HBV infection in countries where blood is not screened for HBsAg especially in developing countries (2). Sexual transmission of HBV accounts for a high proportion of new hepatitis B infection among adolescents and adults in countries with low and intermediate endemicity of chronic HBV infection (17). In countries where the infection is highly endemic, sexual transmission does not account for a significant percentage of cases because most people are already infected during childhood. In these high endemic areas the lifetime risk of HBV infection is more than 60% and most infection are acquired from perinatal and child-to-child transmission (2), when the risk of the developing chronic infection is greatest. The implication here is that the prevalence of acute hepatitis B infection in these areas would be very low since most perinatal and early childhood infections are asymptomatic. However, rate of liver cirrhosis and cancer in adults would be very high. In areas of intermediate endemicity, the lifetime risk of infection is 20-60%, and infections occur in all age groups. Whereas, in low endemicity, the lifetime risk of infection is less than 20%, but most of the HBV infections occur in adults in relatively defined risk groups (2).

CONTROL OF HEPATITIS B INFECTION

Most of the burden associated with HBV infection is related to the chronic condition of the disease resulting from carrier state, and the highest carrier rate occurs in children. These children are not only at risk themselves for the long term complications of HBV but also constitute an important reservoir and source of hepatitis B infection within the community at large. Therefore they constitute important target group that must be focused in any HBV control

programme. The following levels of disease prevention and control measures will be useful in reducing the public health problem associated with the disease.

1. **Health promotion** through health education is an important strategy in the control of HBV infection (particularly in children). The general public, especially the parents, and the health workers should be targeted in order to create awareness on the disease and solicit necessary support and cooperation required for intervention. The key message of the health education should highlight the mode of transmission, people at risk of infection, the burden of the disease on individual and the nation and the importance of safe injection and blood transfusion practices and use of sterile Sharps. This would go a long way to help parents understand the disease and take measure to protect their children against HBV infection (2).

The role of health education in the control of this disease is evident by the result of a study conducted in Poland where hepatitis B prevention through health education programme was evaluated. The study showed that health education has impact in the reduction of the incidence of HBV infection (18).

2. **Specific protection** of children and other at risk groups through vaccination is the most useful strategy. Vaccination against HBV has been well documented to be cost-effective in the prevention and control of the disease (2,19,20). Routine infant hepatitis B vaccination should be a high priority for all countries regardless of the degree of endemicity of the disease, while vaccination for other groups at risk of infection will depend on the epidemiological patterns, economic factors, cultural and sexual practices of each country (2). Infant immunization prevents chronic infections acquired during early childhood, thus preventing the serious consequences of HBV infection. Strategies targeting adolescents and adults risk groups have failed to control HBV adequately because of the difficulty of immunizing people in many risk groups before they initiate high risk behaviours and also due to the fact that infections can occur among people with no identified high risk factor (2). Therefore, routine immunization of infants is most desirable in the control of the disease.

The World Health Organization (WHO) has recommended that hepatitis B vaccine be included in routine immunization schedules for all children in all countries of the world (21), and many countries

have complied by incorporating, through policy and or practice, the vaccine in to their routine immunization programmes (2, 22-24). However, an acceptable universal coverage is yet to be achieved (2,24,25). Hepatitis B vaccines are available in monovalent formulation that protect only against hepatitis B and in combination formulations that protect against hepatitis B and other diseases (e.g. DPT-Hep B, DPT-Hep B+Hib, Hib-Hep B). The movovalent hepatitis B vaccine has an advantage over the combination vaccines because it can be used for birth dose unlike the combination vaccines that have other components that cannot be administered at birth.

In pre-exposure immunization, a course of 3 doses of hepatitis B vaccine induces protective levels of antibody to HBsAg (Anti-HBs) in over 95% of healthy infants and children when given in a variety of schedules (26,27). Children who respond to the vaccine are protected against acute hepatitis B and chronic infection. Studies have shown that those who respond to a 3-dose hepatitis B immunization series are protected for as long as 15 years (28). Long-time protection relies on immunological memory that allows a protective anamnestic antibody response after exposure to HBV (29). Therefore, booster doses are not recommended (2, 30). Post-exposure immunization, beginning at birth with either hepatitis B vaccine alone or combine with hepatitis B immune globuline (HBIG) can prevent the spread of more than 90% of HBV infections from mother to baby. However, the efficacy of giving recombinant hepatitis B vaccine alone is similar to that of giving hepatitis B vaccine with HBIG. (28). Hence, the use of HBIG may not be necessary. Optimum efficacy in preventing perinatal HBV infections in newborn can be achieved when the vaccine is given within 24hours after birth /exposure. There is no evidence of protection against perinatal transmission if the first dose of vaccine is given more than 7 days after birth (2). Administration of HBIG confers protection to individual accidentally exposed following needle stick injuries or sexual partners of acute cases.

3. Early diagnosis/case detection: Prenatal screening of all pregnant women for HBsAg and HBeAg is useful in identifying mothers at risk of transmitting the infection to the newborn. This requires a lot of resources to screen these women and track infants of ected mothers in order to ensure completion of the vaccine series, especially in developing countries where some pregnant women do not go for antenatal care or where many

deliveries are conducted by unskilled person or take place out side the health facility. Prenatal screening should therefore be used to supplement routine childhood immunization (2). Every country should also adopt policy of safe blood transfusion by ensuring that all blood are screened for HBsAg before transfusion. All blood bank services should be strengthened in this area.

