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HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVITY IN AFRICAN PATIENTS PRESENTING TO THE EYE CLINIC - A PRELIMINARY TO PREVENTION OF OCCUPATIONAL EXPOSURE

*** Adeoti C.O, ** Ashaye A.O, * Isawumi M.A.,**

***Department of Ophthalmology, LAUTECH Teaching Hospital, Osogbo Osun state, Nigeria.**
****Department of Ophthalmology University College Hospital, Ibadan.**

Correspondence: DR. (MRS.) C. O. Adeoti, P.O.Box 979, Osogbo, Nigeria.
E-mail: caroladeoti2001@ yahoo.co.uk; Phone number: 234 8033742827

ABSTRACT

A seroprevalence study of Human immunodeficiency virus (HIV) infection in new patients attending the eye clinic of LAUTECH Teaching Hospital in Osogbo, Osun State, Nigeria showed that twenty-nine patients 2.7%) were positive to HIV1. No patient was positive to HIV 2. There were 21 males (72.4%) and 8 females (27.6%). The clinical diagnosis in the HIV positive patients was as shown in Table 1. Cataract was found in nine cases (31.03%), herpes zoster 4 (13.79%), glaucoma, optic atrophy (non-glaucomatous) and corneal abscess were responsible for 3 (10.35%) of cases each; presbyopia, bacterial conjunctivtis 2 (6.89%) while maculopathy, orbital cellulitis and adherent leucoma were found in 1 (3.45%) patient each. These findings suggest that, patients with ocular disorders and who are otherwise healthy looking may infact be HIV seropositive and as such it may be necessary to observe all rules relating to HIV transmission so as to prevent occupational exposure and cross infection in our clinics and operating theatres. Necessary measures to reduce occupational HIV infection and post exposure treatment if exposure occurs are discussed.

KEYWORDS: Nigeria, ophthalmological disorders, occupational exposure, cross infection.

INTRODUCTION

An estimated 42 million people worldwide are now infected with the human immunodeficiency virus (HIV), (1) the causative agent of the acquired immunodeficiency syndrome compared with 30million people that were infected in 1997(2). Ninety per cent (90%) of these live in developing countries.

Reports from the 14th International AIDS Conference (Barcelona, Spain) project that, in the absence of an expanded prevention effort, there will be 45 million new HIV infections by 2010. It is estimated that 29 million of these infections could be prevented with the expansion of existing prevention strategies (3). Ocular involvement in HIV infection has been reported in large numbers in industrialized countries with comparatively little report from developing countries where most affected people live. In the health care setting, exposures occur through needle sticks or cuts from other sharp instruments contaminated with an infected patient's blood (percutaneous exposures) or, less frequently, after infected blood gets into a worker's open cut or a mucous membrane such as the eyes or inside the nose.

Some people with HIV remain asymptomatic and these constitute an important source of transmission of the virus (4). HIV has been isolated from the tear fluid, conjunctiva of HIV positive but asymptomatic individuals (5). There have also been reports of health care workers who seroconverted following infected blood splash onto their mucous membranes and non-intact skin (6). The conjunctiva and cornea have also been recognized as a potential route for transmission of infection in the operating room personne (7). These findings show that there is a risk of being infected while treating an unsuspected HIV positive patient.

In a study in the Eastern part of Nigeria, 5.3% of new patients seen in the eye clinic were HIV positive (8).

This study will estimate the prevalence of HIV infection among eye patients in this area to be able to plan and provide preventive measures that will reduce cross infection within the staff and patients. It will also attempt to discuss the various measures necessary to reduce occupational HIV infection and post exposure treatment if there is exposure.

MATERIALS AND METHOD

This was a prospective study conducted in the eye clinic of LAUTECH Teaching Hospital and a private specialist clinic in Osogbo, Osun State, Nigeria. One thousand and sixty six (1066) new patients seen between July 2004 and May 2007 were included in the study. Osun state is situated in South Western Nigeria. It is bounded on the West and North West by Oyo State, on the East by Ondo State and on the South by Ogun State. Only new patients were included in the study. They were investigated using enzyme linked immuno sorbent assay (ELISA) technique after thorough pretest counseling. They were tested for both HIV 1 and 2 using immunocomb. Those who tested positive had confirmatory test using immunocomb II HIV 1 AND 2 Comb firm. Other information obtained included the age, sex, diagnosis and whether or not the patients needed surgery. Data obtained were presented in tables.

RESULTS

One thousand and sixty six (1066) new patients were studied. Males predominate with a male to female ratio of 1.2: 1. Their ages ranged between 6 months and 90 years with a mean age of 47.53 years.

Twenty-nine patients (2.7%) were positive to HIV1. No patient was positive to HIV 2. There were 21 males (72.4%) and 8 females (27.6%). The clinical diagnosis in the HIV positive patients was as shown in Table 1. Cataract was found in nine cases (31.03%), herpes zoster 4 (13.79%), glaucoma, optic atrophy (non-glaucomatous) and corneal abscess were responsible for 3 (10.35%) of cases each; presbyopia, bacterial conjunctivtis 2 (6.89%) while maculopathy, orbital cellulitis and adherent leucoma were found in 1 (3.45%) patient each.

TABLE 1: HIV Positive Cases By Diagnosis

Diagnosis	no of cases	%
Cataract	9.00	31.03
Herpes zoster	4.00	13.79
Glaucoma	3.00	10.35
Maculopathy	1.00	3.45
Non-glaucomatous		
Optic atrophy	3.00	10.35
Orbital cellulites	1.00	3.45
Corneal abscess	3.00	10.35
Adherent leucoma	1.00	3.45
Presbyopia	2.00	6.89
Bacterial conjunctivitis	2.00	6.89
Total	29.00	100.00

DISCUSSION

According to the World Health Organization, the estimated total number of HIV-positive people worldwide has increased from 39.4 million in 2004 to 40.3 million and by the end of year 2005, there were between 1.7M to 4.2M Nigerians living with HIV/AIDS.

Reports from the 14th International AIDS Conference (Barcelona, Spain) project that, in the absence of an expanded prevention effort, there will be 45 million new HIV infections by 2010. It is estimated that 29 million of these infections could be prevented with the expansion of existing prevention strategies (3).

This study shows that patients with eye disorders may have been infected with HIV as twenty nine (2.7%) out of 1066 new patients who attended the eye clinic for one thing or the other were HIV

positive. This is lower than what was obtained in the eastern part of this country (8) and a previous study in this hospital (9) possibly due to the larger population in this study (1066) compared with 76 and 241 in those studies. Several workers who studied non-ophthalmic patients had prevalence rates of 5.4% in sexually transmitted disease and antenatal clinic attendees in Oyo State (10), and 2.4% in tuberculous patients in Lagos (11).

Male to female ratio in HIV positive patients was 1.2: 1. This contrasts with Nwosu's (8) study in which all HIV positive patients were males. The ages ranged between 20 and 61 years. 13 (44.8%) patients were aged 20-40 years. This supports previous findings in Western Nigeria (10) where the highest prevalence for HIV was found between 20 –39years. The 4 patients that had herpes zoster ophthalmicus were HIV positive and they were aged 22 and 43 years. This further confirms previous reports that Herpes zoster ophthalmicus in apparently healthy young adults is a marker of HIV in Africa (12).

Nine patients (37%) were cataract surgical patients and 3 patients had glaucoma. This is in contrast to the study in the Eastern part of Nigeria[8] where all the surgical patients were HIV negative.
It is therefore necessary that ophthalmic workers must observe all the rules for preventing HIV transmission during routine patient examination and surgical operations (5, 13). As a matter of fact, it may be necessary to screen routinely all patients going for surgery for HIV.

Young adults with Herpes zoster infection must be assumed positive to HIV until proven otherwise and rules for preventing HIV transmission to staff and other patients must be observed.

The increasing number of HIV positive people who are asymptomatic in this environment also calls for high index of suscipicion and so appropriate steps need be taken to avoid cross infection in clinical practice.

PREVENTIVE STRATEGIES

All healthcare personnel should assume that blood and other body fluids (such as tears especially when contaminated with discharges or blood) from all patients are potentially infectious. They should therefore follow infection control precautions at all times. These precautions include:

1. The routine use of barriers (such as gloves and/or goggles) when anticipating contact with blood or body fluids
2. Washing hands and other skin surfaces immediately after contact with blood or body fluids, and
3. The careful handling and disposing of sharp instruments during and after use. There are safety devices which, if used properly may reduce the risk of exposure to HIV. Many percutaneous injuries are related to sharps disposal. Strategies for safer disposal, including safer design of disposal containers and placement of containers, are being developed.

Using universal precautions, along with personal protective equipment, engineering controls and other work practice controls, reduces employee exposure to blood borne pathogens. However, personal protective equipment may provide a barrier to protect skin and mucous membranes from contact with blood and other potentially infectious material, but most of them such as gloves can easily be penetrated by needles. There is therefore the need to prevent needle stick injuries. These are caused by unsafe needle devices rather than careless use by health care workers.[14] Safer needle devices that incorporate engineering controls have been shown to significantly reduce the incidence of accidental needle stick injuries and exposure to potentially fatal blood borne illnesses. The term, "safer needle device," is broad and

includes many different types of devices such as those that have a protective shield over the needle, self re-sheathing needles, blunted surgical needles) and those that do not use needles at all. The common feature of effective safer needle devices is that they reduce the risk of needle stick injuries for health care workers before, during, or after use through built-in safety features. Although all major medical device manufacturers market devices with safety features, no standard criteria exist for evaluating the safety claims of these features. Employers implementing needle stick prevention programs should evaluate the effectiveness of various devices in their specific settings. Also, the introduction of new devices must be accompanied by intensive staff education and training.

Despite precautions and safety devices, occupational exposures to HIV will continue to occur. Planning for such incidents and knowing how to treat exposed healthcare workers are paramount.

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THE SENSITIVITY OF DIAZO TEST IN THE DIAGNOSIS OF ENTERIC FEVERS

K.B. Tanyigna and J.O. Ogar

Department of Microbiology Faculty of Natural Sciences
University of Jos. P.M.B. 2084 Jos, Nigeria.

All correspondences to Dr. K.B. Tanyigna

ABSTRACT

To ascertain the sensitivity of Diazo (chemical) test in comparison to the Widal (serological) test in the diagnosis of enteric fevers, blood specimens from101 patients suspected of having enteric fevers were collected. 54.5% (55) of the patients were significantly seropositive. Fifteen urine specimens from these 55 seropositive patients were positive for Diazo tests, giving a sensitivity of about 27%. *Salmonella typhi* was the predominant serotype causing typhoid/paratyphoid fevers, followed by *S. paratypi* A; *S. paratyphi* C and *S. paratyphi* B respectively. Although Diazo test does not appear to be reliable, it could still be useful alongside with Widal agglutination test in endemic rural or urban areas where electricity and facilities are absent or inadequate for routine laboratory investigations.

INTRODUCTION

Isolation of *Salmonella typhi*, *Salmonella paratyphi* A, B, and C remains the confirmatory test for the diagnosis of typhoid fevers. This is usually after a presumptive test which is a serology test known as Widal agglutination test has been carried out and found positive. These enteric fever diseases have clinical features that include fever, chills, diarrhea, headache, anorexia, abdominal pain etc (1; 2).

In developing countries however, and in areas where the disease is endemic, such as Nigeria, a single Widal test on serum during acute illness together with compatible clinical features is used for diagnosis but usually when a baseline titer has been established for the community (3; 4).

Diagnosis of enteric fevers is being made difficult as a result of myriad of problems including indiscriminate use of antibiotics and false positive results. Different comparisons of diagnostic methods have been made in the past (5). However, this is only where laboratory facilities are available. This has brought to the fore front a need to examine other chemical methods which could be used in rural areas of many economically poor countries where electricity and laboratory facilities may be inadequate or completely absent.

The Diazo test of urine, a chemical test for typhoid illness has elucidated many claims to the level that it is the most valuable single test in the diagnosis of typhoid fever. Some authors have stated that it is 30-90% positive in typhoid cases (6; 7).

It does appear however, that there are no data to portray these claims in this part of central Nigeria. The study was therefore undertaken to provide data on the Diazo test as an alternative or otherwise to Widal test in typhoid endemic rural and even urban areas where laboratory facilities may be inadequate or absent.

MATERIALS AND METHODS

Subjects.

Urine and blood samples were collected from 101 patients clinically diagnosed as having typhoid fevers. These patients were attending Jos University Teaching Hospital (JUTH) and Plateau Hospital, both in Jos metropolis of Plateau State, Nigeria.

Preparation of Diazo Reagent

The Diazo reagent was made from two stock solutions, A and B. Solution A was composed of sulphuric acid (0.5g), concentrated hydrochloric acid (5ml) and distilled water (100ml), while solution B was composed of sodium nitrate (0.5g), and distilled water (100ml). Forty parts of Solution A was mixed with one part of Solution B to make the Diazo reagent (7).

Collection of Urine Sample

Early morning urine specimens of patients were collected using clean-dry sterile universal bottles and then appropriately labeled. Samples were immediately processed, and where this was not possible, they were preserved in the refrigerator at 4c before being processed.

The Diazo Test

Equal amount of the urine specimen was mixed with equal amount of the Diazo reagent and a few drops of 30% ammonium hydroxide was added. This was shaken with a positive result showing a red or pinkish coloration of the urine's froth. A negative test showed no colour change.

Collection of Blood Samples

Blood samples of about 3mls each was collected by venepuncture into clean plain containers and allowed to clot. The blood was then centrifuged for 5 minutes at 3000rpm. The serum was separated using Pasteur pipette. Serum not processed immediately were stored at 2-8c before being processed.

Widal Agglutination Test

Two drops of undiluted serum were placed on 2 circles of the test tile. Serum kept in the refrigerator was first brought to room temperature before used. A drop of the appropriate well shaken suspension of somatic and flagella antigens was added to each circle. The contents of each circle was then mixed with a disposable stirrer and spread over the area. The slide was gently rocked by hand for 2 minutes and observed for agglutination.

Widal Titration Test

The tube agglutination test employed the Cromatest stained bacterial suspension antigens. Ten clean dry tubes for both O and H antigens were placed in a rack and labeled 1-10. Using a pipette, 1.9ml of 0.85% saline was dispensed into tube 1 and 0.1ml of the saline into the remaining tubes. 0.1ml of the patient's serum was dispensed into the 1st t tube. This was properly mixed and 1.0ml of the mixture from the 1st tube added into the 2nd tube. The 2nd tube was mixed properly and the serial doubling dilution was continued to the 8th tube to give dilutions of the reciprocals of 20, 40, 80, 160, 320, 640, 1280, and 2560 respectively. Tube 9 contained 1.0ml of the normal saline and 2 drops of the positive control while tube 10 contained 1.0ml of the normal saline only which served as negative control. The tubes were incubated in water bath at 48-50c for 4 hours for somatic antigens and 2 hours for flagella antigens after which tubes were examined macroscopically for agglutination.

RESULTS

There were 54.5% (55) patients with significant Widal agglutination titres (i.e. O $\geq$ 160; H $\geq$ 320) out of the 101 suspected of having typhoid fevers. This was based on taking the normal baseline titres of somatic (O) and flagella (H) antigens as the reciprocals of 40 and 80 respectively (4).

Salmonella typhi was the most prevalent with 69.1% (38) positive cases while 27.3% of those with significant Widal agglutination titres were Diazo positive (Table 1).

Table 1: Significant antibody titer and positive Diazo test in relation to different Salmonella serotypes

	Widal test		**Diazo test**	
Salmonella group	No. With signt. ab. Titre	%	+ve	%
A	7	12.7	2	13.3
B	4	7.2	1	6.7
C	6	10.9	1	6.7
D	38	69.1	11	73.3
Total	**55**	**100**	**15**	**100**

DISCUSSION

The result of the present study showing 54.5% patients who have symptoms of typhoid fevers with significant titres tally with the result of Mandal (8) who reported that 46.9% cases of typhoid showed positive Widal test. Caution should however be taken in relaying on Widal test for the diagnosis because it can be non-specific and even give significant reading in healthy carriers, post clinical infections, false positives and immunization with typhoid vaccines.

Our result which showed Diazo test to be sensitive in 27.3% of Widal positive cases does not agree with the those of Manson-Bahr & Apted (6) and Cheesbrough (7) who stated that Diazo test is positive in 80% of typhoid cases within the 5th and 14th day of illness. The difference in result may however not be unrelated to the strength of the Diazo reagent used or possible occurrence of some irregularities during the running of the test. Our result however agrees with that of Boosma (9) who found Diazo test not to be helpful in a prospective study of clinical aspect of typhoid fever carried out in two rural Nigerian hospitals.

The present result and that of others especially that of Onile and Odugbemi (10), showed that *Salmonella typhi* is the dominant serotype that causes typhoid fever in Nigeria and some parts of Africa are not at variance with each other.

The paper concludes by recommending that Diazo test does not appear to be reliable but for quick diagnosis of typhoid fever, it could still be used as an adjunct to Widal agglutination test in rural areas where electricity and laboratory facilities are not available. However, since only about 27% of typhoid cases are sensitive to Diazo test, arrangement should be made immediately to transfer the specimens to locations where facilities are available for the usual confirmatory tests.