4. Treatment of acute hepatitis B infection is mainly supportive. Since chronic infection accounts for the burden associated with the disease, treatment of chronic hepatitis B infection using interferon or antiviral analogues would be an important control measure. Many studies have reported some degree of safety of the interferon when use in the treatment of chronic condition. The effectiveness, however, is in the range of 33-43% when use in the treatment of chronic hepatitis infection in children (31-33). However, when you consider the large number of infected people worldwide, vaccination remained the most cost-effective option in the disease (2,31).

5. Surveillance system in HBV infection involves an exercise of continuous scrutiny over the occurrence and spread of the disease and related consequences with such accuracy and completeness to provide basis for effective control. It involves systematic data collection and consolidation, analysis and evaluation in order to discern long term trends and epidemiological patterns resulting from the control measures put in place. Reporting of acute hepatitis B infection can be integrated into the existing notification and surveillance system for easy data generation since children do not usually show symptoms on acquiring the infection, but tend to develop chronic infection, data on occurrence of chronic liver diseases (cirrhosis and liver cancer) must also be collected to be able to estimate the magnitude and burden of the disease over time. However, the interpretation of the data generated must take into consideration other aetiological factors that can lead to chronic liver diseases (2).

ACKNOWLEDGEMENT

I am grateful to Dr. G. O. Oyeyinka for his useful comments and contribution during the preparation of this manuscript.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1596-689 Vol 4 No 2
AJCEM/2002073/2325

PREVALENCE OF *TRICHOMONAS VAGINALIS* AMONG THE SEXUAL PARTNERS OF WOMEN WITH TRICHOMONIASIS IN IBADAN, NIGERIA.

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This study was conducted to determine the prevalence of *Trichomonas vaginalis* among the sexual partners of women with Trichomoniasis. While 103 female patients were found to have *T. vaginalis* using both direct wet mount microscopy and culture, only 44(42.7%) male contacts reported for screening. *Trichomonas vaginalis* was isolated from 20(45.5%) of the 44 male contact investigated, whilst there was no laboratory evidence of the agent in 13(29.6%) of the study group. Amongst the 60 control subjects, *T. vaginalis* was recovered from 7(11.7%) of them. The difference in the occurrence of *T. vaginalis* between the patients and the control group was highly statistically significant ($p<0.001$). This implies that in most cases, infestation with *T. vaginalis* in the male is silent and might represent a carrier state. Eight (40%) of the 20 men with *Trichomonas vaginalis* and 43.2% of the 44 men investigated were between 20 and 29 years and this parallels that of other sexually transmitted diseases. While only 6(30%) of the men with *T.vaginalis* were diagnosed by direct wet mount preparation, all the 20 (100%) positive cases were diagnosed by culture. This however, indicated the general superiority of culture over fresh smears but it is desirable to use both methods in the diagnosis of Trichomoniasis since they complement each other.

Key Words: Trichomonas vaginalis, Prevalence, Sexual Partners, Trichomoniasis, Ibadan.

INTRODUCTION

Trichomonas vaginalis has been incriminated as one of the common sexually transmitted genital pathogens and is found to be associated with approximately 20% of all cases of non-gonococal urenthritis (NGU) (1). Trichomoniasis is widely distributed all over the world and remains a common infection among female patients attending sexually transmitted disease clinics. However, in spite of the fact that the presence of *Trichomonas vaginalis* in the male urinary tract was discovered as early as 1883 by Kuntler, there is scanty information on it's prevalence in the male patients asymptomatic cases and self-limited nature of the infection (2). While majority of female patients harbouring this organism present with vaginal discharge, which is usually frothy, greenish-yellow and offensive, the infection in the male may give rise to no symptom or present as low-grade non-gonococcal urethritis (2). Unlike pre-pubertal gonococcal vulvo-vaginitis cases occasionally encounterd in Ibadan, Trichomoniasis among this age group is very rare (3). Dunlop and Wisdom (4) found no *T. vaginalis* even in post pubertal virgins from the study done in 1965, which confirms sexual contact as the main mode of transmission. The fact that vaginitis due to *T.vaginalis* continues to cause considerable

and prolonged distress to many women and a high incidence of relapse even after a considerable period of freedom from symptoms because of re-infection by coitus, the study of the infected male becomes a matter of considerable importance (5). Trichomoniasis has been frequently found among the sexual partners of patients with proven infection and infact, the parasite has been demonstrated in 30-40% of male sexual partners of infected women (6-11). A prevalence of 70% was observed among men who had sexual contact with infected women within the previous 48hr. and only 33% of men were infected if their last contact was 2 weeks previously (12). Several studies have suggested an increased cure rate in women following treatment of their regular sexual partner (13-16). Apart from the retrospective studies done in Ibadan by Osoba (17) and Alausa (18) in 1972 and 1974 respectively, no detailed study has been conducted in our environment to determine the prevalence of *Trichomonas vaginalis* among the male sexual partners of infected women even though these sexual contacts are being treated epidemiologically. The aim of the present study was to determine the prevalence of *Trichomonas vaginalis* among the sexual partners of women with Trichomoniasis