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PROTEINURIA AND OCCURRENCE OF ONCHOCERCA VOLVULUS MICROFILARIAE IN SKIN, URINE AND BLOOD OF ONCHOCERCIASIS PATIENTS AFTER IVERMECTIN TREATMENT IN ADIKPO, BENUE STATE, NIGERIA

K.B. Tanyigna[1]; J.A. Onah[2]; E.U Amuta[3]; C.O.E. Onwuliri[3]; and I.A.O. Ujah[4]

[1, 3, 4]Departments of Microbiology, Zoology and Obstetrics & Gyneacology, University of Jos, P.M.B. 2084 Jos, Nigeria.
[2]Department of Parasitology, Faculty of Vetenary Medicine, University of Agriculture, Makurdi.

Correspondence to: Dr. K.B. Tanyigna

ABSTRACT

Fifty adult patients in Adikpo, Benue State, Nigeria having an average of 50 microfilaria/skin snip (mflss) and 2 microfilaria/milliliter (mflml) in skin and urine respectively were given a single treatment of Ivermectin at a dose rate between 150 – 200mg/kg. Five of the patients also had microfilariae in their blood. Seven days after this treatment, there was a decrease of the microfilaria from pretreatment level to 17.8% and 10% in the skin and urine respectively (i.e.82% and 90% clearance). There was 100% clearance in the blood. Trace proteinuria shown in 30 (60%) patients before treatment was increased to 45 (90%) patients after treatment. This study has revealed that Ivermectin has a great efficacy in clearing microfilaria of *Onchocerca volvulus* in the skin, urine and blood of individuals. The increase in the number of patients with mild proteinuria after treatment and its implication require further investigation. It may not mean that the increase in protein content after treatment is due to Ivermectin.

INTRODUCTION

Onchocerciasis or River blindness disease is caused by the infection of filarial nematode worm *Onchocerca volvulus*. The disease affects over 80 million people culminating in causing blindness with a "lion look" and visual impairment in 1 – 2 million people in rural communities of Africa (1, 2). Other clinical manifestations include palpable onchocercal nodules (predominantly in the pelvic region, "Leopard skin", elephantiasis of the genitalia, hanging groin and hernias (2).

The disease has been reported in all parts of Nigeria (3) including Benue and Plateau States of Nigeria (4, 5, 6). Onchocerciasis has remained both a significant public health and socio-economic problem interfering with Government plans and programmes. This has especially made "Health for all by the year 2000" a dream rather than a reality (7). The importance of this disease has made it imperative to assess the efficacy of the "new drug" – Ivermectin (Mectizan) in an onchocerciasis endemic area of Adikpo in Benue State – Nigeria. Other drugs in the use before Ivermectin emergence have shown many adverse side effects beside the long periods of administration. Ivermectin, a microfilaricidal drug, on the other hand can be effective with a single treatment, it is safe and it has a synergistic effect on the other intestinal helminthes (8). Efficacy of Ivermectin may have been assessed but not in Adikpo as literature survey has revealed.

MATERIALS AND METHODS

Ivermectin (Mectizan) were administered at a dose of between 150 – 200mg/mg to 50 patients that previously tested positive for *Onchocerca volvulus* infection by skin snip method. 20 other individuals that tested negative for *Onchcerca volvulus* infection were also treated with the drug at the same dose rate. After seven days of the oral

administration of the drug, parasitological examination were carried out on the skin snip, blood (both thin and thick smear preparation of the peripheral blood) and urine as described by Anderson *et al,* (9). The bloodless skin snip was taken from the Iliac Crest region of the body using a 2mm bite Holth type Corneo-scteral punch. The protein in the urine was detected using the Albustix (AMES multiple reagent strips, Great Britain). The strips were dropped into freshly voided urine which was read immediately as described by Greene *et al,* (10).

RESULTS

The mean microfilaria in the skin snips and urine of the 50 patients were 50mf/ss and 2mf/ml respectively. The mean microfilaria after treatment with Ivermectin was 8.9mg/ss (17.8%) and 0.2mf/ml (10%) in the skin and urine respectively. This represents an average clearance of 82.2% and 90% from skin and urine respectively (Table 1). Trace proteinuria in onchocerciasis patients (n=50) showed that 30 (60%) of them were positive while 20 (40%) were negative. Those persons with no microfilariae in the skin serving as control were 20 (100%). After treatment, the proteinurial level increased to 45 (90%) in those positive with microfilariae. The control group however remained negative even after the administration of the drug with placebo (Table 2).

Table 1: Microfilariae level in skin and urine of onchocerciasis patients (n=50)

	Skin (mf/ss)	% +ve	Urine (mf/ml)	% +ve
Average No. before treatment	50	100	2.0	100
Average No. after treatment	8.9	17.8	0.2	10
Average clearance	41.1	82.2	1.8	90

Table 2: Trace proteinuria in onchocerciasis patients

	Onchocerciasis patients (n=50)		No. Onchocerciasis (i.e control) (n=20)
Before treatment	+ve (%)	-ve (%)	-ve (%)
	30 (60)	20 (40)	
After treatment	45 (90)	5 (10)	20 (100) 20 (100)

DISCUSSION

A rapid decrease of the skin microfilariae compare to the pretreatment level as noted in the present study has also been observed by other workers (11, 12). The reverse has however been the case with increase microfilariae in blood and urine after Diethyl Carbamazine (DEC) treatment (13).

As in the present work, proteinuria although transient has been recorded in a proportion of Onchocerciasis patients and also among individuals receiving anti-filaria treatment (10, 14, 15). The number of patients with mild proteinuria agrees with the finding of Anderson *et al,* (9), who also reported that most Onchocerciasis patients had mild proteinuria (i.e. 30mg protein/100ml for those who do intense physical exercise). The reason for mild proteinuria cannot easily be linked up with their occupation (farming), which is a sort of intense exercise since their counterparts in the control group showed negative proteinuria. These observations may suggest some associations between Onchocerciasis disease and proteinuria. The increase in the number of cases with mild proteinuria after treatment may also not be linked up with Ivermectin, for the same reason that treatment with the drug in the control group remained unchanged. This may need further researches for better elucidation.

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EVALUATION OF THE QUALITY OF LOCALLY MANUFACTURED ANTIMICROBIAL SUSCEPTIBILITY TESTING DISCS USED IN SOUTH EASTERN NIGERIA

Emmanuel O. Ekundayo and Olorunshola D. Omodamiro

Department of Microbiology, College Of Biological and Physical Sciences,
Michael Okpara University of Agriculture, Umudike,
MOUAU P.O Box 7, Umudike, Abia State, Nigeria.

ABSTRACT

Locally manufactured antimicrobial susceptibility discs are becoming increasingly used in both private and government hospital laboratories in Nigeria. Data on the quality of these locally manufactured antimicrobial discs are not available. In order to provide some data, we evaluated the quality of three brands of locally manufactured antimicrobial susceptibility discs in common use in southeastern Nigeria. The three brands are Brodisk, Jirehdisk and Optudisc. The performances of these brands in agar disc diffusion assay against clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were compared with that of Abtek, the imported brand. Un-interpretable zones of inhibition defined as large zones of inhibition that merged together or complete wiping out of bacterial growth at the time of reading of the plate, were common with Optudisc and Jiredisk brands. The imported brand, Abtek, did not produce any such results. While Gentamycin on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16mm against *S. aureus, P. aeruginosa* and *E. coli* respectively, the same antibiotic disc with the same stated potency on Brodisk an Jiredisk produced no zone of inhibition against the test bacteria. Amoxylin and Augmentin discs on Abtek produced zones of inhibition of 13mm and 21mm against *S. aureus* respectively. Amoxylin disc on Brodisk did not produce any zone of inhibition against the bacterial strain while Augmentin disc on Jirehdisk produced un-interpretable result. Of the three locally made brands, Brodisk is the only one that conformed to the international standard of not having more than eight discs per 90mm plate. In our opinion, Brodisk can be recommended for clinical use in Nigeria with further improvement. The outcome of this study indicates the need for improved standardization in the production of these locally manufactured susceptibility discs.

INTRODUCTION

Antimicrobial susceptibility testing results provide guidance in the choice of antimicrobial agents in patient care. They also serve as a major source of data for surveillance of drug resistance. As such the accuracy of the results is of utmost priority (1, 2). The accuracy of results of antimicrobial susceptibility testing can be affected by multiple factors which include the media, antimicrobial discs or preparations, inoculum's size, plate reading and incubation conditions (3) and the competence of the Medical Laboratory personnel (2). For the results to be reliable, there is need for careful control and standardization of the various steps and components of the testing procedures (3).

Two groups of manual method commonly used for antimicrobial susceptibility testing are the Dilution and Disc diffusion methods. The Disc diffusion testing based on the Kirby-Bauer method is the simpler method and is therefore the most widely used (4). When this method is performed with strict adherence to the standard procedures in accordance to National Committee for Clinical Laboratory Standards (NCCLS) Method, it gives reliable results and can predict clinical efficacy of the antibiotics tested (1). One of the most critical components of the Disc Diffusion method is the quality of the antimicrobial discs. The potency and the accuracy of the antimicrobial content of the discs must be ensured. Antimicrobial discs need to be manufactured within strict control limits and handle correctly within the laboratory, otherwise, they cannot meet the quality and performance standards required.

In the developed countries, it is believed that these conditions are adequately met (4). In the

developing countries, this may not usually be the case (1). In Nigeria, for instance, imported susceptibility discs were in common use. However, due to the high cost of importation, imported discs became expensive and scarce. Some clinical laboratories resorted to in-house preparation of their antibiotic discs. With this development, some private laboratories have ventured into commercial production of antimicrobial susceptibility discs. These locally manufactured discs are considerably cheaper than the imported discs. They are also more readily available. As a result, they are becoming widely used in both private and government hospitals and laboratories. There is little or no data on the quality of these locally manufactured discs. This is the major reason for our decision to evaluate the quality and performance of the three common brands of locally manufactured antimicrobial susceptibility discs used in South-eastern Nigeria in comparison with an imported brand.

This paper presents the results of our evaluation of the performance characteristics of three brands of locally manufactured susceptibility discs. We highlight the need for improvement in the standardization of the manufacture of these local brands of susceptibility discs.

MATERIALS AND METHODS.

Brands of Antibiotic discs.

Four brands of antibiotic susceptibility discs were used in this study. One imported brand, Abtek (manufactured by Abtek Biologicals Ltd, U.K) and three locally manufactured brands namely Optudisc (manufactured by Optun Laboratories, Nigeria Ltd, Aba), Brodisk (Bronila Diagnostic Systems, Enugu, Nigeria) and Jirehdisk (produced by Jireh Laboratories, Nigeria). Abtek multidiscs were purchased from C.C. Obi (Nig) Ltd, in Lagos, Brodisk from the manufacturer, Bronila Diagnostic Systems, in Enugu, Optudisc from MEDLABCOP, a Medical Laboratory supply group, in Port Harcourt, and Jirehdisk from IG Enterprises, in Aba. All discs were stored at 2-8^{0}C throughout the period of the study as recommended by the manufacturers. The study was done between August and December 2005.

I l Strains

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from Microbiology Laboratory of the Federal Medical Centre (FMC), Umuahia, Abia State, Nigeria. The bacterial strains were subsequently maintained on Nutrient Agar slants at the Department of Microbiology Laboratory, sichael Okpara University of Agriculture, Umudike. Culture media used were Nutrient Agar (International Diagnostics Groups, Plc, Bury, Lancashire, U.K), and Mueller Hinton Agar (Oxoid Limited, Basingstoke, Hampshire, England). Culture media were prepared according to the instructions of the manufacturers.

The preparation of McFarland turbidity standard (Tube 0.5) and the inocula of the bacterial strains were prepared as described by Cheesbrough (5). Disc Diffusion susceptibility test as originally described by Bauer et al (7) was carried out according to the procedures of the National Committee for Clinical Laboratory Standards (NCCLS) methods (6). Briefly, a sterilized wire loop was used to transfer 3-5 isolated colonies from a Nutrient agar plate into a bijou bottle containing about 4ml of physiological saline. The colonies were emulsified in the normal saline to obtain a homogenous suspension of the bacterial cells. The turbidity of the suspension was adjusted visually to that of 0.5 McFarland Standard by adding sterile physiological saline to the suspension. This was used as the inoculum. A sterile swab stick was dipped in the standardized inoculum in a bijou bottle; excess fluid was removed from the swab by pressing it against the

side of the bottle. The surface of a Mueller Hinton agar plate previously dried in an incubator was streaked with the swab. The plate was left on the bench for about 20-30 minutes. The antimicrobial discs were aseptically placed on the inoculated plates. Each disc was gently pressed on the agar surface using a sterilized forceps to ensure proper contact. Plates were inverted within 30 minutes of applying the discs and incubated aerobically at 35-37^{0}C for 12- 18 hours. The diameter of the zone of inhibition around each disc was measured in millimetre (mm) using a plastic transparent ruler.

RESULTS

Three brands of locally manufactured antimicrobial sensitivity discs (Optudisc, Brodisk and Jirehdisk) were evaluated for their quality by comparing their performances with that of a foreign brand, Abtek disc, in an Agar Diffusion antibiotic susceptibility testing assay. Diameter of zone of inhibition measured in millimetres (mm) was used as a parameter for evaluation of the performance of the antibiotic discs. Table 1 presents the product presentation and packaging characteristics of the brands of antibiotic sensitivity discs evaluated in the study. The performance of Abtek and Brodisk brands of antibiotic sensitivity discs against the test bacteria are presented in Table 2 and Table 3 respectively. Few antibiotics are common to both brands. The performances of discs of these antibiotics are compared in Table 4. Gentamycin discs on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16 mm against *S.aureus*. *P. aeruginosa* and *E. coli* respectively. Gentamycin discs with the same stated antibiotic concentration on Brodisk produced no zone of inhibition against the test organisms. Similarly, Amoxycillin disc on Abtek produce a mean diameter of zone of inhibition of 13mm against *S. aureus* but the same disc on Brodisk did not produce any activity. The other three antibiotics common to both brands namely Nitrofurantoin, Cloxacillin and Erythromycin produced no inhibition zone against the test organisms. The only difference being that the Abtek Nitrofurantoin disc had a potency of 300□g while the Brodisk Nitrofurantoin had a potency of 2000□g.

Table 5 presents the results of the performance of the Optudisc brand and Table 6 presents that of Jirehdisk. Most of the discs on Optudisc brand produced un-interpretable zones of inhibition against the test bacteria. The zone of inhibition was regarded as un-interpretable when it was too large and either merged together or the whole plate was wiped out before the incubation period was reached. When this occurred in at least 2 out of 3 plates tested, it was recorded as un-interpretable. This occurred most frequently with Gram Positive discs of Optudisc tested against *S. aureus*

We compared the performances of discs of the same antibiotics common to both Abtek and Optudisc. The results are presented in Table 7. Seven antibiotics are common to Abtek and Jirehdisk. Comparing the performance of discs of these antibiotics (Table 8), Augmentin disc on Abtek produced a mean diameter zone of inhibition of 21mm against *S. aureus* while the disc of the same antibiotic on Jirehdisk produced un-interpretable results. On the other hand while Gentamycin disc against Gram Positive produced a similar result against S. *aureus* for both brands, the discs of the antibiotic on the Gram Negative pack produced different results. Abtek discs produced zone of inhibition of 16 mm and 19 mm against *E.coli* and *P. aeruginosa* respectively while the Jirehdisk produced no zone of inhibition against these organisms. Ofloxacin (Tarivid) and Tetracycline discs produced similar results against *P.aeruginosa* (see Table 8). Out of the three local brands, Optudisc produced the greatest number of un-interpretable results followed by Jirehdisk. Brodisk performed closest to the Abtek indicating that it appeared to be the most standardized of the locally manufactured antibiotic sensitivity discs.

Table 1: Product Presentation and Packaging Characteristics of the Different Brands of Antibiotic Sensitivity Discs Evaluated

Brand Name	Country of Manufacture	Lot No.	Format and Characteristics of disc.	No. of discs/plate (90 mm)	Total No. of antibiotics in each brand (G+ve & G-ve)
Abtek	UK.	FC05/P FC06/P	Multidisc with centre cut out for growth control. Neatly cut discs.	8	11
Brodisk	Nigeria	None	Multidisc with solid centre, no provision for growth control. Fairly neatly cut discs.	8	11
Optudisc	Nigeria	None	Mutidisc with centre cut out for growth control. Discs are rough and unequal in size.	10	17
Jirehdisk	Nigeria	None	Mutidisc with centre cut out for growth control. Discs are rough and uneven in outline.	10	14

Table 2: Diameter of Zones of Inhibition (mm) Produced by Abtek Brand of Antibiotic Sensitivity Discs against the Test Organisms.