MATERAL AND METHODS

Study population

The study population comprised all sexual contacts of women with confirmed diagnosis of *Trichomonas vaginalis* infection who were invited for screening. These represented a highly selected group of men who had undoubtedly been exposed frequently to *Trichomonas vaginalis*. Each patient was questioned as regards his marital status, previous genito-urinary symptoms, data of last coitus and other sexual consorts (whether casual or regular). The previous antimicrobial drugs used before attending the clinic were noted. A clinical examination of the lower genito-urinary tract for signs of infection such as urethral discharge and the nature of the discharge was carried out.

Control group

Sixty male patients randomly selected among the male patients that attended Special Treatment Clinic, University Collage Hospital, Ibadan, but without symptoms during the period of study were entered into the study. This group represented a cross-section of presumable sexually active male, some of which reported because of fear of venereal disease but had no infection.

Laboratory procedures

The urethral specimens were examined for the presence of *Trichomonas vaginalis* by agitating

the cotton swab in 1ml of saline in a tube and a drop of resulting suspension transferred to a microscope slide. The preparation was covered with a cover slip and then examined at x100 and x400. A Gram-stained smear was also examined for the presence of intracellular Gram-negative diplococci, other bacteria and yeast cells. The urethral specimens were then plated on modified Thayer-Martin medium and incubated using standard method (19). Culture for *T. vaginalis* was performed using Nutrient Broth Glucose Serum medium (20). Examination for presence of *T. vaginalis* was made at 48 hours and 5 days of incubation by making a wet mount of sediment from the bottom of the Bijou bottles containing the medium and motile *T. vaginalis* were searched for.

Data analysis

Statistical analysis was performed using the student t and the chi squared tests.

RESULTS

During the period of study, 103 female patients were found to have *Trichomonas vaginalis* using both direct wet mount microscopy and culture whilst only 44 (42.7%) male contacts of the women reported for screening. While *Trichomonas vaginalis* was isolated from 20 (45.45%) of the 44 male patients investigated, there was no laboratory evidence of the agent found in 13 (29.55%) of the study group (Table1). There was evidence of urethritis in all the *T. vaginalis* positive cases as shown by the presence of increase in the number of polymorpho-nuclear leucocytes in voided urine and urethral swabs examined (>4-5/HPF). Seven (15.9%) of the 44 male patients had non-specific urethritis (NSU) with no evidence of incriminating agent. Amongst the 60 control subjects, *T.vaginalis* was recovered from 7(11.7%) of them. The difference in the occurrence of *T.vaginalis* between the patients and the control group was highly statistically significant ($p<0.001$).

Table 2 shows the age distribution of the 44 male patients investigated, 43.2% were within the age of 20 to 29 years while 11.3% were over 50 years of age. Of the 20 men with *Trichomonas vaginalis*, 8(40%) were between the age of 20 and 29 years. While 39 (37.9%) of the female partners investigated were single, 26 (25.2%) were married, 11(10.7%) separated, 18 (17.5%) divorced and 9(8.7%) widowed (Table 3). Twenty six (59.1%) of the men investigated were single, 26.7% of whom had contact with casual consorts and 73.3% had contact with regular consorts. Of the 18 (40.9%) that were married; only 16.7% had contact with their wives. Table 4

compares the positive diagnostic rates by the different method used. While 6 (30%) of the 20 *T. vaginalis* isolated were diagnosed by direct wet mount microscopy, all the 20 (100%) were isolated on culture. All the men with *Trichomonas vaginalis* were treated and requested to attend for a repeat examination after 2 weeks but seven failed to report.

Table 1: INCIDENCE OF *Trichomonas vaginalis* IN MALE PARTNERS OF INFECTED WOMEN. N=44.

Diagnosis	Number	Percentage
Trichomonas vaginalis	20	45.5
Candida albicans	3	6.8
T.V & Candida	1	2.3
NSU	7	15.9

Table 2: AGE DISTRIBUTION OF MEN INVESTIGATED n=44.

AGE (years)	Number	Percentage	No. of T.V.Positive Pts.	Percentage
10-19	4	9.1	1	5
20-29	19	43.2	8	40
30-39	8	18.2	3	15
40-49	8	18.2	6	30
> 50	5	11.3	2	10
TOTAL	**44**	**100**	**20**	**100**

Table 3: MARITAL STATUS OF FEMALE PARTNERS OF MEN INVESTIGATED

Marital Status	Number	Percentage
Single	39	37.9
Married	26	25.2
Separated	11	10.7
Divorced	18	17.5
Widowed	9	8.7
TOTAL	**103**	**100**

Table 4: COMPARISON OF POSITIVE DIAGNOSTIC RATES BY DIFFERENT METHODS.

Wet mounts Prep.		Culture
No.of Positive n=20	6	20
Percentage Positive	30	100

P<0.001

DISCUSSION

Trichomonas vaginalis has been incriminated as one of the common sexually transmitted pathogens and although appears to be highly prevalent with a widespread geographical distribution, it has not been the focus of intensive study nor of active control programs (3, 21). Most patients with *Trichomonas vaginalis* infection are asymptomatic or mildly symptomatic and hence they are likely to continue to remain sexually active in spite of infection (21,22). While it has been reasonably confirmed in our centre that *T. vaginalis* is a cause of non-gonococcal urethritis (NGU) (3), many of these patients still come for treatment because they are the sexual partners of women with symptomatic infection.

In this study, a significantly high proportion of male that are repeatedly exposed to *T.vaginalis* during sexual contact themselves harbour the agent. While 20 (45.5 %) sexual partners of women with Trichomoniasis had *T. vaginalis* isolated from their urethral swabs, 7(11.7%) of the 60 control subjects were positive. This implies that in most cases, infestation with *T.vaginalis* in the male is silent and might represent a carrier state.

Age distribution of male patients with *Trichomonas vaginalis* parallels that of other sexually transmitted diseases (3, 23-25). A large percentage (43.2 %) of the men investigated as well as 40 % of those with *T. vaginalis* were within the age range of 20-29 years. This is the period of greatest sexual activity and those in this group tend to be promiscuous and are therefore prone to sexually transmitted diseases. Trichomoniasis has been found to be prevalent among patients with higher levels of sexual activity and larger number of different sexual partners (25). Twenty-six (59.1%) of the men investigated were single, 26.7 % of whom had contact with casual consorts and 73.3 percent with regular consorts. This confirms the repeated exposure these men have to *T.vaginalis* during sexual contact. This is in contrast with the findings of the previous study where a large percentage of the men investigated had contact with casual consorts (23). Extramarital exposure to risk of infection by casual contacts among married men also plays an important part in the transmission of infection (17) which may be due to the fact that married men abstain from sexual intercourse with their wives during pregnancy and few years after delivery. In this study, 15(83.3%) of the 18 married men had contact with casual consorts whilst only 16.7% had contact with their wives. The data

on the prevalence of Trichomoniasis vary around the world, depending on the reliability of the diagnostic tests used on them and their response to medical examination. During the period of study, 103 female patients showed cultural evidence of Trichomoniasis and were given contact tracing paper for their partners but only 44 (42.7%) of the male contacts of the women came for screening. This confirmed the attitude of the general population to investigations of sexually transmitted diseases. In the less educated people, it is difficult to separate venereal diseases from genital infection of other kinds (3). To this set of people, any case of urethral discharge is gonorrhoea and genital ulcer is syphilis. In the case of Trichomoniasis that often lacks symptoms and signs, it is almost impossible to convince these people to come for screening. Some of them declined to be examined or investigated and in spite of the strict confidentiality in the clinic, many of those men invited queried the possibility of STI and repeated reassurance and explanation could not remove the lingering doubts. There are conflicting reports on the role of *T.vaginalis* in causing complications in infected male patients. Compared to other sexually transmitted diseases, Trichomoniasis is relatively devoid of late complications. However, a few cases of acute, non-gonococcal epididymitis have been attributed to Trichomonal infection (1, 26-28). In some cases, *T. vaginalis* was observed in epididymal aspirates and it has been isolated from about 10 % of some series of infertile men (1,7), but such association does not imply causality. Some workers have observed phagocytosis of spermatozoa by *T. vaginalis* (7), and others have noted that in the presence of large numbers of the agent in vitro, sperm mobility is decreased (1,11). Ogunbanjo *et al* (29), by retrospective study on infective factors of male infertility among Nigerians, found *T. vaginalis* to be one of the causative agents of conjugal infertility.

Many workers have found cultural method to be superior to other methods such as fresh wet mount examinations and staining (3,23,30). This has been confirmed by this study. While only six (30%) of men with *T. vaginalis* were diagnosed by direct wet mount preparation, all the twenty (100%) positive cases were diagnosed by culture. There is a high significant difference between the two laboratory methods. This however, indicates the general superiority of culture over fresh smears but it is desirable to use both methods in the diagnosis of Trichomoniasis since they complement each other.

ACKNOWLEDGEMENT

The author wishes to acknowledge with gratitude the support and encouragement received from Consultants, Resident Doctors, Nursing and Technical staff of the Special Treatment Clinic, University College Hospital, Ibadan during the period of this study.

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AFRICAN JOURNAL OF CLINICAL & EXPERIMENTAL MICROBIOLOGY JULY. 2003 ISSN1595-689 Vol 4 No 2
AJCEM/2002097/2326

BACTERIOLOGICAL AND PARASITOLOGICAL ASSESSMENT OF VAGINITIS IN PREGNANT WOMEN IN ISEYIN, OYO STATE, NIGERIA.