Antimicrobial Agents	Code	Stated Disc potency		*S.aureus*	**P. aeruginosa**	*E. coli*
		G+ve	G-ve			
Augmentin	Aug	30□g	30□g	21	0	0
Amoxicillin	Amx	25□g	25□g	13	0	0
Erythromycin	Ery	5□g	-	0	-	-
Tetracycline	Tet	10□g	30□g	0	13	0
Cloxacillin	Cxc	5□g	-	0	-	-
Gentamycin	Gen	10□g	10□g	15	19	16
Cotrimoxazole	Cot	25□g	25□g	0	0	0
Chloramphenicol	Chl	30□g	-	20	-	-
Nitrofurantoin	Nit	-	300□g	-	0	0
Nalidixic acid	Nal	-	30□g	-	8	0
Ofloxacin	Ofl	-	30□g	-	31	0

Table 3: Diameter of Zones of Inhibition (mm) produced by Brodisk Brand of Antibiotic Sensitivity Discs against the Test Organisms

Antimicrobial Agent	**Code**	**Stated Disc potency**		***S. aureus***	***P.aeruginosa***	***E. coli***
		G+ve	G-ve			
Ampicillin	AMP/PN	10mcg	25mcg	0	0	0
Cephalexine	Cx	10mcg	25mcg	0	0	0
Cefuroxime	Cxm	-	30mcg	-	0	0
Gentamycin	Gen	10mcg	10mcg	0	0	0
Ciproxin/Ciprofloxacin	Cip	5mcg	10mcg	21	33	0
Nitrofurantoin	F	-	200mcg	-	0	0
Aztreonam	AZM	30mcg	30mcg	0	19	*U (2of3)
Levofloxacin	Lev	-	10mcg	-	26	0
Amoxicillin	AM	20mcg	-	0	-	-
Cloxacilin	OB	10mcg	-	0	-	-
Erythromycin	E	15mcg	-	0	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.
- Not tested, antibiotic not on the G+ve or G-ve pack

Table 4: Comparison of the performance (Diameter of zones of Inhibition (mm) of Brodisk with Abtek discs against the test Organisms.

Antibiotics	Code		Stated disc potency				Test organisms					
			G+ve		**G-ve**		*S. aureus*		*E.coli*		P. aeruginosa	
	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek
Gentamycin	Gen/GEN	Gen	10mcg	10□g	10mcg	10□g	0	15	0	16	0	19
Nitrofurantoin	F	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Amoxycillin	AM	AMX	20mcg	25□g		25□g	0	13	-	0	-	0
Cloxacilin	OB	CXC	10mcg	5□g	-	-	0	0	-	-	-	-
Erythromycin	E	Ery	15mcg	5□g	-	-	0	0	-	-	-	-

Table 5: Diameter of Zone of Inhibition (mm) Produced by Optudisc Brand of Antibiotic sensitivity discs against the test organisms.

Antimicrobial Agent	Code	Stated Disc potency		*Diameter of zone of inhibition (mm) against Test organism*		
		G+ve	G-ve	***S. aureus***	***P.aeruginosa***	*E. coli.*
Tarivid (Ofloxacillin)	OFX	-	10mcg	-	U (2 of 3)*	13
Peflacine	PEF	-	10mcg	-	U (2 of 3)	0
Ciproflox	CPX	10mcg	10mcg	U (3 of 3)	U (2 of 3)	16
Augmentin	AU	-	30mcg	-	0	0
Gentamycin	CN	10mcg	10mcg	U (3 of 3)	U (2 of 3)	U (2 of 3)
Streptomycin	S	-	30mcg	-	U(2 of 3)	18
Ceporex	CEP	-	10mcg	-	0	0
Nalidixic Acid	NA	-	30mcg	-	8	0
Septrin (Co-trimoxazole)	SxT	-	30mcg	-	8	0
Ampicillin	PN	-	30mcg	-	0	0
Norfloxacin	NB	-	30mcg	U (3 of 3)	-	-
Lincocin	LC	-	30mcg	U (3 of 3)	-	-
Rifampin	RD	10mcg	-	U (3 of 3)	-	-
Floxapen	FLX	30mcg	-	U (3 of 3)	-	-
Erythromycin	E	30mcg	-	U (3 of 3)	-	-
Chloramphenicol	CH	20mcg	-	U (3 of 3)	-	-
Ampiclox	APX	30mcg	-	U (3 of 3)	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.
- Not tested, antibiotic not on the G+ve or G-ve pack

Table 6: Comparison of the performance (diameter of zone of inhibition (mm) of Jirehdisk discs with Abtek discs against the test organisms.

Antibiotics	**Code**		**Stated disc potency**				**Test organisms**				***p. aeruginosa***	
			G+ve		**G-ve**		**S. aureus**		**E.coli**			
	Jireh disk	Abtek	Jireh disk	Abtek	Jirehdisk	Abtek	Jirehdisk	Abtek	Jirehdisk	Abtek	Jireh disk	Abtek
Augmentin	AG	Aug	30mcg	30□g	-	30□g	U	21	-	0	-	0
Septrin (cotrimoxazole)	SXT	COT	25mcg	25□g	25mcg	25□g	0	0	0	0	0	0
Gentamycin	CN	Gen	10mcg	10□g	10mcg	10□g	17	15	0	16	0	19
Ofloxacin (Tarivid)	OFX	OFL	10mcg	-	10mcg	30□g	22	-	0	0	0	31
Erythromycin	E	Ery	5mcg	5□g	-	-	0	0	-	-	-	-
Nitrofurantoin	N	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Tetracycline	T	Tet	-	10□g	25mcg	30□g	-	0	0	0	0	13

Table 7: Comparison of the performance (Diameter of zones of Inhibition (mm) of Optudisc with Abtek discs gainst the test bacteria.

Antimicrobial Agents	Code		Stated disc potency G+ve		G-ve		Test organisms S. aureus		E.coli		P. aeruginosa	
	Optu disk	Abt ek	Optudi sc	Abtek	Optudisc	Abtek	Optu disc	Abt ek	Optu disc	Abt ek	Optudi sc	Abt ek
Augumentin	AU	Aug	-	30□g	30mcg	30□g	-	21	0	0	0	0
Gentamycin	CN	Gen	10mcg	10□g	10mcg	10□g	U	15	U	16	U	19
Tarivid (Ofloxacillin)	OFX	Ofl	-	-	10mcg	30□g	-	-	13	0	U	31
Nalidixic acid	NA	Nal	-	-	30mcg	30□g	-	-	0	0	8	0
Septrin (co-trimoxazole)	SXT	Cot	-	25□g	230mcg	30□g	-	0	0	0	8	0
Erythromycin	E	Ery	30mcg	5□g	-	-	U	0	-	-	-	-
Chloramphenicol	CH	Chl	20mcg	30□g	-	-	U	20	-	-	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.

- Not tested, antibiotic not on the G+ve or G-ve pack

DISCUSSION

The results of our evaluation of the three brands of locally manufactured antimicrobial susceptibility discs in common use in the South eastern parts of Nigeria indicate the need for further standardization of these brands of antimicrobial susceptibility discs. The Three local brands contained different types and number of antibiotics on the multidisc panel. Different manufacturers also use different codes and in some cases different concentrations for the same antibiotics. Furthermore, while one brand includes the same antibiotic on the Gram Positive pack, another includes the same antibiotic on the Gram Negative Pack. The situation reflects a state of much confusion, which makes it difficult to make a direct comparison of the performance of the different brands. The situation may further be a reflection of lack of clear policy guidelines on antibiotic usage in the country or the failure of the manufacturers to comply with such guidelines. Antimicrobial agents included for susceptibility testing should conform to a national antibiotic usage policy. Furthermore, the number of discs on a multidisc panel for 90mm Petri dish should conform to International Standard such as the recommendation of the WHO of six or not more than 8 discs per such plate. In the present study, two of the local brands had 10 discs per plate. This probably contributed to the problem of un-interpretable zones of inhibition very common to discs on these brands.

The problem of un-interpretable zone of inhibition produced by some discs on the various local brands needs further attention. In some cases, the zones of inhibition were too large and either merged together or the whole plate was wiped out before the required incubation period. This occurred in at least 2 out of 3 plates tested before it was so recorded. We want to believe that this was due to poor standardization in the preparation of the discs. The discs probably contained antibiotic concentration above the stated potency. In comparison, Abtek, the imported brand did not produce a single case of un-interpretable result. Only a case of such results was observed with

Brodisk's Aztreonam disc against *E. coli* (see Table 3).

The clinical implication of higher concentration of antibiotic beyond the required stated concentration in sensitivity discs is that misleading results might be sent to the clinicians based on the wrong results being produced by the discs. A bacterial strain may be recorded as sensitive while in actual case it is resistant. The wrong result could be used as the basis for antibiotic prescription. This will be of no benefit to the patient and can actually delay treatment with the right drug. It can further contribute to the problem of misuse of antibiotics (8) that favours the emergence of drug resistant strains of bacteria (9).

All the three locally made brands of susceptibility discs evaluated in this study manifested poor quality and performed below expected standard in comparison to the imported brand. Brodisk performed closest to the imported brand and appeared to be the most standardized. With further improvement in quality, this brand can be recommended for use in Nigeria.

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MICROBIOLOGICAL STUDIES OF BLOOD SPECIMEN FROM PRESUMPTIVELY DIAGNOSED TYPHOID FEVER PATIENTS IN ZARIA, NORTHERN NIGERIA.

ADEDARE, T.A., *IBRAHIM, Y.K.E. AND EHINMIDU, J.O.

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaira, Nigeria.

Corresponding Address : Dr. Y.K.E. Ibrahim Department of Pharmaceutics and Pharmaceutical Microbiology, ABU, Zairia, Nigeria.

ABSTRACT

Three hundred and fifteen blood samples were obtained from presumptively diagnosed typhoid patients who were referred for Widal Serological test at four diagnostic centres. The blood samples were subjected to bacteriological investigations. Salmonella and non-Salmonella organisms isolated were identified according to standard identification schemes. The Salmonella serological O - and H - antigen titre values of the patients whose blood samples were cultured, were also collated and compared with the bacteriological findings.

There was generally low correlation between the antigenic (O and H) titre value and cultural isolation of the causative organisms of typhoid fever. However, as the O-antigen titre value increased from 1:20 to 1:160, the percentage of samples in which Salmonella spp. Were isolated, rose from 5.6% to 50%. There was also significant variation in the percentage values among the four diagnostic centers (varying from 46% to 83% at O-tire value of 1:160). Beside Salmonella spp. Other organisms comprising mostly members of the Enterobacteriaceae Family, Psuedmonas spp. and Streptococcus were isolated from the blood of patients presenting high O-antigen titre values.

KEY WORDS: Typhoid Fever Diagnosis

INTRODUCTION

Typhoid fever is a debilitating systemic infection caused by *Salmonella typhi* and *paratyphi* with a contagious incidence of as much as50% (1). It is ogften fatal if allowed to progress for long, undetected and untreated. It has continued to pose serious epidemiological problems due to its high mortality and morbidity rates as well as its adverse economic effects in countries where it is endemic. Prognosis is good once the infection is diagnosed early and prompt treatment is commenced. Proper management of the infection largely depends on its early and prompt diagnosis. Many of the clinical symptoms presented in typhoid such as continuous fever, headache, malaise, bradycardia and early constipation, at the early state of the infection, closely resembles those presented by other similar feverish infections like malaria, hence other identification methods are usually employed to aid its diagnosis in patients.

Proper diagnosis and confirmation of clinical symptoms presented by typhoid suspected patients is only achieved by isolating and identifying the causative organisms coupled with serological examination of antigenic properties. Diagnostic methods currently in use are broadly classified into two: conventional methods and rapid methods. The conventional methods involve the isolation and identification of the causative organisms by culturing in non-selective, selective enrichment and differential media (cultural method), followed by serolohical confirmation. Cultural method is highly time-consuming and may constitute a problem where prompt and early institution of therapy of the

infection is urgently desired. Generally, presumptive result takes about 3-4 days while definite positive result is obtained only after 5-6 days. The serological test, which examines the patient' serum for salmonella antibodies is a rapid tool in the diagnosis of enteric fever, but can afford an indirect evidence of infection and can not differentiate between recent infection from past exposure or previous treatment with TAB (typhi, paratyphi A and B) vaccine.

The rapid methods which provide reliable and precise results within 24-27 hours, still lay emphasis on the detection of the causative organisms of typhoid. They involve the amplification of the target organisms as the case with immunomagnetic separation (IMS), bacteriophage amplification, enzyme-linked immunosorbent assay (2,3,4,), or amplification of the DNA of the causative organism, for example polymerase chain reaction, pulsed field field gel electrophoresis and hybridization (5,6,7). Most of the rapid methods especially the molecular ones require expensive materials, specialized facilities and trained personnel to be carried out, which are not readily available in developing countries like Nigeria.

In Nigeria and many other developing countries, bacteriological culturing and serological tests are the only available diagnostic methods employed in typhoid fever confirmation. In the recent times, morbidity and mortality from typhoid has been on the increase in (8). Most of the deaths that have resulted from typhoid in Nigeria have largely been attributed to incorrect diagnosis and/or improper treatment. Analysis of patient medical records in hospitals in zaria as well as responses distributed to the public in the same environment showed that Widal serological test is virtually the only diagnostic tool used to confirm clinical symptoms presented by typhoid suspected patients in Zaria and other major towns in Northern Nigeria. This is because it yields results within a few hours (9). This work report on the significance of Widal serological diagnostic test in relationship to the cultural method on blood samples from presumptively diagnosed typhoid patients in Zaria with a view of assessing the proper role of serological test in typhoid diagnosis in this environment

MATERIALS AND METHODS

Blood Sample Collection

Blood samples of patient presumptively diagnosed for typhoid fever by physicians and referred for Widal serological test, were obtained from four laboratories where Widal serological test were being performed; two hospitals with equipped microbiology laboratories and two private medical laboratories, all located within Zaria town of Kaduna State, Nigeria. The blood samples were aseptically collected into sterile bijou bottles containing sodium citrate anti-coagulant solution (10), mixed and stored in cold packs for transport to the laboratories for culturing. A total of 315 blood samples (one sample from each patient) were collected for analysis

Serological Test

Widal serological diagnosis was carried at the four collection centers by staffs of the respective laboratories. The tube agglutination method in which various dilutions of patient's serum are mixed with drops of either O or H-antigen of *Salm. Typhi, salm. Paratyphi* A, *Salm. Paratyphi* B or *Salm paratyphi* C (11) was employed in all the four laboratories. The titre values obtained in these laboratories were collected and compared with the corresponding results of the bacteriological cultures. Stained Bacterial Antigen suspensions used in this test were products of Biotec Laboratories (Suffolk, UK) and Antec Diagnostic Products, also of U.k.

Preparation of Bacteriological Culture Media

For the culturing, isolation and eventual identification of organisms from the blood samples, various bacteriological media were used. Bismuth sulfite agar, Koser citrate, MRVP broth, Peptone water, Salmonella-Shigella agar an tetrathionate broth media were Oxoid products (Oxoid Ltd, Basingstoke, England). Casein peptone soya peptone(CASO) broth and agar were obtained from Biotec Laboratories (Surrey, U. K.). Urea broth was from Difco Laboratories (Detroit, USA).

Appropriate quantities of the dehydrated media were reconstituted in freshly distilled water, distributed in desired amounts and sterilized as specified by the manufacturers. The sterilized media were stored in refrigerator until required.

Bacteriological Examination

Collected blood samples were cultured into sterile peptone water, for tetrathionate broths. Growth from these broth cultures were sub-cultured onto surfaces of selective and differential agar media of Bismuth sulfite, Deoxycholate citrate, MacConkey and Salmonella Shigella, incubated at 37^0C for 24-48 hours. Where necessary, growths were also inoculated onto other selective and diagnostic media such as Pseudocel (for Pseudomonas aeruginosa) and Kligler iron ager media. Biochemical test were carried out as recommended in some monographs and textbooks (12,13). Eventual identification of the various isolates were achieved by comparing the morphological characteristics of resulting growths (microscopic and macroscopic) and their biochemical profiles with thos stated in the individual media monograph in the Oxoid manual and literatures (1,11,14,15).

RESULTS

Bacteriological culturing of blood samples from the 315 presumptive diagnosed typhoid patients yielded organisms in 237 samples. Of these numbers, 112 of them were Salmonella organisms (Table 1). The relationship between antigen titre values and isolation of Salmonella spp and other organisms from typhoid patients is also illustrated in this table. Generally, higher proportions of organisms were isolated at the higher O-antigen titre values. For example, at O-antigen titre value of 1:20, only 6 of the 54 samples yielded organisms (i.e., 11%) compared with 107 organisms out of 108 blood samples at titre value of 1:160. Similarly, percentage of samples in which Salmonella organisms were isolated increased with increasing O antigen tire values. For example, percentage of blood sample in which salmonella organisms were isolated were 5.6%, 23.64%, 42.42% and 50% at titre values of 1:20, 1:40, 1:80 and 1:60 respectively. Analysis of the data based on the flagella (H) antigen showed similar trend with the O-antigen. Highest percentages of Salmonella organisms were obtained at H-antigen titre values of 1:80 and 1:60 (6.3% at 1:20 and 29% at 1;40, compared with 49% at 1:80 and at 1:160).

Analysis of other organisms isolated at the different 0_antigen titre values shows that organisms mostly belonging to the Enterobacteriaceae, pseudomonas and Streptococcus Families were also isolated at O-antigen titre values normally considered as indicative of typhoid infection (Table 2). Of the 35 other non-Enterobacteriaceae organisms (27 of them were sugar fermenting and 8 others non-sugar fermenting gram negative organisms), 29 were isolated at high 0-antigen titre values.

Table 3 shows that the percentage of samples in which Salmonella organisms were isolated, varied significantly from one diagnostic laboratory to another: 58% in center A (a 50-bed hospital in Samaru, Zaria) to 31.3% in center D (a private medical laboratory). He variation in the distribution of isolates among the various centers is more obvious at relatively high O-antigen titre values. For example, at O-antigen titre value of 1:160,

83.3% samples screened in Center A yielded Salmonella organisms, which dramatically dropped to 47% in center C.