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Specimens of High Vaginal Swabs (HVS) of 135 pregnant women were examined to determine the cause of vaginitis in pregnant women in Iseyin, Oyo State, Nigeria between August and October 1999. Study subjects were selected from patient attending selected antenatal clinics in public, private and mission hospitals/clinics in Iseyin. Samples were collected from subject in lithotomy position using sterile cuscos bivalve speculum. Sample were analysed by using standard technique as described. A structured questionnaire was also administered in order to obtain vital epidemiological information necessary for the study as described. The data analysis was done using chi square test. Results shows that 45 (33.3%) were positive for *Candida spp*, 15 (11.1%) for *Gardnerella vaginalis* and 5 (3.7%) for *Trichomonas vaginalis*. Sexual activities of individual have no significant effect on prevalence of vaginitis while symptomatology was a major indicator of infection. The effect of educational attainment and religion on infection rate was discussed. Infection decreased with age of patients while infection distribution by age of pregnancy gave a confusing pattern and the factors responsible for this were discussed. Since vaginitis could be asymptomatic most time, the screening of all pregnant women with risk factors for preterm labour and premature rupture of membranes must be undertaken. Prompt treatment of cases is also recommended.

Key Words: Pregnant women, vaginitis, aetiologic agents, prevalence, Iseyin.

INTRODUCTION

Vaginitis is inflammation of the vaginal, a disease entity first described by Willinson (1). Abnormal vaginal discharge and related symptoms are frequent complaints in patients attending obsterics and gynaecology clinics and some form of vaginitis inducing organism, such as *Candida albicans* or Bacterial Vaginosis (BV) are commonly diagnosed. In acute vaginitis, the squamous epithelial lining of the vaginal wall is invaded and inflammed causing discomfort, pruritus, or pain in addition to discharge. There is no doubt that the microorganisms associated with cases of vaginitis frequently are isolated from asymptomatic women (2).

During pregnancy, the vaginal is more susceptible to infection, resulting in a higher incidence of colonization and sypmptomatic vaginitis. The clinical attack rate is increased maximally during the third trimester and symptomatic recurrence is also more common. Vaginitis has been observed in 61-90% of pregnant vaginal carriers as shown by Carrel *et al* (3).

Bacterial vaginosis (BV) is an entity receiving extensive attention

in pregnancy and needs greater focus on the part of the clinicians. It is found in 15 to 23% of pregnant women with up to 50% of patients being asymptomatic (4,5,6.). The vulnerability of pregnant women to these infections is as a result of increased levels of oestrogen during pregnancy, which creates a climate for the growth of these agents (7).

Whereas so much work has been done on vaginitis and its aetiologic agents in other parts of the world, because of the importance of the subject, little or no work has been reported in Nigeria. Therefore this study is aimed at determining the prevalence of the aetiologic agents of vaginitis among pregnant women in Iseyin, Nigeria with a view to having a data base for planning effective case management and control of the problem.

MATERIALS AND METHODS

Study area

The study was conducted in Iseyin in Iseyin Local Government Area of Oyo State between August and October 1999. Iseyin, with a population of about 79,838 (national census 1991) predominantly Yoruba, is located on the longitude 4°15E and latitude 8°N, about 98 kilometers North of Ibadan, the Oyo State capital. Their main stay of economy is farming and trading with a reputation for the production of native fabrics called 'Aso Oke'. The town is endowed with many educational and health institutions both at primary and secondary levels. It is a community of mixed religions.

Study subjects

For the purpose of the study and sample collection, the hospitals and health institutions in the study area were grouped into 3-viz mission, public and private. The hospitals from each group were then randomly selected by balloting using lottery method. One hospital from each category was thus selected. The chosen hospitals for the study were: Our lady catholic hospital (mission), central maternity centre (public) and faith foundation hospital (private) respectively. The target population was pregnant women in Iseyin and those included in the study were those with symptom of vaginitis as recommended by Thomason *et al* (8). Most of the women who presented themselves in the hospital were in the third trimester.

Sample collection

One hundred and thirty five high vaginal swab samples were collected from pregnant women attending the antenatal clinic in the three hospitals enlisted in the study. Non- pregnant women on postnatal visit were included in the

study as controls. Specimens were collected with the assistance of clinical staff, with patents in lithotomy position using sterile coscos bivalve speculum. A structured questionnaire sheet was used to obtain information such as age, educational level, trimester, religion, occupation, marital status, number of sexual partners, symptoms and other important questions about their social life.

Sample analysis

Cultures were done on modified Brain Heart Haeme agar, a multipurpose medium by using the conventional rolling method and incubated anaerobically at 37°C for 48 hours. All isolates were identified using the method described by Cruickshank *et al* (9) and characterized using the criteria of Cowan and Steel (10). Wet mount preparation for isolation of protozoa was done. The slide was prepared by suspending the discharge on the swab in a drop or two saline previously placed on the glass and examined microscopically using x10 and x40 objectives to scan at least ten fields for protozoa, *Trichomonas vaginalis* and yeast cells.

Data analysis

Data were analysed statistically using the Chi-square test to determine the significance of some variable where applicable; a p value of <0.05 was considered significant.