Table 1: Distribution of Organisms isolated from Presumptively diagnosed Typhoid Patients According to O-antigen Titre

O-antigen titre	No of Samples screened	No of samples in which organisms were Isolated	No of samples in which Salmonella spp were Isolated
1:20	54	6	3 (5.6)*
1:40	55	29	13 (23.10
1:80	98	95	42 (42.4)
1:160	108	107	54 (50.0)
Total	315	237	112

***Figures in parenthesis represent the percentage of the isolates that were identified as* Salmonella spp.**

Table 2: Distribution of Non-Salmonella Organisms from Blood Samples of Suspected typhoid Patients According

Isolated Organisms	No of antigen 1:20	Organisms titre Values 1:40	Isolated of: 1:80	At O- 1:160
A. Gram Negative Rods				
I. **Enterobacteriaceae**				
i. Citrobacter spp	0	0	4	1
ii. Enterobacter spp	0	2	3	8
iii. Klebsiella spp	0	1	2	5
iv. Proteus spp	0	0	3	5
v. Seratia spp	0	1	1	3
vi. Shigella spp	0	2	1	2
Non-Enterobacteriaceae				
i. *Ps. aeruginosa*	0	1	3	5
ii. other Pseudomonas spp	0	0	4	2
iii. others (e.g. Acinetobacter, Aetomonas)	1	5	16	13
B. Gram Positive Bacteria				
i. Staphylococci spp	0	0	2	1
ii. Streptococci spp	0	0	6	4
TOTAL	**1**	**12**	**45**	**49**

Table 3: Distribution of Organisms Isolated from Presumptively Diagnosed Typhoid Patients According to Serological Diagnostic Centres.

Diagnostic Centres	No of Samples screened	No of *Salmonella spp* isolated	Percentage of Samples in which *Salmonella spp* were isolated at O-antigen titres of	
			1:80	1:160
A	31	18	72.7	83.3
B	34	9	25.0	56.6
C	151	54	31.0	47.0
D	99	31	55.0	50.0

DISCUSSIONS

The higher percentages of Salmonella organisms isolated at high O – and H –antigen titre values in this study is in agreement with previous findings: in a study on the usefulness of Widal test for diagnosing typhoid fever in Lebanon (16), Widal test was mostly discriminative at O-titre values of at least 1/160, having a sensitivity of 67.9%. Though there is a positive and direct relationships between increasing serum antigen titre values and probability of isolating Salmonella organisms, high serum O-antigen titre value should not be taken alone as indicative of active typhoid infections state, as organisms other than *Salmonella sp.* May be responsible for such infections as shown in this study. It has also been reported that correlation between serological findings and isolation of causative organisms of typhoid fever is often low (1); it is dependent on the stage of infection at which the sample is collected and the type of sample obtained for bacteriological culturing. For example, while *Salmonella sp.* Can be isolated from blood in 90% of cases in the first week of an infection, the probability decreases substantially to about 30-50% in the third week (1, 17). In contrast, the serological titre values continue to rise as long as the infectious organism is not cleared from the body system. Conversely, the frequency of isolation of organism in feaces increases from 40-50% in the first week to 80% in the third week. It should be realized also that O-antigen titre value may be very low (e.g 1:20) in patients infected by *Salmonella sp.* Possessing virulent (Vi) antigens which usually masks the O-antigen and prevent agglutination of such organisms by patient' serum. As observed in this study, in most clinical typhoid infections states, high O-antigen titre values are usually associated with high H-antigen titres, hence such patients are often contagious.

The isolation of non-Salmonella organisms in patient presenting relatively high O- and H-antigen titre values indicates that organisms beside *Salmonella sp.* may also be responsible for elevation of O-antigen titre values. This observation is in agreement with the findings of a similar study carried out in the Eastern part of Nigeria (8). This might be due to the fact that organisms belonging to *Enterobacteriaceae* and *Pseudomonas* Families possess similar outer membranes with Salmonella organisms (18): O-antigen is a lipopolysacchacide polypeptide complex, present in the outer membranes of most gram negative bacteria. It has also been reported that there is cross-reactivity between Salmonella O-antigen and other *Enterobacteriaceae* organisms particularly *Citrobacter, E. coli, Serratia and Enterobacter spp.* (19, 20). Cross-reactivity between O-antigens of *Pseudomonas aeruginosa* and *Salmonella* spp. had also been observed (18). The isolation of organisms which are not negative bacteria and therefore do not possess common outer membrane structures with Salmonella oranissm at high O-angiten titres may not be particularly strange as cross reactivity of O-antigen of Salmonella with *Saccharomysces cerevisiaw* has been reported (18). The presence of *Streptococcus pneumoniae* and *H. influenzae* at high Widal O-antigen titres may be due to the pathological conditions caused by these organisms (20). Even malaria infections is reported to increase Salmonella O-antigen titre values 918). The isolation of non Salmonella organisms from patients presumptively diagnosed for typhoid fever is worrisome as it has been shown that non-typhi Salmonella bacteria can occur with high incidences of morbidity and mortality rates (21).

The wide variation in the distribution of isolates among the various centers and the relatively low correlation between the antigenic titre values and cultural isolation of Salmonella organisms from the blood samples might be attributed to the non-

adherenceof of the laboratory personnel to recommended dianostic procedures. Investigation carried out during the study revealed that in some private medical laboratories, the O-antigen suspensions provided in the Widal test kits were often pooled together for use in the determination of antigen titres, as way of maximizing profit. This could lead to non-specificity in the reaction and may account for the non-isolation of salmonella organisms at the high O-antigen titre values the pooling together of O-antigen suspensions may also be responsible for the differences in the percentage of samples that yielded Salmonella sp. at high O-titre values among the hospitals and medical laboratories where the serological tests were performed.

Widal serological test should as much as possible be complemented with isolation of the causative organisms of typhoid fever. Cultural isolation, biochemical characterization and sero-typing are essential for complete identification of salmonella organisms, since no matter how well-defined a serological laboratory may be, serological procedures do not supercede bacteriological culturing.

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HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVITY IN AFRICAN PATIENTS PRESENTING TO THE EYE CLINIC - A PRELIMINARY TO PREVENTION OF OCCUPATIONAL EXPOSURE

*** Adeoti C.O, ** Ashaye A.O, * Isawumi M.A.,**

***Department of Ophthalmology, LAUTECH Teaching Hospital, Osogbo Osun state, Nigeria.**
****Department of Ophthalmology University College Hospital, Ibadan.**

Correspondence: DR. (MRS.) C. O. Adeoti, P.O.Box 979, Osogbo, Nigeria.
E-mail: caroladeoti2001@ yahoo.co.uk; Phone number: 234 8033742827

ABSTRACT

A seroprevalence study of Human immunodeficiency virus (HIV) infection in new patients attending the eye clinic of LAUTECH Teaching Hospital in Osogbo, Osun State, Nigeria showed that twenty-nine patients 2.7%) were positive to HIV1. No patient was positive to HIV 2. There were 21 males (72.4%) and 8 females (27.6%). The clinical diagnosis in the HIV positive patients was as shown in Table 1. Cataract was found in nine cases (31.03%), herpes zoster 4 (13.79%), glaucoma, optic atrophy (non-glaucomatous) and corneal abscess were responsible for 3 (10.35%) of cases each; presbyopia, bacterial conjunctivtis 2 (6.89%) while maculopathy, orbital cellulitis and adherent leucoma were found in 1 (3.45%) patient each. These findings suggest that, patients with ocular disorders and who are otherwise healthy looking may infact be HIV seropositive and as such it may be necessary to observe all rules relating to HIV transmission so as to prevent occupational exposure and cross infection in our clinics and operating theatres. Necessary measures to reduce occupational HIV infection and post exposure treatment if exposure occurs are discussed.

KEYWORDS: Nigeria, ophthalmological disorders, occupational exposure, cross infection.

INTRODUCTION

An estimated 42 million people worldwide are now infected with the human immunodeficiency virus (HIV), (1) the causative agent of the acquired immunodeficiency syndrome compared with 30million people that were infected in 1997(2). Ninety per cent (90%) of these live in developing countries.

Reports from the 14th International AIDS Conference (Barcelona, Spain) project that, in the absence of an expanded prevention effort, there will be 45 million new HIV infections by 2010. It is estimated that 29 million of these infections could be prevented with the expansion of existing prevention strategies (3). Ocular involvement in HIV infection has been reported in large numbers in industrialized countries with comparatively little report from developing countries where most affected people live. In the health care setting, exposures occur through needle sticks or cuts from other sharp instruments contaminated with an infected patient's blood (percutaneous exposures) or, less frequently, after infected blood gets into a worker's open cut or a mucous membrane such as the eyes or inside the nose.

Some people with HIV remain asymptomatic and these constitute an important source of transmission of the virus (4). HIV has been isolated from the tear fluid, conjunctiva of HIV positive but asymptomatic individuals (5). There have also been reports of health care workers who seroconverted following infected blood splash onto their mucous membranes and non-intact skin (6). The conjunctiva and cornea have also been recognized as a potential route for transmission of infection in the operating room personne (7). These findings show that there is a risk of being infected while treating an unsuspected HIV positive patient.

In a study in the Eastern part of Nigeria, 5.3% of new patients seen in the eye clinic were HIV positive (8).

This study will estimate the prevalence of HIV infection among eye patients in this area to be able to plan and provide preventive measures that will reduce cross infection within the staff and patients. It will also attempt to discuss the various measures necessary to reduce occupational HIV infection and post exposure treatment if there is exposure.

MATERIALS AND METHOD

This was a prospective study conducted in the eye clinic of LAUTECH Teaching Hospital and a private specialist clinic in Osogbo, Osun State, Nigeria. One thousand and sixty six (1066) new patients seen between July 2004 and May 2007 were included in the study. Osun state is situated in South Western Nigeria. It is bounded on the West and North West by Oyo State, on the East by Ondo State and on the South by Ogun State. Only new patients were included in the study. They were investigated using enzyme linked immuno sorbent assay (ELISA) technique after thorough pretest counseling. They were tested for both HIV 1 and 2 using immunocomb. Those who tested positive had confirmatory test using immunocomb II HIV 1 AND 2 Comb firm. Other information obtained included the age, sex, diagnosis and whether or not the patients needed surgery. Data obtained were presented in tables.

RESULTS

One thousand and sixty six (1066) new patients were studied. Males predominate with a male to female ratio of 1.2: 1. Their ages ranged between 6 months and 90 years with a mean age of 47.53 years.

Twenty-nine patients (2.7%) were positive to HIV1. No patient was positive to HIV 2. There were 21 males (72.4%) and 8 females (27.6%). The clinical diagnosis in the HIV positive patients was as shown in Table 1. Cataract was found in nine cases (31.03%), herpes zoster 4 (13.79%), glaucoma, optic atrophy (non-glaucomatous) and corneal abscess were responsible for 3 (10.35%) of cases each; presbyopia, bacterial conjunctivtis 2 (6.89%) while maculopathy, orbital cellulitis and adherent leucoma were found in 1 (3.45%) patient each.

TABLE 1: HIV Positive Cases By Diagnosis

Diagnosis	no of cases	%
Cataract	9.00	31.03
Herpes zoster	4.00	13.79
Glaucoma	3.00	10.35
Maculopathy	1.00	3.45
Non-glaucomatous		
Optic atrophy	3.00	10.35
Orbital cellulites	1.00	3.45
Corneal abscess	3.00	10.35
Adherent leucoma	1.00	3.45
Presbyopia	2.00	6.89
Bacterial conjunctivitis	2.00	6.89
Total	29.00	100.00

DISCUSSION

According to the World Health Organization, the estimated total number of HIV-positive people worldwide has increased from 39.4 million in 2004 to 40.3 million and by the end of year 2005, there were between 1.7M to 4.2M Nigerians living with HIV/AIDS.

Reports from the 14th International AIDS Conference (Barcelona, Spain) project that, in the absence of an expanded prevention effort, there will be 45 million new HIV infections by 2010. It is estimated that 29 million of these infections could be prevented with the expansion of existing prevention strategies (3).

This study shows that patients with eye disorders may have been infected with HIV as twenty nine (2.7%) out of 1066 new patients who attended the eye clinic for one thing or the other were HIV

positive. This is lower than what was obtained in the eastern part of this country (8) and a previous study in this hospital (9) possibly due to the larger population in this study (1066) compared with 76 and 241 in those studies. Several workers who studied non-ophthalmic patients had prevalence rates of 5.4% in sexually transmitted disease and antenatal clinic attendees in Oyo State (10), and 2.4% in tuberculous patients in Lagos (11).

Male to female ratio in HIV positive patients was 1.2: 1. This contrasts with Nwosu's (8) study in which all HIV positive patients were males. The ages ranged between 20 and 61 years. 13 (44.8%) patients were aged 20-40 years. This supports previous findings in Western Nigeria (10) where the highest prevalence for HIV was found between 20 –39years. The 4 patients that had herpes zoster ophthalmicus were HIV positive and they were aged 22 and 43 years. This further confirms previous reports that Herpes zoster ophthalmicus in apparently healthy young adults is a marker of HIV in Africa (12).

Nine patients (37%) were cataract surgical patients and 3 patients had glaucoma. This is in contrast to the study in the Eastern part of Nigeria[8] where all the surgical patients were HIV negative.
It is therefore necessary that ophthalmic workers must observe all the rules for preventing HIV transmission during routine patient examination and surgical operations (5, 13). As a matter of fact, it may be necessary to screen routinely all patients going for surgery for HIV.

Young adults with Herpes zoster infection must be assumed positive to HIV until proven otherwise and rules for preventing HIV transmission to staff and other patients must be observed.

The increasing number of HIV positive people who are asymptomatic in this environment also calls for high index of suscipicion and so appropriate steps need be taken to avoid cross infection in clinical practice.

PREVENTIVE STRATEGIES

All healthcare personnel should assume that blood and other body fluids (such as tears especially when contaminated with discharges or blood) from all patients are potentially infectious. They should therefore follow infection control precautions at all times. These precautions include:

1. The routine use of barriers (such as gloves and/or goggles) when anticipating contact with blood or body fluids
2. Washing hands and other skin surfaces immediately after contact with blood or body fluids, and
3. The careful handling and disposing of sharp instruments during and after use. There are safety devices which, if used properly may reduce the risk of exposure to HIV. Many percutaneous injuries are related to sharps disposal. Strategies for safer disposal, including safer design of disposal containers and placement of containers, are being developed.

Using universal precautions, along with personal protective equipment, engineering controls and other work practice controls, reduces employee exposure to blood borne pathogens. However, personal protective equipment may provide a barrier to protect skin and mucous membranes from contact with blood and other potentially infectious material, but most of them such as gloves can easily be penetrated by needles. There is therefore the need to prevent needle stick injuries. These are caused by unsafe needle devices rather than careless use by health care workers.[14] Safer needle devices that incorporate engineering controls have been shown to significantly reduce the incidence of accidental needle stick injuries and exposure to potentially fatal blood borne illnesses. The term, "safer needle device," is broad and

includes many different types of devices such as those that have a protective shield over the needle, self re-sheathing needles, blunted surgical needles) and those that do not use needles at all. The common feature of effective safer needle devices is that they reduce the risk of needle stick injuries for health care workers before, during, or after use through built-in safety features. Although all major medical device manufacturers market devices with safety features, no standard criteria exist for evaluating the safety claims of these features. Employers implementing needle stick prevention programs should evaluate the effectiveness of various devices in their specific settings. Also, the introduction of new devices must be accompanied by intensive staff education and training.

Despite precautions and safety devices, occupational exposures to HIV will continue to occur. Planning for such incidents and knowing how to treat exposed healthcare workers are paramount.

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THE SENSITIVITY OF DIAZO TEST IN THE DIAGNOSIS OF ENTERIC FEVERS

K.B. Tanyigna and J.O. Ogar

Department of Microbiology Faculty of Natural Sciences
University of Jos. P.M.B. 2084 Jos, Nigeria.

All correspondences to Dr. K.B. Tanyigna

ABSTRACT

To ascertain the sensitivity of Diazo (chemical) test in comparison to the Widal (serological) test in the diagnosis of enteric fevers, blood specimens from101 patients suspected of having enteric fevers were collected. 54.5% (55) of the patients were significantly seropositive. Fifteen urine specimens from these 55 seropositive patients were positive for Diazo tests, giving a sensitivity of about 27%. *Salmonella typhi* was the predominant serotype causing typhoid/paratyphoid fevers, followed by *S. paratypi* A; *S. paratyphi* C and *S. paratyphi* B respectively. Although Diazo test does not appear to be reliable, it could still be useful alongside with Widal agglutination test in endemic rural or urban areas where electricity and facilities are absent or inadequate for routine laboratory investigations.

INTRODUCTION

Isolation of *Salmonella typhi*, *Salmonella paratyphi* A, B, and C remains the confirmatory test for the diagnosis of typhoid fevers. This is usually after a presumptive test which is a serology test known as Widal agglutination test has been carried out and found positive. These enteric fever diseases have clinical features that include fever, chills, diarrhea, headache, anorexia, abdominal pain etc (1; 2).

In developing countries however, and in areas where the disease is endemic, such as Nigeria, a single Widal test on serum during acute illness together with compatible clinical features is used for diagnosis but usually when a baseline titer has been established for the community (3; 4).

Diagnosis of enteric fevers is being made difficult as a result of myriad of problems including indiscriminate use of antibiotics and false positive results. Different comparisons of diagnostic methods have been made in the past (5). However, this is only where laboratory facilities are available. This has brought to the fore front a need to examine other chemical methods which could be used in rural areas of many economically poor countries where electricity and laboratory facilities may be inadequate or completely absent.