RESULTS

Table 1 shows the distribution of isolates from pregnant women in Iseyin. Of 135 subjects examined, 45(33.3%) were positive for *Candida albicans*, 15 (11.1%) for *Gardnerella vaginalis* and 5(3.7%) for *Trichomonas vaginalis*. Data revealed that infection rate with these agents differ significantly with age of the patients ($p<0.05$). Generally, there were mixed infections with some other bacterial agents. Table 2 shows the distribution of infective agents by number of pregnancies. Result reveals that infection decreases with number of pregnancies. There was a significant difference when rates were analysed using the chi-square test ($p<0.05$). Fig 1 shows infection rate by sexual activities. There was no significant difference in infection rate between subjects with single and those with multiple sexual partners ($p>0.05$). Also women who had intercourse during pregnancy were more infected than those who did not. However, the difference was not statistically significant ($p>0.05$). Table 3 shows infection rate by trimester. Data revealed that the age of pregnancy did not affect infection rate distribution ($p>0.05$). Table 4 shows distribution by symptomatology and actiological agents. There were higher infection rates among

women with symptoms of discharge and itching than those without any symptoms. The chi-square test revealed significant difference in infection rate between patients that presented with symptoms and the control group without any symptom ($p<0.05$). Table 5 shows distribution by marital status. Infection rates were higher among the married and polygamous than all other groups. Statistically however, the difference was not significant ($p>0.05$). The distribution of agents of vaginitis by educational level is shown in Table 6. Marginally there is a decrease in infection rate with educational level, and there was a zero percent infectivity of all the agents of vaginitis among women with tertiary education. However, the difference was not statistically significant ($p>0.05$). The result of the distribution of infective agents by occupation is presented in Table 7. There is a variation in prevalence with no definite pattern. The statistical analysis revealed that there was no significant difference in the infection occurrence rate between the occupational groups ($p>0.05$). Table 8 shows distribution of infection by religion. There was 48% infectivity among muslims and 34% infectivity among christians. The Chi-square test revealed no statistical significant difference between the two ($p>0.05$).

Table 1: Distribution of agents of vaginitis and other bacterial isolates by age.

		Isolate distribution						
Age (yrs)	Number Examined	Candida spp %	G. vaginalis	T.vaginalis	Strept. Spp.	Kleb spp	E.coli	Staph spp
≤20	30	11(36.7%)	2 (6.7%)	0 (0)	0 (0)	9(30%)	5(16.7%)	25(83.3%)
21-25	55	17(30.9%)	8(14.6)	2(3.63%)	5(9.1%)	13(23.6%)	11(20%)	43(78.2)
26-30	38	13(34.2)	13(10.5)	3(7.89%)	2(5.3%)	7(18.4%)	10(26.3%)	30(78.9%)
31-35	10	3(30.%)	1(10%)	0 (0)	0 (0)	2(20%)	3(30%)	9(90%)
36 & above	2	1(80%)	0 (0)	0 (0)	0 (0)	2(100%)	0(0%)	2(100%)
Total	135	45(33.3%)	15(11.1%)	5(3.7%)	7(5.2%)	33(24.4%)	29(21.5%)	109(80.7%)

P<0.05

Table 2: Distribution of infection by number of pregnancy

No of pregnancy	Total No examined (%)	Candida spp	G.vaginalis	T. vaginalis	Total No infected
1	45(33.3%)	15(33.3%)	4(8.9%)	1(2.2%)	20(44.4%)
2.	41(30.4%)	16(39.9%)	4(9.8%)	1(2.4%)	21(51.2%)
3.	16(11.9%)	5(31.3%)	1(6.3%)	0 (0)	6(37.5%)
≥4	15(11.1%)	3(20%)	4(26.7%)	0 (0)	7(46.7%)

P>0.05

Table 3: Distribution by Trimester

Trimester	Total No examined (%)	Candida spp	G.vaginalis	T. vaginalis	Total No infected
First	4(2.9%)	3(75%)	0 (0)	0 (0)	3(75%)
Second	29(21.5%)	6(20.7%)	3(10.3%)	1(3.4%)	10(34.5%)
Third	102(75.5%)	36(35.3%)	12(11.8%)	4(3.9%)	52(50.9%)

P>0.05

Table 4: Distribution by symptomatology and aetiologic agents

Symptoms	Total No examined = 135(%)	Candida (%)	G.vaginalis (%)	T. vaginalis (%)	Total No infected (%)
Discharge	127(94.1%)	44(34.6%)	12(9.4%)	5(3.9%)	61(48.0%)
No discharge	8(5.9%)	1(12.5%)	3(32.5%)	0(0)	4(50%)
Itching	57(42.2%)	25(43.9)	3(5.3%)	5(8.8%)	33(57.9%)
No itching	78(57.8)	20(25.6%)	12(15.4%)	0(0)	32(41.1%)
Itching+ discharge	57(42.2%)	27(47.4%)	3(5.3%)	5(88%)	35(61.4%)
Discharge + no itching	70(51.9)	18(25.7%)	10(14.3%)	0 (0)	28(40%)
Control (Neg)	8(5.9)	1(12.5%)	3(37.5%)	0 (0)	4(50%)

P>0.05

Table 5: Distribution of agents of vaginitis by marital status

Marital Status	Total No examined (%)	Candida spp (%)	G.vaginalis (%)	T. vaginalis (%)	Total No infected
Married & polygamous	49(36.3%)	20(40.8%)	7(14.3%)	0(0)	27(55%)
Married & monogamous	58(42.9%)	17(29.3%)	4(6.9%)	1(3.4%)	22(37.9%)
Single	28(20.7.5%)	8(28.6%)	4(14.3%)	0(0)	12(42.9%)

P>0.05

Table 6: Distribution of agents of vaginitis by educational level

Educational level	Total No examined (%)	Candida (%)	G.vaginalis (%)	T. vaginalis (%)	Total No infected (%)
Non-formal	37(27.4%)	12(32.4%)	6(16.2%)	2(5.4%)	20(54.1%)
Primary	54(40%)	12(38.9%)	2(3.7%)	2(3.7%)	25(46.3%)
Secondary	41(30.4%)	12(29.3%)	7(17.1%)	1(2.4%)	20(48.8%)
Tertiary	3(2.2%)	0 (0)	0 (0)	0 (0)	0 (0)

P>0.05

Table 7: Distribution of agents of vaginitis by occupation

Occupation	Total No examined (%)	Candida (%)	G.vaginalis (%)	T. vaginalis (%)	Total No infected (%)
Trader	97(71.9%)	35(36.1%)	12(12.4%)	5(5.15%)	52(53.6%)
Civil servant	5(3.7%)	1(20%)	1(20%)	0(0)	2(40%)
Farmer	5(3.7%)	1(20%)	0(0)	0(0)	1(20%)
Artisan	24(17.8%)	7(29.2%)	1(4.2%)	0 (0)	8(33.3%)
Housewife	4(2.9%)	1(25%)	2(25%)	0 (0)	2(50%)

P>0.05

Table 8: Distribution of agents of vaginitis by Religion

Religion	Total No examined	Candida spp	G.vaginalis	T. vaginalis	Total No infected
Christian	23(17.0%)	6(26.1%)	2(8.7%	0 (0)	8(34.8%)
Muslim	112(82.9%)	39(11.6%)	13(11.6%)	5(4.5%)	57(48.2%)

P>0.05

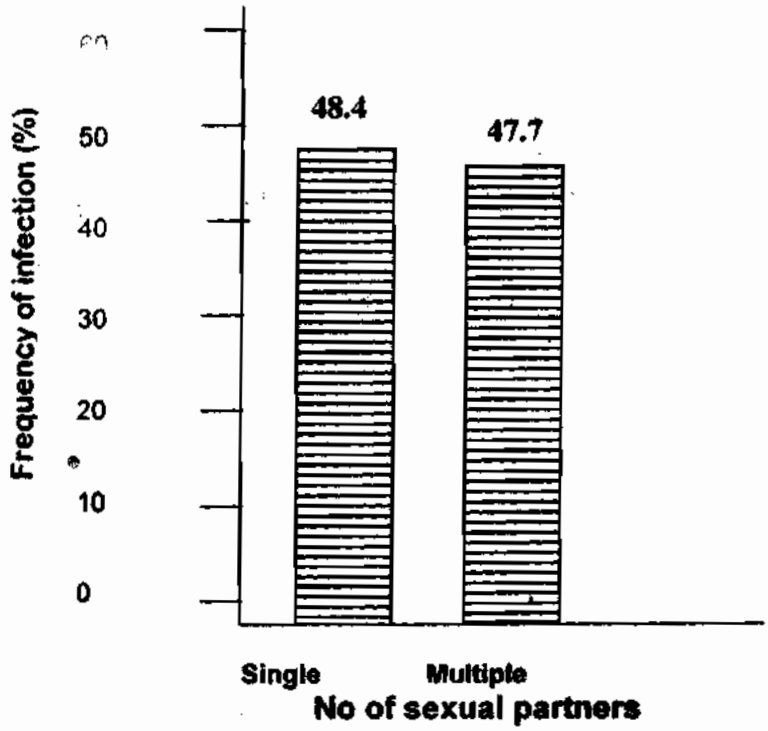

Figure 2a: Infection rate by sexual activities (Number of sexual partners)

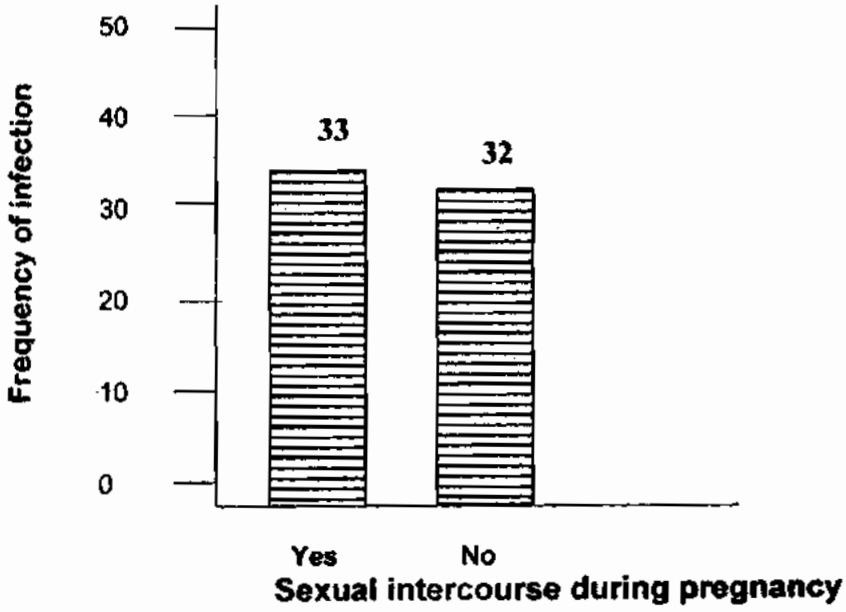

Figure 2b: Infection rate by sexual activities (intercourse during pregnancy)