The Diazo test of urine, a chemical test for typhoid illness has elucidated many claims to the level that it is the most valuable single test in the diagnosis of typhoid fever. Some authors have stated that it is 30-90% positive in typhoid cases (6; 7).

It does appear however, that there are no data to portray these claims in this part of central Nigeria. The study was therefore undertaken to provide data on the Diazo test as an alternative or otherwise to Widal test in typhoid endemic rural and even urban areas where laboratory facilities may be inadequate or absent.

MATERIALS AND METHODS

Subjects.

Urine and blood samples were collected from 101 patients clinically diagnosed as having typhoid fevers. These patients were attending Jos University Teaching Hospital (JUTH) and Plateau Hospital, both in Jos metropolis of Plateau State, Nigeria.

Preparation of Diazo Reagent

The Diazo reagent was made from two stock solutions, A and B. Solution A was composed of sulphuric acid (0.5g), concentrated hydrochloric acid (5ml) and distilled water (100ml), while solution B was composed of sodium nitrate (0.5g), and distilled water (100ml). Forty parts of Solution A was mixed with one part of Solution B to make the Diazo reagent (7).

Collection of Urine Sample

Early morning urine specimens of patients were collected using clean-dry sterile universal bottles and then appropriately labeled. Samples were immediately processed, and where this was not possible, they were preserved in the refrigerator at 4c before being processed.

The Diazo Test

Equal amount of the urine specimen was mixed with equal amount of the Diazo reagent and a few drops of 30% ammonium hydroxide was added. This was shaken with a positive result showing a red or pinkish coloration of the urine's froth. A negative test showed no colour change.

Collection of Blood Samples

Blood samples of about 3mls each was collected by venepuncture into clean plain containers and allowed to clot. The blood was then centrifuged for 5 minutes at 3000rpm. The serum was separated using Pasteur pipette. Serum not processed immediately were stored at 2-8c before being processed.

Widal Agglutination Test

Two drops of undiluted serum were placed on 2 circles of the test tile. Serum kept in the refrigerator was first brought to room temperature before used. A drop of the appropriate well shaken suspension of somatic and flagella antigens was added to each circle. The contents of each circle was then mixed with a disposable stirrer and spread over the area. The slide was gently rocked by hand for 2 minutes and observed for agglutination.

Widal Titration Test

The tube agglutination test employed the Cromatest stained bacterial suspension antigens. Ten clean dry tubes for both O and H antigens were placed in a rack and labeled 1-10. Using a pipette, 1.9ml of 0.85% saline was dispensed into tube 1 and 0.1ml of the saline into the remaining tubes. 0.1ml of the patient's serum was dispensed into the 1st t tube. This was properly mixed and 1.0ml of the mixture from the 1st tube added into the 2nd tube. The 2nd tube was mixed properly and the serial doubling dilution was continued to the 8th tube to give dilutions of the reciprocals of 20, 40, 80, 160, 320, 640, 1280, and 2560 respectively. Tube 9 contained 1.0ml of the normal saline and 2 drops of the positive control while tube 10 contained 1.0ml of the normal saline only which served as negative control. The tubes were incubated in water bath at 48-50c for 4 hours for somatic antigens and 2 hours for flagella antigens after which tubes were examined macroscopically for agglutination.

RESULTS

There were 54.5% (55) patients with significant Widal agglutination titres (i.e. O $\geq$ 160; H $\geq$ 320) out of the 101 suspected of having typhoid fevers. This was based on taking the normal baseline titres of somatic (O) and flagella (H) antigens as the reciprocals of 40 and 80 respectively (4).

Salmonella typhi was the most prevalent with 69.1% (38) positive cases while 27.3% of those with significant Widal agglutination titres were Diazo positive (Table 1).

Table 1: Significant antibody titer and positive Diazo test in relation to different Salmonella serotypes

	Widal test		**Diazo test**	
Salmonella group	No. With signt. ab. Titre	%	+ve	%
A	7	12.7	2	13.3
B	4	7.2	1	6.7
C	6	10.9	1	6.7
D	38	69.1	11	73.3
Total	**55**	**100**	**15**	**100**

DISCUSSION

The result of the present study showing 54.5% patients who have symptoms of typhoid fevers with significant titres tally with the result of Mandal (8) who reported that 46.9% cases of typhoid showed positive Widal test. Caution should however be taken in relaying on Widal test for the diagnosis because it can be non-specific and even give significant reading in healthy carriers, post clinical infections, false positives and immunization with typhoid vaccines.

Our result which showed Diazo test to be sensitive in 27.3% of Widal positive cases does not agree with the those of Manson-Bahr & Apted (6) and Cheesbrough (7) who stated that Diazo test is positive in 80% of typhoid cases within the 5th and 14th day of illness. The difference in result may however not be unrelated to the strength of the Diazo reagent used or possible occurrence of some irregularities during the running of the test. Our result however agrees with that of Boosma (9) who found Diazo test not to be helpful in a prospective study of clinical aspect of typhoid fever carried out in two rural Nigerian hospitals.

The present result and that of others especially that of Onile and Odugbemi (10), showed that *Salmonella typhi* is the dominant serotype that causes typhoid fever in Nigeria and some parts of Africa are not at variance with each other.

The paper concludes by recommending that Diazo test does not appear to be reliable but for quick diagnosis of typhoid fever, it could still be used as an adjunct to Widal agglutination test in rural areas where electricity and laboratory facilities are not available. However, since only about 27% of typhoid cases are sensitive to Diazo test, arrangement should be made immediately to transfer the specimens to locations where facilities are available for the usual confirmatory tests.

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PROTEINURIA AND OCCURRENCE OF ONCHOCERCA VOLVULUS MICROFILARIAE IN SKIN, URINE AND BLOOD OF ONCHOCERCIASIS PATIENTS AFTER IVERMECTIN TREATMENT IN ADIKPO, BENUE STATE, NIGERIA

K.B. Tanyigna[1]; J.A. Onah[2]; E.U Amuta[3]; C.O.E. Onwuliri[3]; and I.A.O. Ujah[4]

[1, 3, 4]Departments of Microbiology, Zoology and Obstetrics & Gyneacology, University of Jos, P.M.B. 2084 Jos, Nigeria.
[2]Department of Parasitology, Faculty of Vetenary Medicine, University of Agriculture, Makurdi.

Correspondence to: Dr. K.B. Tanyigna

ABSTRACT

Fifty adult patients in Adikpo, Benue State, Nigeria having an average of 50 microfilaria/skin snip (mflss) and 2 microfilaria/milliliter (mflml) in skin and urine respectively were given a single treatment of Ivermectin at a dose rate between 150 – 200mg/kg. Five of the patients also had microfilariae in their blood. Seven days after this treatment, there was a decrease of the microfilaria from pretreatment level to 17.8% and 10% in the skin and urine respectively (i.e.82% and 90% clearance). There was 100% clearance in the blood. Trace proteinuria shown in 30 (60%) patients before treatment was increased to 45 (90%) patients after treatment. This study has revealed that Ivermectin has a great efficacy in clearing microfilaria of *Onchocerca volvulus* in the skin, urine and blood of individuals. The increase in the number of patients with mild proteinuria after treatment and its implication require further investigation. It may not mean that the increase in protein content after treatment is due to Ivermectin.

INTRODUCTION

Onchocerciasis or River blindness disease is caused by the infection of filarial nematode worm *Onchocerca volvulus*. The disease affects over 80 million people culminating in causing blindness with a "lion look" and visual impairment in 1 – 2 million people in rural communities of Africa (1, 2). Other clinical manifestations include palpable onchocercal nodules (predominantly in the pelvic region, "Leopard skin", elephantiasis of the genitalia, hanging groin and hernias (2).

The disease has been reported in all parts of Nigeria (3) including Benue and Plateau States of Nigeria (4, 5, 6). Onchocerciasis has remained both a significant public health and socio-economic problem interfering with Government plans and programmes. This has especially made "Health for all by the year 2000" a dream rather than a reality (7). The importance of this disease has made it imperative to assess the efficacy of the "new drug" – Ivermectin (Mectizan) in an onchocerciasis endemic area of Adikpo in Benue State – Nigeria. Other drugs in the use before Ivermectin emergence have shown many adverse side effects beside the long periods of administration. Ivermectin, a microfilaricidal drug, on the other hand can be effective with a single treatment, it is safe and it has a synergistic effect on the other intestinal helminthes (8). Efficacy of Ivermectin may have been assessed but not in Adikpo as literature survey has revealed.

MATERIALS AND METHODS

Ivermectin (Mectizan) were administered at a dose of between 150 – 200mg/mg to 50 patients that previously tested positive for *Onchocerca volvulus* infection by skin snip method. 20 other individuals that tested negative for *Onchcerca volvulus* infection were also treated with the drug at the same dose rate. After seven days of the oral

administration of the drug, parasitological examination were carried out on the skin snip, blood (both thin and thick smear preparation of the peripheral blood) and urine as described by Anderson *et al,* (9). The bloodless skin snip was taken from the Iliac Crest region of the body using a 2mm bite Holth type Corneo-scteral punch. The protein in the urine was detected using the Albustix (AMES multiple reagent strips, Great Britain). The strips were dropped into freshly voided urine which was read immediately as described by Greene *et al,* (10).

RESULTS

The mean microfilaria in the skin snips and urine of the 50 patients were 50mf/ss and 2mf/ml respectively. The mean microfilaria after treatment with Ivermectin was 8.9mg/ss (17.8%) and 0.2mf/ml (10%) in the skin and urine respectively. This represents an average clearance of 82.2% and 90% from skin and urine respectively (Table 1). Trace proteinuria in onchocerciasis patients (n=50) showed that 30 (60%) of them were positive while 20 (40%) were negative. Those persons with no microfilariae in the skin serving as control were 20 (100%). After treatment, the proteinurial level increased to 45 (90%) in those positive with microfilariae. The control group however remained negative even after the administration of the drug with placebo (Table 2).

Table 1: Microfilariae level in skin and urine of onchocerciasis patients (n=50)

	Skin (mf/ss)	% +ve	Urine (mf/ml)	% +ve
Average No. before treatment	50	100	2.0	100
Average No. after treatment	8.9	17.8	0.2	10
Average clearance	41.1	82.2	1.8	90

Table 2: Trace proteinuria in onchocerciasis patients

	Onchocerciasis patients (n=50)		No. Onchocerciasis (i.e control) (n=20)
Before treatment	+ve (%)	-ve (%)	-ve (%)
	30 (60)	20 (40)	
After treatment	45 (90)	5 (10)	20 (100) 20 (100)

DISCUSSION

A rapid decrease of the skin microfilariae compare to the pretreatment level as noted in the present study has also been observed by other workers (11, 12). The reverse has however been the case with increase microfilariae in blood and urine after Diethyl Carbamazine (DEC) treatment (13).

As in the present work, proteinuria although transient has been recorded in a proportion of Onchocerciasis patients and also among individuals receiving anti-filaria treatment (10, 14, 15). The number of patients with mild proteinuria agrees with the finding of Anderson *et al,* (9), who also reported that most Onchocerciasis patients had mild proteinuria (i.e. 30mg protein/100ml for those who do intense physical exercise). The reason for mild proteinuria cannot easily be linked up with their occupation (farming), which is a sort of intense exercise since their counterparts in the control group showed negative proteinuria. These observations may suggest some associations between Onchocerciasis disease and proteinuria. The increase in the number of cases with mild proteinuria after treatment may also not be linked up with Ivermectin, for the same reason that treatment with the drug in the control group remained unchanged. This may need further researches for better elucidation.

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EVALUATION OF THE QUALITY OF LOCALLY MANUFACTURED ANTIMICROBIAL SUSCEPTIBILITY TESTING DISCS USED IN SOUTH EASTERN NIGERIA

Emmanuel O. Ekundayo and Olorunshola D. Omodamiro

Department of Microbiology, College Of Biological and Physical Sciences,
Michael Okpara University of Agriculture, Umudike,
MOUAU P.O Box 7, Umudike, Abia State, Nigeria.

ABSTRACT

Locally manufactured antimicrobial susceptibility discs are becoming increasingly used in both private and government hospital laboratories in Nigeria. Data on the quality of these locally manufactured antimicrobial discs are not available. In order to provide some data, we evaluated the quality of three brands of locally manufactured antimicrobial susceptibility discs in common use in southeastern Nigeria. The three brands are Brodisk, Jirehdisk and Optudisc. The performances of these brands in agar disc diffusion assay against clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were compared with that of Abtek, the imported brand. Un-interpretable zones of inhibition defined as large zones of inhibition that merged together or complete wiping out of bacterial growth at the time of reading of the plate, were common with Optudisc and Jiredisk brands. The imported brand, Abtek, did not produce any such results. While Gentamycin on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16mm against *S. aureus, P. aeruginosa* and *E. coli* respectively, the same antibiotic disc with the same stated potency on Brodisk an Jiredisk produced no zone of inhibition against the test bacteria. Amoxylin and Augmentin discs on Abtek produced zones of inhibition of 13mm and 21mm against *S. aureus* respectively. Amoxylin disc on Brodisk did not produce any zone of inhibition against the bacterial strain while Augmentin disc on Jirehdisk produced un-interpretable result. Of the three locally made brands, Brodisk is the only one that conformed to the international standard of not having more than eight discs per 90mm plate. In our opinion, Brodisk can be recommended for clinical use in Nigeria with further improvement. The outcome of this study indicates the need for improved standardization in the production of these locally manufactured susceptibility discs.

INTRODUCTION

Antimicrobial susceptibility testing results provide guidance in the choice of antimicrobial agents in patient care. They also serve as a major source of data for surveillance of drug resistance. As such the accuracy of the results is of utmost priority (1, 2). The accuracy of results of antimicrobial susceptibility testing can be affected by multiple factors which include the media, antimicrobial discs or preparations, inoculum's size, plate reading and incubation conditions (3) and the competence of the Medical Laboratory personnel (2). For the results to be reliable, there is need for careful control and standardization of the various steps and components of the testing procedures (3).

Two groups of manual method commonly used for antimicrobial susceptibility testing are the Dilution and Disc diffusion methods. The Disc diffusion testing based on the Kirby-Bauer method is the simpler method and is therefore the most widely used (4). When this method is performed with strict adherence to the standard procedures in accordance to National Committee for Clinical Laboratory Standards (NCCLS) Method, it gives reliable results and can predict clinical efficacy of the antibiotics tested (1). One of the most critical components of the Disc Diffusion method is the quality of the antimicrobial discs. The potency and the accuracy of the antimicrobial content of the discs must be ensured. Antimicrobial discs need to be manufactured within strict control limits and handle correctly within the laboratory, otherwise, they cannot meet the quality and performance standards required.

In the developed countries, it is believed that these conditions are adequately met (4). In the

developing countries, this may not usually be the case (1). In Nigeria, for instance, imported susceptibility discs were in common use. However, due to the high cost of importation, imported discs became expensive and scarce. Some clinical laboratories resorted to in-house preparation of their antibiotic discs. With this development, some private laboratories have ventured into commercial production of antimicrobial susceptibility discs. These locally manufactured discs are considerably cheaper than the imported discs. They are also more readily available. As a result, they are becoming widely used in both private and government hospitals and laboratories. There is little or no data on the quality of these locally manufactured discs. This is the major reason for our decision to evaluate the quality and performance of the three common brands of locally manufactured antimicrobial susceptibility discs used in South-eastern Nigeria in comparison with an imported brand.

This paper presents the results of our evaluation of the performance characteristics of three brands of locally manufactured susceptibility discs. We highlight the need for improvement in the standardization of the manufacture of these local brands of susceptibility discs.

MATERIALS AND METHODS.

Brands of Antibiotic discs.

Four brands of antibiotic susceptibility discs were used in this study. One imported brand, Abtek (manufactured by Abtek Biologicals Ltd, U.K) and three locally manufactured brands namely Optudisc (manufactured by Optun Laboratories, Nigeria Ltd, Aba), Brodisk (Bronila Diagnostic Systems, Enugu, Nigeria) and Jirehdisk (produced by Jireh Laboratories, Nigeria). Abtek multidiscs were purchased from C.C. Obi (Nig) Ltd, in Lagos, Brodisk from the manufacturer, Bronila Diagnostic Systems, in Enugu, Optudisc from MEDLABCOP, a Medical Laboratory supply group, in Port Harcourt, and Jirehdisk from IG Enterprises, in Aba. All discs were stored at 2-8^{0}C throughout the period of the study as recommended by the manufacturers. The study was done between August and December 2005.

I l Strains

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from Microbiology Laboratory of the Federal Medical Centre (FMC), Umuahia, Abia State, Nigeria. The bacterial strains were subsequently maintained on Nutrient Agar slants at the Department of Microbiology Laboratory, sichael Okpara University of Agriculture, Umudike. Culture media used were Nutrient Agar (International Diagnostics Groups, Plc, Bury, Lancashire, U.K), and Mueller Hinton Agar (Oxoid Limited, Basingstoke, Hampshire, England). Culture media were prepared according to the instructions of the manufacturers.

The preparation of McFarland turbidity standard (Tube 0.5) and the inocula of the bacterial strains were prepared as described by Cheesbrough (5). Disc Diffusion susceptibility test as originally described by Bauer et al (7) was carried out according to the procedures of the National Committee for Clinical Laboratory Standards (NCCLS) methods (6). Briefly, a sterilized wire loop was used to transfer 3-5 isolated colonies from a Nutrient agar plate into a bijou bottle containing about 4ml of physiological saline. The colonies were emulsified in the normal saline to obtain a homogenous suspension of the bacterial cells. The turbidity of the suspension was adjusted visually to that of 0.5 McFarland Standard by adding sterile physiological saline to the suspension. This was used as the inoculum. A sterile swab stick was dipped in the standardized inoculum in a bijou bottle; excess fluid was removed from the swab by pressing it against the

side of the bottle. The surface of a Mueller Hinton agar plate previously dried in an incubator was streaked with the swab. The plate was left on the bench for about 20-30 minutes. The antimicrobial discs were aseptically placed on the inoculated plates. Each disc was gently pressed on the agar surface using a sterilized forceps to ensure proper contact. Plates were inverted within 30 minutes of applying the discs and incubated aerobically at 35-37^{0}C for 12- 18 hours. The diameter of the zone of inhibition around each disc was measured in millimetre (mm) using a plastic transparent ruler.

RESULTS

Three brands of locally manufactured antimicrobial sensitivity discs (Optudisc, Brodisk and Jirehdisk) were evaluated for their quality by comparing their performances with that of a foreign brand, Abtek disc, in an Agar Diffusion antibiotic susceptibility testing assay. Diameter of zone of inhibition measured in millimetres (mm) was used as a parameter for evaluation of the performance of the antibiotic discs. Table 1 presents the product presentation and packaging characteristics of the brands of antibiotic sensitivity discs evaluated in the study. The performance of Abtek and Brodisk brands of antibiotic sensitivity discs against the test bacteria are presented in Table 2 and Table 3 respectively. Few antibiotics are common to both brands. The performances of discs of these antibiotics are compared in Table 4. Gentamycin discs on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16 mm against *S.aureus*. *P. aeruginosa* and *E. coli* respectively. Gentamycin discs with the same stated antibiotic concentration on Brodisk produced no zone of inhibition against the test organisms. Similarly, Amoxycillin disc on Abtek produce a mean diameter of zone of inhibition of 13mm against *S. aureus* but the same disc on Brodisk did not produce any activity. The other three antibiotics common to both brands namely Nitrofurantoin, Cloxacillin and Erythromycin produced no inhibition zone against the test organisms. The only difference being that the Abtek Nitrofurantoin disc had a potency of 300□g while the Brodisk Nitrofurantoin had a potency of 2000□g.

Table 5 presents the results of the performance of the Optudisc brand and Table 6 presents that of Jirehdisk. Most of the discs on Optudisc brand produced un-interpretable zones of inhibition against the test bacteria. The zone of inhibition was regarded as un-interpretable when it was too large and either merged together or the whole plate was wiped out before the incubation period was reached. When this occurred in at least 2 out of 3 plates tested, it was recorded as un-interpretable. This occurred most frequently with Gram Positive discs of Optudisc tested against *S. aureus*

We compared the performances of discs of the same antibiotics common to both Abtek and Optudisc. The results are presented in Table 7. Seven antibiotics are common to Abtek and Jirehdisk. Comparing the performance of discs of these antibiotics (Table 8), Augmentin disc on Abtek produced a mean diameter zone of inhibition of 21mm against *S. aureus* while the disc of the same antibiotic on Jirehdisk produced un-interpretable results. On the other hand while Gentamycin disc against Gram Positive produced a similar result against S. *aureus* for both brands, the discs of the antibiotic on the Gram Negative pack produced different results. Abtek discs produced zone of inhibition of 16 mm and 19 mm against *E.coli* and *P. aeruginosa* respectively while the Jirehdisk produced no zone of inhibition against these organisms. Ofloxacin (Tarivid) and Tetracycline discs produced similar results against *P.aeruginosa* (see Table 8). Out of the three local brands, Optudisc produced the greatest number of un-interpretable results followed by Jirehdisk. Brodisk performed closest to the Abtek indicating that it appeared to be the most standardized of the locally manufactured antibiotic sensitivity discs.

Table 1: Product Presentation and Packaging Characteristics of the Different Brands of Antibiotic Sensitivity Discs Evaluated

Brand Name	Country of Manufacture	Lot No.	Format and Characteristics of disc.	No. of discs/plate (90 mm)	Total No. of antibiotics in each brand (G+ve & G-ve)
Abtek	UK.	FC05/P FC06/P	Multidisc with centre cut out for growth control. Neatly cut discs.	8	11
Brodisk	Nigeria	None	Multidisc with solid centre, no provision for growth control. Fairly neatly cut discs.	8	11
Optudisc	Nigeria	None	Mutidisc with centre cut out for growth control. Discs are rough and unequal in size.	10	17
Jirehdisk	Nigeria	None	Mutidisc with centre cut out for growth control. Discs are rough and uneven in outline.	10	14

Table 2: Diameter of Zones of Inhibition (mm) Produced by Abtek Brand of Antibiotic Sensitivity Discs against the Test Organisms.

Antimicrobial Agents	Code	Stated Disc potency		*S.aureus*	**P. aeruginosa**	*E. coli*
		G+ve	G-ve			
Augmentin	Aug	30□g	30□g	21	0	0
Amoxicillin	Amx	25□g	25□g	13	0	0
Erythromycin	Ery	5□g	-	0	-	-
Tetracycline	Tet	10□g	30□g	0	13	0
Cloxacillin	Cxc	5□g	-	0	-	-
Gentamycin	Gen	10□g	10□g	15	19	16
Cotrimoxazole	Cot	25□g	25□g	0	0	0
Chloramphenicol	Chl	30□g	-	20	-	-
Nitrofurantoin	Nit	-	300□g	-	0	0
Nalidixic acid	Nal	-	30□g	-	8	0
Ofloxacin	Ofl	-	30□g	-	31	0

Table 3: Diameter of Zones of Inhibition (mm) produced by Brodisk Brand of Antibiotic Sensitivity Discs against the Test Organisms

Antimicrobial Agent	**Code**	**Stated Disc potency**		***S. aureus***	***P.aeruginosa***	***E. coli***
		G+ve	G-ve			
Ampicillin	AMP/PN	10mcg	25mcg	0	0	0
Cephalexine	Cx	10mcg	25mcg	0	0	0
Cefuroxime	Cxm	-	30mcg	-	0	0
Gentamycin	Gen	10mcg	10mcg	0	0	0
Ciproxin/Ciprofloxacin	Cip	5mcg	10mcg	21	33	0
Nitrofurantoin	F	-	200mcg	-	0	0
Aztreonam	AZM	30mcg	30mcg	0	19	*U (2of3)
Levofloxacin	Lev	-	10mcg	-	26	0
Amoxicillin	AM	20mcg	-	0	-	-
Cloxacilin	OB	10mcg	-	0	-	-
Erythromycin	E	15mcg	-	0	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.
- Not tested, antibiotic not on the G+ve or G-ve pack

Table 4: Comparison of the performance (Diameter of zones of Inhibition (mm) of Brodisk with Abtek discs against the test Organisms.

Antibiotics	Code		Stated disc potency				Test organisms					
			G+ve		**G-ve**		*S. aureus*		*E.coli*		P. aeruginosa	
	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek
Gentamycin	Gen/GEN	Gen	10mcg	10□g	10mcg	10□g	0	15	0	16	0	19
Nitrofurantoin	F	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Amoxycillin	AM	AMX	20mcg	25□g		25□g	0	13	-	0	-	0
Cloxacilin	OB	CXC	10mcg	5□g	-	-	0	0	-	-	-	-
Erythromycin	E	Ery	15mcg	5□g	-	-	0	0	-	-	-	-

Table 5: Diameter of Zone of Inhibition (mm) Produced by Optudisc Brand of Antibiotic sensitivity discs against the test organisms.

Antimicrobial Agent	Code	Stated Disc potency		*Diameter of zone of inhibition (mm) against Test organism*		
		G+ve	G-ve	***S. aureus***	***P.aeruginosa***	*E. coli.*
Tarivid (Ofloxacillin)	OFX	-	10mcg	-	U (2 of 3)*	13
Peflacine	PEF	-	10mcg	-	U (2 of 3)	0
Ciproflox	CPX	10mcg	10mcg	U (3 of 3)	U (2 of 3)	16
Augmentin	AU	-	30mcg	-	0	0
Gentamycin	CN	10mcg	10mcg	U (3 of 3)	U (2 of 3)	U (2 of 3)
Streptomycin	S	-	30mcg	-	U(2 of 3)	18
Ceporex	CEP	-	10mcg	-	0	0
Nalidixic Acid	NA	-	30mcg	-	8	0
Septrin (Co-trimoxazole)	SxT	-	30mcg	-	8	0
Ampicillin	PN	-	30mcg	-	0	0
Norfloxacin	NB	-	30mcg	U (3 of 3)	-	-
Lincocin	LC	-	30mcg	U (3 of 3)	-	-
Rifampin	RD	10mcg	-	U (3 of 3)	-	-
Floxapen	FLX	30mcg	-	U (3 of 3)	-	-
Erythromycin	E	30mcg	-	U (3 of 3)	-	-
Chloramphenicol	CH	20mcg	-	U (3 of 3)	-	-
Ampiclox	APX	30mcg	-	U (3 of 3)	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.
- Not tested, antibiotic not on the G+ve or G-ve pack

Table 6: Comparison of the performance (diameter of zone of inhibition (mm) of Jirehdisk discs with Abtek discs against the test organisms.

Antibiotics	**Code**		**Stated disc potency**				**Test organisms**				***p. aeruginosa***	
			G+ve		**G-ve**		**S. aureus**		**E.coli**			
	Jireh disk	Abtek	Jireh disk	Abtek	Jirehdisk	Abtek	Jirehdisk	Abtek	Jirehdisk	Abtek	Jireh disk	Abtek
Augmentin	AG	Aug	30mcg	30□g	-	30□g	U	21	-	0	-	0
Septrin (cotrimoxazole)	SXT	COT	25mcg	25□g	25mcg	25□g	0	0	0	0	0	0
Gentamycin	CN	Gen	10mcg	10□g	10mcg	10□g	17	15	0	16	0	19
Ofloxacin (Tarivid)	OFX	OFL	10mcg	-	10mcg	30□g	22	-	0	0	0	31
Erythromycin	E	Ery	5mcg	5□g	-	-	0	0	-	-	-	-
Nitrofurantoin	N	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Tetracycline	T	Tet	-	10□g	25mcg	30□g	-	0	0	0	0	13

Table 7: Comparison of the performance (Diameter of zones of Inhibition (mm) of Optudisc with Abtek discs gainst the test bacteria.

Antimicrobial Agents	Code		Stated disc potency G+ve		G-ve		Test organisms S. aureus		E.coli		P. aeruginosa	
	Optu disk	Abt ek	Optudi sc	Abtek	Optudisc	Abtek	Optu disc	Abt ek	Optu disc	Abt ek	Optudi sc	Abt ek
Augumentin	AU	Aug	-	30□g	30mcg	30□g	-	21	0	0	0	0
Gentamycin	CN	Gen	10mcg	10□g	10mcg	10□g	U	15	U	16	U	19
Tarivid (Ofloxacillin)	OFX	Ofl	-	-	10mcg	30□g	-	-	13	0	U	31
Nalidixic acid	NA	Nal	-	-	30mcg	30□g	-	-	0	0	8	0
Septrin (co-trimoxazole)	SXT	Cot	-	25□g	230mcg	30□g	-	0	0	0	8	0
Erythromycin	E	Ery	30mcg	5□g	-	-	U	0	-	-	-	-
Chloramphenicol	CH	Chl	20mcg	30□g	-	-	U	20	-	-	-	-

***U** - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.

- Not tested, antibiotic not on the G+ve or G-ve pack

DISCUSSION

The results of our evaluation of the three brands of locally manufactured antimicrobial susceptibility discs in common use in the South eastern parts of Nigeria indicate the need for further standardization of these brands of antimicrobial susceptibility discs. The Three local brands contained different types and number of antibiotics on the multidisc panel. Different manufacturers also use different codes and in some cases different concentrations for the same antibiotics. Furthermore, while one brand includes the same antibiotic on the Gram Positive pack, another includes the same antibiotic on the Gram Negative Pack. The situation reflects a state of much confusion, which makes it difficult to make a direct comparison of the performance of the different brands. The situation may further be a reflection of lack of clear policy guidelines on antibiotic usage in the country or the failure of the manufacturers to comply with such guidelines. Antimicrobial agents included for susceptibility testing should conform to a national antibiotic usage policy. Furthermore, the number of discs on a multidisc panel for 90mm Petri dish should conform to International Standard such as the recommendation of the WHO of six or not more than 8 discs per such plate. In the present study, two of the local brands had 10 discs per plate. This probably contributed to the problem of un-interpretable zones of inhibition very common to discs on these brands.

The problem of un-interpretable zone of inhibition produced by some discs on the various local brands needs further attention. In some cases, the zones of inhibition were too large and either merged together or the whole plate was wiped out before the required incubation period. This occurred in at least 2 out of 3 plates tested before it was so recorded. We want to believe that this was due to poor standardization in the preparation of the discs. The discs probably contained antibiotic concentration above the stated potency. In comparison, Abtek, the imported brand did not produce a single case of un-interpretable result. Only a case of such results was observed with

Brodisk's Aztreonam disc against *E. coli* (see Table 3).

The clinical implication of higher concentration of antibiotic beyond the required stated concentration in sensitivity discs is that misleading results might be sent to the clinicians based on the wrong results being produced by the discs. A bacterial strain may be recorded as sensitive while in actual case it is resistant. The wrong result could be used as the basis for antibiotic prescription. This will be of no benefit to the patient and can actually delay treatment with the right drug. It can further contribute to the problem of misuse of antibiotics (8) that favours the emergence of drug resistant strains of bacteria (9).

All the three locally made brands of susceptibility discs evaluated in this study manifested poor quality and performed below expected standard in comparison to the imported brand. Brodisk performed closest to the imported brand and appeared to be the most standardized. With further improvement in quality, this brand can be recommended for use in Nigeria.

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ORIGINAL ARTICLE

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MICROBIOLOGICAL STUDIES OF BLOOD SPECIMEN FROM PRESUMPTIVELY DIAGNOSED TYPHOID FEVER PATIENTS IN ZARIA, NORTHERN NIGERIA.

ADEDARE, T.A., *IBRAHIM, Y.K.E. AND EHINMIDU, J.O.

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaira, Nigeria.

Corresponding Address : Dr. Y.K.E. Ibrahim Department of Pharmaceutics and Pharmaceutical Microbiology, ABU, Zairia, Nigeria.

ABSTRACT

Three hundred and fifteen blood samples were obtained from presumptively diagnosed typhoid patients who were referred for Widal Serological test at four diagnostic centres. The blood samples were subjected to bacteriological investigations. Salmonella and non-Salmonella organisms isolated were identified according to standard identification schemes. The Salmonella serological O - and H - antigen titre values of the patients whose blood samples were cultured, were also collated and compared with the bacteriological findings.

There was generally low correlation between the antigenic (O and H) titre value and cultural isolation of the causative organisms of typhoid fever. However, as the O-antigen titre value increased from 1:20 to 1:160, the percentage of samples in which Salmonella spp. Were isolated, rose from 5.6% to 50%. There was also significant variation in the percentage values among the four diagnostic centers (varying from 46% to 83% at O-tire value of 1:160). Beside Salmonella spp. Other organisms comprising mostly members of the Enterobacteriaceae Family, Psuedmonas spp. and Streptococcus were isolated from the blood of patients presenting high O-antigen titre values.

KEY WORDS: Typhoid Fever Diagnosis

INTRODUCTION

Typhoid fever is a debilitating systemic infection caused by *Salmonella typhi* and *paratyphi* with a contagious incidence of as much as50% (1). It is ogften fatal if allowed to progress for long, undetected and untreated. It has continued to pose serious epidemiological problems due to its high mortality and morbidity rates as well as its adverse economic effects in countries where it is endemic. Prognosis is good once the infection is diagnosed early and prompt treatment is commenced. Proper management of the infection largely depends on its early and prompt diagnosis. Many of the clinical symptoms presented in typhoid such as continuous fever, headache, malaise, bradycardia and early constipation, at the early state of the infection, closely resembles those presented by other similar feverish infections like malaria, hence other identification methods are usually employed to aid its diagnosis in patients.

Proper diagnosis and confirmation of clinical symptoms presented by typhoid suspected patients is only achieved by isolating and identifying the causative organisms coupled with serological examination of antigenic properties. Diagnostic methods currently in use are broadly classified into two: conventional methods and rapid methods. The conventional methods involve the isolation and identification of the causative organisms by culturing in non-selective, selective enrichment and differential media (cultural method), followed by serolohical confirmation. Cultural method is highly time-consuming and may constitute a problem where prompt and early institution of therapy of the

infection is urgently desired. Generally, presumptive result takes about 3-4 days while definite positive result is obtained only after 5-6 days. The serological test, which examines the patient' serum for salmonella antibodies is a rapid tool in the diagnosis of enteric fever, but can afford an indirect evidence of infection and can not differentiate between recent infection from past exposure or previous treatment with TAB (typhi, paratyphi A and B) vaccine.

The rapid methods which provide reliable and precise results within 24-27 hours, still lay emphasis on the detection of the causative organisms of typhoid. They involve the amplification of the target organisms as the case with immunomagnetic separation (IMS), bacteriophage amplification, enzyme-linked immunosorbent assay (2,3,4,), or amplification of the DNA of the causative organism, for example polymerase chain reaction, pulsed field field gel electrophoresis and hybridization (5,6,7). Most of the rapid methods especially the molecular ones require expensive materials, specialized facilities and trained personnel to be carried out, which are not readily available in developing countries like Nigeria.