DISCUSSION

The study of microorganism incriminated as aetiologic agents of vaginitis serves to emphasis this complicated interaction that may exist between these microorganisms and the host. Studies have shown that organisms associated with vaginitis are part of the host's own microflora or exogenous microorganisms that must interact with species present as part of the host's indigenous flora (2). It has been shown in this study that the main organisms incriminated with vaginitis in pregnancy are *Trichomonas vaginalis, Candida albicans and Gardnerella vaginalis.* This confirms the observation of some authors (10). *Candida albicans* with the occurrence rate of 33.3% in Iseyin has been shown to be the commonest cause of vaginitis among the pregnant women in the area, a report, which is in consonance with the 30-40% prevalence reported by Hurley (12). This study has shown that *G. vaginalis* with 11.1% is second to *Candida albicans* in prevalence. Although this figure does not fall into the range (15.2%) reported by Amsel *et al* (4), there is agreement in the position the parasitic infection occupies as the second most prevalent cause of vaginitis in pregnant women. Our report that *Trichomonas vaginalis* occurs less frequently is not controverted by other workers (13,14). Mixed infections have remained a common occurrence in the study area. This is in conformity with the report of Lossick (14) who also agree that infection with these agents differ significantly with age of the patients ($p<0.05$). Garber *et al* (15) also agree that there is an increased incidence of *Trichomonas vaginalis* in pregnancy. This study has shown that the occurrence rate of infective agents shows no definite pattern with the number of pregnancy ($p>0.05$). This may not be unconnected with the level of health education among the populace. The subjects may have learnt from experience the need for prompt treatment as symptoms of infection manifest.

Result in this study has shown that age of pregnancy has no significant relevance in the distribution of infection ($p>0.05$). This is at variance with the reports of Monton *et al* (16) and Hopsu-Havu (7), who showed that symptomatic disease is developed in about 10% of women during the first trimester and 36-55% during the third trimester. This disagreement in the report may be as a result of the attitude of the patients in our study area. Most of them usually attend clinic only during the third trimester. This has brought about the imbalance in the numbers in the respective stages of

pregnancy, which may have accounted for the inference.

It has been shown in this study that sexual activities of the subjects have no significant impact on the level of infection. There was no significant difference in the level of infection between women who had single sexual partner and those with multiple partners. This is an aberration from the belief that promiscuity and / or unfaithfulness premised on infidelity are a major epidemiological factor of disease diffusion. The fact of the case may be due in part to regular case treatment, or those partners do not operate outside the regulars who may be bacteriologically clean. It was also observed in this study that sexual intercourse during pregnancy does not necessarily promote infection, as there is no statistical difference in the infection rate between those pregnant women who claimed to engage in sexual intercourse during gestation, and those who denied. Therefore, it could be safe to assume that when sexual activity is restricted faithfully to one partner (that is zero grazing) the health of the individual is not thrown into jeopardy. However, the incidence of infection was significantly higher in women married into polygamous setting than those in monogamous homes. On the contrary the data in Table 5 revealed that there is no significant difference between married women and the single pregnant women. This revelation presents a paradoxical picture as opposed to the general belief that sexual promiscuity among partners do expose them more to risk of infection (14). Besides, these patients are at greater risk of contacting HIV. This is a subject of future investigation.

This study has shown that symptomatology and infection rates are linearly related. There were higher infection rates among subjects with vaginal itching and discharge than those without these symptoms. The factors responsible had been enunciated by many workers (15, 17) who incriminated surface protease action on mucous membrane of the vaginal in cases of Trichonomoniasis. It has been shown that the level of education of women has effect on the incidence of vaginitis in the study area. The uneducated and illiterate were mostly infected. Although the infection rate decreases with the level of education, the difference was not statistically significant ($p>0.05$). This study also showed that religious belief and occupation had no effect on the distribution of infection among the study population ($p>0.05$).

Whereas the infection could be either symptomatic or asymptomatic, many workers

(11,12,18,19) had documented the pathogenesis and sequelae of the infection. The disease could lead to premature rupture of membranes in pregnancy, pre-term labour and procure abortion. The infection may predispose the patients to HIV infection and other opportunistic infections with grave consequences. Therefore, it is recommended that pregnant women must register in the antenatal clinic within the first trimester for adequate and timely management. It is of paramount importance for the pregnant women to undergo periodic screening in order to forestall pre-term labour and premature rupture of membranes.

ACKNOWLEDGEMENT

We are greatly indebted to Dr. Ojo of Faith Foundation Hospital, Iseyin for being our linkman to other hospitals in Iseyin. We also appreciate the hospital workers for their support during the study.

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