In Nigeria and many other developing countries, bacteriological culturing and serological tests are the only available diagnostic methods employed in typhoid fever confirmation. In the recent times, morbidity and mortality from typhoid has been on the increase in (8). Most of the deaths that have resulted from typhoid in Nigeria have largely been attributed to incorrect diagnosis and/or improper treatment. Analysis of patient medical records in hospitals in zaria as well as responses distributed to the public in the same environment showed that Widal serological test is virtually the only diagnostic tool used to confirm clinical symptoms presented by typhoid suspected patients in Zaria and other major towns in Northern Nigeria. This is because it yields results within a few hours (9). This work report on the significance of Widal serological diagnostic test in relationship to the cultural method on blood samples from presumptively diagnosed typhoid patients in Zaria with a view of assessing the proper role of serological test in typhoid diagnosis in this environment

MATERIALS AND METHODS

Blood Sample Collection

Blood samples of patient presumptively diagnosed for typhoid fever by physicians and referred for Widal serological test, were obtained from four laboratories where Widal serological test were being performed; two hospitals with equipped microbiology laboratories and two private medical laboratories, all located within Zaria town of Kaduna State, Nigeria. The blood samples were aseptically collected into sterile bijou bottles containing sodium citrate anti-coagulant solution (10), mixed and stored in cold packs for transport to the laboratories for culturing. A total of 315 blood samples (one sample from each patient) were collected for analysis

Serological Test

Widal serological diagnosis was carried at the four collection centers by staffs of the respective laboratories. The tube agglutination method in which various dilutions of patient's serum are mixed with drops of either O or H-antigen of *Salm. Typhi, salm. Paratyphi* A, *Salm. Paratyphi* B or *Salm paratyphi* C (11) was employed in all the four laboratories. The titre values obtained in these laboratories were collected and compared with the corresponding results of the bacteriological cultures. Stained Bacterial Antigen suspensions used in this test were products of Biotec Laboratories (Suffolk, UK) and Antec Diagnostic Products, also of U.k.

Preparation of Bacteriological Culture Media

For the culturing, isolation and eventual identification of organisms from the blood samples, various bacteriological media were used. Bismuth sulfite agar, Koser citrate, MRVP broth, Peptone water, Salmonella-Shigella agar an tetrathionate broth media were Oxoid products (Oxoid Ltd, Basingstoke, England). Casein peptone soya peptone(CASO) broth and agar were obtained from Biotec Laboratories (Surrey, U. K.). Urea broth was from Difco Laboratories (Detroit, USA).

Appropriate quantities of the dehydrated media were reconstituted in freshly distilled water, distributed in desired amounts and sterilized as specified by the manufacturers. The sterilized media were stored in refrigerator until required.

Bacteriological Examination

Collected blood samples were cultured into sterile peptone water, for tetrathionate broths. Growth from these broth cultures were sub-cultured onto surfaces of selective and differential agar media of Bismuth sulfite, Deoxycholate citrate, MacConkey and Salmonella Shigella, incubated at 37^0C for 24-48 hours. Where necessary, growths were also inoculated onto other selective and diagnostic media such as Pseudocel (for Pseudomonas aeruginosa) and Kligler iron ager media. Biochemical test were carried out as recommended in some monographs and textbooks (12,13). Eventual identification of the various isolates were achieved by comparing the morphological characteristics of resulting growths (microscopic and macroscopic) and their biochemical profiles with thos stated in the individual media monograph in the Oxoid manual and literatures (1,11,14,15).

RESULTS

Bacteriological culturing of blood samples from the 315 presumptive diagnosed typhoid patients yielded organisms in 237 samples. Of these numbers, 112 of them were Salmonella organisms (Table 1). The relationship between antigen titre values and isolation of Salmonella spp and other organisms from typhoid patients is also illustrated in this table. Generally, higher proportions of organisms were isolated at the higher O-antigen titre values. For example, at O-antigen titre value of 1:20, only 6 of the 54 samples yielded organisms (i.e., 11%) compared with 107 organisms out of 108 blood samples at titre value of 1:160. Similarly, percentage of samples in which Salmonella organisms were isolated increased with increasing O antigen tire values. For example, percentage of blood sample in which salmonella organisms were isolated were 5.6%, 23.64%, 42.42% and 50% at titre values of 1:20, 1:40, 1:80 and 1:60 respectively. Analysis of the data based on the flagella (H) antigen showed similar trend with the O-antigen. Highest percentages of Salmonella organisms were obtained at H-antigen titre values of 1:80 and 1:60 (6.3% at 1:20 and 29% at 1;40, compared with 49% at 1:80 and at 1:160).

Analysis of other organisms isolated at the different 0_antigen titre values shows that organisms mostly belonging to the Enterobacteriaceae, pseudomonas and Streptococcus Families were also isolated at O-antigen titre values normally considered as indicative of typhoid infection (Table 2). Of the 35 other non-Enterobacteriaceae organisms (27 of them were sugar fermenting and 8 others non-sugar fermenting gram negative organisms), 29 were isolated at high 0-antigen titre values.

Table 3 shows that the percentage of samples in which Salmonella organisms were isolated, varied significantly from one diagnostic laboratory to another: 58% in center A (a 50-bed hospital in Samaru, Zaria) to 31.3% in center D (a private medical laboratory). He variation in the distribution of isolates among the various centers is more obvious at relatively high O-antigen titre values. For example, at O-antigen titre value of 1:160,

83.3% samples screened in Center A yielded Salmonella organisms, which dramatically dropped to 47% in center C.

Table 1: Distribution of Organisms isolated from Presumptively diagnosed Typhoid Patients According to O-antigen Titre

O-antigen titre	No of Samples screened	No of samples in which organisms were Isolated	No of samples in which Salmonella spp were Isolated
1:20	54	6	3 (5.6)*
1:40	55	29	13 (23.10
1:80	98	95	42 (42.4)
1:160	108	107	54 (50.0)
Total	315	237	112

***Figures in parenthesis represent the percentage of the isolates that were identified as* Salmonella spp.**

Table 2: Distribution of Non-Salmonella Organisms from Blood Samples of Suspected typhoid Patients According

Isolated Organisms	No of antigen 1:20	Organisms titre Values 1:40	Isolated of: 1:80	At O- 1:160
A. Gram Negative Rods				
I. **Enterobacteriaceae**				
i. Citrobacter spp	0	0	4	1
ii. Enterobacter spp	0	2	3	8
iii. Klebsiella spp	0	1	2	5
iv. Proteus spp	0	0	3	5
v. Seratia spp	0	1	1	3
vi. Shigella spp	0	2	1	2
Non-Enterobacteriaceae				
i. *Ps. aeruginosa*	0	1	3	5
ii. other Pseudomonas spp	0	0	4	2
iii. others (e.g. Acinetobacter, Aetomonas)	1	5	16	13
B. Gram Positive Bacteria				
i. Staphylococci spp	0	0	2	1
ii. Streptococci spp	0	0	6	4
TOTAL	**1**	**12**	**45**	**49**

Table 3: Distribution of Organisms Isolated from Presumptively Diagnosed Typhoid Patients According to Serological Diagnostic Centres.

Diagnostic Centres	No of Samples screened	No of *Salmonella spp* isolated	Percentage of Samples in which *Salmonella spp* were isolated at O-antigen titres of	
			1:80	1:160
A	31	18	72.7	83.3
B	34	9	25.0	56.6
C	151	54	31.0	47.0
D	99	31	55.0	50.0

DISCUSSIONS

The higher percentages of Salmonella organisms isolated at high O – and H –antigen titre values in this study is in agreement with previous findings: in a study on the usefulness of Widal test for diagnosing typhoid fever in Lebanon (16), Widal test was mostly discriminative at O-titre values of at least 1/160, having a sensitivity of 67.9%. Though there is a positive and direct relationships between increasing serum antigen titre values and probability of isolating Salmonella organisms, high serum O-antigen titre value should not be taken alone as indicative of active typhoid infections state, as organisms other than *Salmonella sp.* May be responsible for such infections as shown in this study. It has also been reported that correlation between serological findings and isolation of causative organisms of typhoid fever is often low (1); it is dependent on the stage of infection at which the sample is collected and the type of sample obtained for bacteriological culturing. For example, while *Salmonella sp.* Can be isolated from blood in 90% of cases in the first week of an infection, the probability decreases substantially to about 30-50% in the third week (1, 17). In contrast, the serological titre values continue to rise as long as the infectious organism is not cleared from the body system. Conversely, the frequency of isolation of organism in feaces increases from 40-50% in the first week to 80% in the third week. It should be realized also that O-antigen titre value may be very low (e.g 1:20) in patients infected by *Salmonella sp.* Possessing virulent (Vi) antigens which usually masks the O-antigen and prevent agglutination of such organisms by patient' serum. As observed in this study, in most clinical typhoid infections states, high O-antigen titre values are usually associated with high H-antigen titres, hence such patients are often contagious.

The isolation of non-Salmonella organisms in patient presenting relatively high O- and H-antigen titre values indicates that organisms beside *Salmonella sp.* may also be responsible for elevation of O-antigen titre values. This observation is in agreement with the findings of a similar study carried out in the Eastern part of Nigeria (8). This might be due to the fact that organisms belonging to *Enterobacteriaceae* and *Pseudomonas* Families possess similar outer membranes with Salmonella organisms (18): O-antigen is a lipopolysacchacide polypeptide complex, present in the outer membranes of most gram negative bacteria. It has also been reported that there is cross-reactivity between Salmonella O-antigen and other *Enterobacteriaceae* organisms particularly *Citrobacter, E. coli, Serratia and Enterobacter spp.* (19, 20). Cross-reactivity between O-antigens of *Pseudomonas aeruginosa* and *Salmonella* spp. had also been observed (18). The isolation of organisms which are not negative bacteria and therefore do not possess common outer membrane structures with Salmonella oranissm at high O-angiten titres may not be particularly strange as cross reactivity of O-antigen of Salmonella with *Saccharomysces cerevisiaw* has been reported (18). The presence of *Streptococcus pneumoniae* and *H. influenzae* at high Widal O-antigen titres may be due to the pathological conditions caused by these organisms (20). Even malaria infections is reported to increase Salmonella O-antigen titre values 918). The isolation of non Salmonella organisms from patients presumptively diagnosed for typhoid fever is worrisome as it has been shown that non-typhi Salmonella bacteria can occur with high incidences of morbidity and mortality rates (21).

The wide variation in the distribution of isolates among the various centers and the relatively low correlation between the antigenic titre values and cultural isolation of Salmonella organisms from the blood samples might be attributed to the non-

adherenceof of the laboratory personnel to recommended dianostic procedures. Investigation carried out during the study revealed that in some private medical laboratories, the O-antigen suspensions provided in the Widal test kits were often pooled together for use in the determination of antigen titres, as way of maximizing profit. This could lead to non-specificity in the reaction and may account for the non-isolation of salmonella organisms at the high O-antigen titre values the pooling together of O-antigen suspensions may also be responsible for the differences in the percentage of samples that yielded Salmonella sp. at high O-titre values among the hospitals and medical laboratories where the serological tests were performed.

Widal serological test should as much as possible be complemented with isolation of the causative organisms of typhoid fever. Cultural isolation, biochemical characterization and sero-typing are essential for complete identification of salmonella organisms, since no matter how well-defined a serological laboratory may be, serological procedures do not supercede bacteriological culturing.

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A COMPARATIVE STUDY ON THE PREVALENCE OF MALARIA PARASITE AMONG HIV SERO – POSITIVE AND SERO- NEGATIVE INDIVIDUALS IN ABAKALIKI NIGERIA.

IROHA I. R.,[1, 2] ESIMONE C. O[1], AMADI E. S.[2]

[1]Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria.
[2]Department of Applied Microbiology, Ebonyi State University Abakaliki

Corresponding author: Dr. Charles O. Esimone Department of Pharmaceutics, University of Nigeria, Nsukka.
E-mail: coesimone@yahoo.com

KEY WORDS: HIV, Malaria parasitaemia, sero positive, sero negative.

ABSTRACT

A total of 300 blood samples collected from patients at the Federal Medical Centre Abakaliki were examined for HIV infection and malaria parasite using TRI-Dot Immunoassay, capillus and genie Assay and Thick film techniques. The overall prevalence of malaria infection was 59% for HIV sero positive and 41% for HIV sero negative individuals respectively. Demographically, the prevalence was shown to be highest amongst those living in urban areas (71%) compared to those living in rural areas (31%). The prevalence between sex showed that females were more affected (59%) than males (41%); people within the age range of 21 – 30 have the highest prevalence (33%) while the elderly ones between 61 – 70 years of age were least affected (3%). The data also indicated that married people were more infected (60%) than the unmarried (40%). Statistical analysis indicates that there is a significant difference in malaria parasitaemia amongst HIV infected and non-infected individuals with respect to sex, age and marital status.

INTRODUCTION

Malaria is one of the most widely spread infectious diseases that causes

major public health problems, particularly in Sub-Sahara Africa (1 -3). Studies in some health facilities in Africa like in Nigeria showed that malaria constitutes 20 – 60% of all out- patient consultations, 10% of hospital admissions and 60% of infant mortality (3 -4). The epidemiological situation with respect to malaria has worsened in Africa over the last decade, and the disease has occurred in areas previously free of malaria because of changes in the climatic conditions (4). Although it is largely confined to tropical and sub tropical zones, air travels has led to its increased frequency in this country. It is estimated that about 75% of the total population is at risk of the infection and its transmission occurs usually at altitude above sea level (6).

Human immunodeficiency virus (HIV) is a worldwide disease that kills millions of people every year with two distinctive types (HIV I and HIV 2). HIV 2 occurs most commonly in West Africa and occasionally infections have occurred in East Africa (7). However HIV I transmission is slightly less easy and the progression of HIV II infection to AIDS may be slower (8). At the end of 2002, there were an estimated 42 million adults and children living with HIV or AIDS. Of these, 28.5 million (65%) were living in sub sahara Africa (8 - 9). The region has the highest HIV sero positive prevalence and occurs in people between the ages of 15 – 49 years.

The degree of malaria parasites infections is increasing among HIV patients as an opportunistic infection; Also, malaria and HIV are the world

most important tropical diseases that kill more people than any other communicable diseases with the exception of tuberculosis (9 -11.)

This research was conducted in Abakaliki, Nigeria, based on a report of high incidence of HIV infection amongst antenatal mothers and individuals presenting for pre marital HIV screening test. Given that malaria parasite has been shown to be an opportunistic infection that increases the rate of HIV transmission (12 -14.), we decided to investigate incidence of malaria parasite in HIV sero positive and sero negative individuals in Abakaliki.

MATERIALS AND METHODS

Sampling Procedure:

The study population comprises 300 HIV sero-positive and sero-negative patients who visited the Federal Medical Centre (FMC) Abakaliki, Ebonyi State, Nigeria from February-May 2005 with respect to individual reasons, which include pre-marital HIV screening test, antenatal care, symptoms suspected to be related to HIV infections that were recommended for HIV screening test, some individuals who previously tested HIV positive (and were therefore recommended for confirmatory test). Blood samples (2.5 – 5mls) of the patients were collected from anticubital veins using sterile disposable needles and syringes. The HIV sero status of the 300 patients were evaluated with HIV sero positive numbering 150 and HIV sero negative numbering 150. The HIV samples that are positive were confirmed using two commercial kits viz: capillus assay analysis (Biosystem comp. Ltd Austria); TRI-Dot immunoassay (Abbot Laboratories Comp. Ltd) was also used for initial test before confirmatory test. Samples collected were screened for malaria parasites within 2 – 3 hours of collection.

EXAMINATION OF BLOOD FOR HIV/AIDS VIRUS

(a) ***TRI-DOT Immunoassay techniques of screening for HIV.***

HIV TRIDOT is a flow through dot membrane immunoassay that uses recombinant HIV –1 and HIV – 2 protein antigens, immobilized on a porous immuno filtration membrane to detect separately antibodies to HIV-1 and HIV-2 in serum or plasma. The membrane was moistened with 3 drops of buffer solution, and then a drop of the patient's sample was added. Another 5 drops of buffer solution was added with 2 drops of gold conjugate. Then an additional 5 drops of buffer solution was added after which the reaction was visualized. Pink dots in the test areas were interpreted as been reactive for anti HIV – 1, HIV- 2, or both HIV- 1 and HIV -2 according to the manufacturer's specifications.

CAPILLUS ASSAY TECHNIQUE OF SCREENING FOR HIV

CapilIus HIV- 1 and HIV- 2 is a simple and rapid (3 minutes) latex agglutination test that uses recombinant antigen derived from HIV- 1 and HIV- 2 envelop genes to detect antibody to HIV- 1 or HIV- 2 in serum, plasma and whole blood. The test was performed in a plastic capillary slide consisting of a well in which the latex antigen reagent is mixed with the sample, a channel along which the mixture flows by capillary attraction and a viewing chamber at the end of the channel.

After dispensing latex reagent, the patient's sample was added and mixed well with the reagent. The mixture was dragged to the capillary channel and the reaction was visualized. A reactive positive test is shown by agglutination of the HIV antigen coated latex particles while no agglutination indicates "non reactive".

EXAMINATION OF BLOOD FOR MALARIA PARASITE

Thin blood film technique

A thin drop of the patient's blood was dropped on a free grease slide and was gently spread on the slide and left for 1 – 2 mins. to dry. The thin blood was fixed by swabbing a drop of alcohol on it and left for 1 – 2 minutes. A 1:10 dilution of giemsa stain was made and was used to stain for 30 minutes, then washed off in buffer solution, air- dried and examined using X10 and X40 oil – immersion /objective lens.

Thick blood film technique

A 1:10 dilution of giemsa stain was made using buffer solution and distilled water at pH 7.2. A thick blood film was dropped on a clean free grease slide, spread to 0.5-inch diameter, left to dry, and rapidly dipped in clean distilled water. The slide was stained with giemsa stain for 30 minutes, washed off in buffer, allowed to air – dry and was examined using x10 and x40 oil immersion objective lens. An average of 10 fields was counted and the malaria parasites were converted against leucocytes (white blood cells). The density of the malaria parasite was calculated using the WHO standard as shown below:

(i) $$\frac{\text{Total parasites in the fields on a slide}}{\text{Total no. of field on the slide}}$$

(ii) $$\frac{\text{6000 x Parasite counted against 100 WBC} = \text{Parasite density/}ml}{100}$$

Statistical analysis

The data were analyzed using the Student's t-test Parasitaemia level between HIV-positive and HIV-negative individuals were compared. Differences in mean between paired observations were accepted as significant at $P<0.05$.

RESULTS

The results in table 1 and 11 show that malaria parasite were more prevalent in HIV sero-positive individuals than in sero-negative individuals. In males, the average number of parasites present varies with the ages of individuals. The lowest number of parasites was observed in males within age range ≤10 while the highest number of parasites was observed within the age range of 31-40. In females, a similar result was obtained with the lowest number of parasite density gotten from patients within the age ranges of ≤ 10 and the highest within the ranges of 21-30. The results also show that the rate of Malaria infection is more in females HIV sero-negative individuals with respect to age than in males. In males, the parasite is more in patients within the age range of 21-30 while in females it is more in patients within the age range of 31-40. Also in males, the least average number of parasites was observed in patients within the age range of ≤10 but in females it is within the age range of 61-70.

Table 111 reveals that malaria parasite is more in HIV sero- positive patients (59%) than in HIV sero-negative patients (41%). From Table IV which shows the result of demographic distribution of malaria parasites among HIV sero-positive individual, it was observed that malaria parasites were more prevalent in females (59%) than in males (41%); more in married (60%) than unmarried (40%) and also more in urban (71%) than in rural areas (29%). The overall results of demographic distribution show that malaria parasites were most prevalent in urban areas (71%) than in all other groups.

Table I: Malaria Parasite Density Between Male And Female Hiv Sero-Positive Individuals

Age range	No of patients	Average no of parasite (%)	parasite density /*ml*
MALE			
≤10	0	0(0%)	0
11 – 12	7	320(4%)	18 120
21 – 30	15	721 (10.4%)	43 260
31 –40	18	814 (11%)	48 840
41 – 50	5	231 (3%)	13 860
51 – 60	6	230 (3%	13 800
61 – 70	3	170 (2%)	10 200
Total	54	2468	
FEMALE			
≤10	5	2.53 (3%)	15 180
11 – 20	12	571 (8%)	34 260
21 – 30	13	1643 (23%)	98 580
31 –40	30	1512 (21%)	90 720
41 – 50	5	243 (4%)	14 580
51 – 60	10	511 (7%)	30 660
61 – 70	1	38 (1%)	2 280
Total	96	4771	

Table II Comparison Of Rate Of Infection Of Malaria Parasite Between Males And Females With Respect To Age In Hiv Sero-Negative Individuals

Age range	No of patients	Average no of parasite (%)	Parasite density/*ml*
MALE			
≤10	2	63(1%)	3 780
11-12	7	276(5%)	16 560
21-30	27	745(15%)	14 4700
31-40	15	602(12%)	36 120
41-50	13	409(8%)	24 540
51-60	5	167(3%)	10 020
61-70	10	299(7%)	17 940
FEMALE			
≤10	3	128 (3%)	7 680
11 – 20	10	357 (7%)	21 420
21 – 30	21	732 (14%)	43 920
31 –40	20	787 (15%)	47 220
41 – 50	90	324 (6%)	19 440
51 – 60	6	217 (4%)	13 020
61 – 70	2	7 (0. 1%)	420

TABLE III: Difference In Malaria Parasitic Load Between Hiv Sero-Positive And Sero-Negative Individuals

Group	*No of Patients*	*Average No of Parasite (%)*	*Total No of Parasite density /*ml
HIV positive	***150***	***7239 (59%)***	***43 440***
HIV Negative	***150***	***5113 (41%)***	***30 6780***
Total	***300***	***12352 (100%)***	***74 1120***

Table IV: Demographic Distribution Of Malaria Parasites Amongst Hiv Sero Positive Individuals

GROUPS	AVERAGE NO OF PARASITES (%)
MALE	5 029 (41%)
FEMALE	7 323 (59%)
MARRIED	7 431 (60%)
UNMARRIED	4 921 (40%)
URBAN	8 710 (71%)
RURAL	3 612 (29%)

DISCUSSION

Most HIV patients posses a lower immune status and as a result of this patients are always at risk of getting infected with a wide variety of diseases (8, 15). In HIV patients, it was observed that the parasitaemia level was high as compared to non HIV patients. This could be attributed to the low immunity which they posses. This results in the multiplication and spread of the parasites at a higher rate as the immune system that as supposed to fight against infection spread has been destroyed (16-18). Because people at a younger age practice active sex, they are mostly responsible for the transmission of HIV. As observed in this study, this age group was the mostly affected. Individuals above 50 years are less active in making sexual contacts and so are not likely to acquire HIV infections and other immune reducing diseases (18). There is also a high risk of HIV infections within the schooling age (tertiary institution) and/or

early working ages because individual in this group practice active sex when compared at an earlier age of 11-20 (11,19).

The high prevalence of malaria parasites infection observed in females (23%) than in males (11%) could be because females engage themselves more in commercial sex and most unmarried females usually go for abortion and other risky behaviors. Also, young girls are particularly vulnerable to infection as an immature cervix and low vaginal mucus production provides less of barriers to HIV. However, older women who have passed menopause are also more vulnerable due to the thinning and drying of vaginal mucosa hence a weaker barrier. The younger ages are the most affected with higher incidence in females than in males (20-21), because during sexual relationship, the receptive partner (the Peron who is penetrated) is more at risk than the incentive partners and it becomes more risky when bruises occur (22). The HIV virus reduces the immune system and creates way for other opportunistic infections to thrive. Malaria being the most common disease in the tropics infects HIV patients more and this could the reason for the high malaria parasites density observed in this study.

The high presence of malaria parasite density in married females could be as a result of reduction of the immune system during pregnancy which makes them more vulnerable to malaria parasite (22-23). The presence of high parasite density in the urban area could be attributed to some epidemiological factors such as poor sanitation (dirty gutters and stagnant water on the street), deplorable hygienic practice (where every corner within the urban area is taken as a refuge site), quarry industry holes, (after crushing stones some of its particles are left in the holes which later form ditched and turn to small lakes and breeding environment for mosquitoes). Introduction of electricity has increased outdoor recreation for those living in the urban areas at night and the action further exposes urban dwellers to Mosquito bites (24). Importantly, the present study shows that malaria parasite density was significantly higher in HIV sero-positive individuals (59%) than in HIV sero-negative individual (41%). As earlier highlighted, these could be attributed to poor immune status of the patients.

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IN VITRO EFFECT OF SOME QUINOLONE ANTIBIOTICS ON STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM A HOSPITAL ENVIRONMENT.

***Obi , R.K[1] and Nwanebu, F.C[1]**

Dept of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

***Author for correspondence: robertobi_2003@yahoo.ca**

ABSTRACT

A total of 30 different strains of *Staphylococcus aureus* were isolated from some selected wards of Madonna University Teaching Hospital (MUTH), Elele, Nigeria, using blood agar and nutrient agar. All the isolates were subjected to some selected quinolones (ciprofloxacin, pefloxacin, ofloxacin, norfloxcin and sparfloxacin) to determine their antibiotic susceptibility pattern using the disk diffusion method. Ofloxacin had the highest percentage susceptibility of 93.3%, followed by ciprofloxacin with 73.3%; pefloxacin was next with 70%, sparfloxacin 63.3%, while norfloxacin recorded the lowest percentage of 50%. The minimum inhibitory concentration (MIC) of the quinolones to the isolates was also determined. The results show that all the tested quinolones had an MIC ranging from 2.5-10μg/ml.

Key words: Staphylococcus aureus, quinolones, hospital environment

INTRODUCTION

Staphylococcus aureus, the most common cause of staphylococcal infections, is a Gram positive, coagulase and catalase positive, spherical bacterium frequently living on the skin or in the nose of a person, that can cause a range of illnesses from minor skin infections (such as folliculitis, impetigo, cellulitis and abscesses), to life threatening diseases such as pneumonia, meningitis endocarditis, toxic shock syndrome (TSS) and septicemia [1]. In addition, 40% of all cases of urinary tract infections (UTI) are due to *Staphylococcus aureus* [2], while in infants the organism causes a severe disease known as staphylococcal scalded skin syndrome (SSSS) [3].

Staphylococcus aureus is one of the few organisms associated with nosocomial infections in health care institutions. In actual fact, they account for as much as one-third of all cases of nosocomial infections in many hospitals. The infections are mostly asymptomatic with human carriers presenting a much more stable problem, being a frequent source of confusion to infection controllers and healthcare providers. This is because most patients serve as formidable reservoirs of antibiotic – resistant pathogens that are responsible for cross-infections in health care institutions [4, 5, 6].

Staphylococcus aureus infection can be spread through contact with discharge from an infected wound, skin-to-skin contact with an infected person, and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person [7].

Treatment of *Staphylococcus aureus* infection is normally carried out with antimicrobial agents. However, the organism has been known to develop resistance to many of the commonly used antibiotics. This resistance, especially to penicillin, is mediated by penicillinase (β – lactamase) production, which is an enzyme that breaks down the β -lactam ring of the penicillin molecule. To overcome this problem, penicillinase resistant penicillins such as methicillin, oxacillin,

cloxacillin, dicloxacillin and flucloxacillin were developed to treat penicillin resistant *Staphylococcus aureus* infections. Methicillin was the first antibiotic in this class to be used, having been introduced in 1959; but two years later, the first case of methicillin–resistant *Staphylococcus aureus* (MRSA) was reported, reaching its peak in the 1980s when there was an explosion in MRSA prevalence in hospitals where it is now endemic [8, 7, 9].

After the destruction of the efficacy of methicillin by the microorganism, vancomycin became the drug of choice for treating MRSA infections. However, treatment failures, adverse side effects and emergence of vancomycin-resistant MRSA led to urgent requirements for alternative anti-MRSA therapies. In view of this, linezolid (a new agent) was recently developed for Gram-positive bacterial infections, including MRSA. However, resistance to this drug is already developing, thus necessitating the need for the development of more superior anti-MRSA drugs [10, 11, 12].

It is important in this fight to overcome the menace of MRSA, to develop/test for drugs that will target specifically, and inhibit the more aggressive virulent factors of the organism. This need has led to the manufacture of a variety of antimicrobial agents and antibiotics, one of which is a group of new drugs known as quinolones.

Quinolones are antimicrobial agents effective in the treatment of selected community-acquired and nosocomial infections. They are usually administered orally, but some can be given intravenously for treatment of serious infections. They are bactericidal and exhibit concentration-dependent killing. The mode of action of all quinolones involves inhibition of bacterial DNA synthesis by blocking of the DNA gyrase and topoisomerase iv enzymes.

Early quinolones such as nalidixic acid, oxolinic acid and cinoxacin had poor systemic distribution and limited antibacterial activity and were only used primarily for treatment of Gram-negative urinary tract infections. The fluorinated derivatives (e.g. ciprofloxacin, ofloxacin, norfloxacin, enofloxacin, pefloxacin, lomefloxacin etc) have greater antibacterial activity with low toxicity and achieve clinically useful levels in blood and tissues [13, 14].

Due to the ability of *Staphylococcus aureus* to resist treatment with the more common antibiotics, search for more efficacious alternatives by medical and allied scientist has continued unabated. In this study therefore, five (5) quinolones, namely, ciprofloxacin, pefloxacin, ofloxacin norfloxacin and sparfloxacin were tested for antibacterial activity against *S. aureus* isolated from a hospital environment, with a view to making appropriate recommendations to infection controllers and healthcare providers.

MATERIALS AND METHODS

Sources and collection of specimen: A total of 30 swab samples were collected at different times from floors, sink taps, tables and toilets in selected wards, at Madonna University Teaching Hospital, Elele, Rivers State, Nigeria using sterile swab sticks (Evapon sterile swab stick). Each collected sample was immediately taken to the laboratory for culture on blood agar and nutrient agar. After 18-24hrs incubation period at 37^0C, *Staphylococcus aureus* isolates were initially identified based on their cultural characteristics on blood agar and nutrient agar. Confirmation of the isolates was carried out through microscopy, catalase, coagulase and motility tests. The confirmed isolates were sub-cultured using peptone water and after another 18hrs incubation at 37^0C, preserved in the refrigerator at 4^0C as a stock culture that will be used for assay of antibacterial activity.

Antibacterial assay: pure cultures of bacterial isolates were subjected to antimicrobial susceptibility using the disk diffusion (or Kirby Bauer) method as applied by [15] Bruner *et al*., (1995). A volume of 0.1ml of purified stock culture of *S. aureus* was transferred to each nutrient agar plates and spread over the surface of the medium using a bent glass rod (or Hockey stick) in duplicates. The surface of the agar plate was allowed to dry. Single discs, each impregnated with standard concentrations of one of the five test quinolones, (ciprofloxacin (CIP), ofloxacin (OFX), pefloxacin (PEF), sparfloxacin (SPAR) and norfloxacin (NORF)) were carefully and asceptically placed on the inoculated agar medium. The plates were then inverted and incubated at 37^0C for 24hrs after which observations were made for emergence of zones of inhibition. Zones measuring 18mm diameter and above were regarded as indicative of susceptibility while those between 13-17mm were regarded as intermediate and the ones less than 12mm resistant.

Determination of Minimum inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the antimicrobial agents was determined by the agar dilution method as adopted by [15] Brauner *et al*., (1995). Serial dilutions of standard concentrations of each of the test drug were carried out and incorporated into 9ml volumes of Diagnostic Sensitivity Test agar broth (oxoid) in test tubes to give final concentrations of 2.5µg/ml, 5.0µg/ml, 7.5µg/ml, 10µg/ml, 20µg/ml and 30µg/ml. As reported by Jawetz et al., (2001), 1ml of standard concentrations of *S. aureus* was added into each tube and incubated for 24hrs at 37^oC. The MIC for each drug was recorded as the lowest concentration of the drug that inhibited visible growth. Microbial growth however was indicated by turbidity presence while clearance indicated 'no growth' or bactericidal activity.

RESULTS

Results of the susceptibility test show that Ofloxacin (OFX) exhibited a greater antibacterial activity than the rest of the drugs. As shown in Table 1, Ofloxacin inhibited 28 of the isolates while only 2 were resistant. It is followed by ciprofloxacin (CIP) which inhibited 22 of the isolates with only 8 showing resistance.

The least susceptibility was achieved with norfloxacin (NORF) which inhibited 15 of the isolates, while the rest of the 15 isolates were resistant.

Table 1: Antibiotic susceptibility pattern of *S. aureus* isolated from MUTH

Isolate	OFX		CIP		PEF		SPAR		NORF	
	S	R	S.	R.	S.	R.	S.	R.	S.	R
S. aureus	28	02	22	08	21	07	19	11	15	15

Key

CIP	**Ciprofloxacin**
OFX	Ofloxacin
PEF	Pefloxacin
SPAR	Sparfloxacin
NORF	Norfloxacin
S.	Susceptible

RESISTANT

Table 2 shows the percentage susceptibility pattern of *Staphylococcus aureus* to the quinolones tested. With ofloxacin 93.3% of the isolates were susceptible, followed by ciprofloxacin, which inhibited 73.3% of the isolates. Pefloxacin was next with 70% susceptible, while sparfloxacin achieved 63.3% susceptibility with the organism. Norfloxacin achieved the least susceptibility of 50% with the isolates.

Table 2: Percentage (%) susceptibility of *Staphylococcus aureus* strains to some selected quinolones

Number of *S. aureus*	OFX	CIP	PEF	SPAR	NORF
	S 28	S 22	S 21	S 19	S 15
30	93.3	73.3	70	63.3	50

Key

S. Sensitive

The results of the tube dilution (Table 3) show that all the strains of Staphylococcus aureus were killed by these drugs at MIC ranging from 2.5-10µg/ml. At MIC of 2.5µglml OFX was active against most Staphylococcus aureus isolated from the sites. At the same concentration, PEF was bactericidal to a lower number of the isolates but showed greater inhibition at MIC 5µg/ml-7.5µg/ml, while SPAR was able to exhibit its antibacterial activity against the isolates at MIC 7.5µg/ml-10µg/ml. The ability to inhibit most of the strains of this organism at MIC of 2.5µg/ml still shows that ofloxacin (Tarivid) is the drug of choice in the treatment of hospital acquired Staphylococcus aureus infections.

Table 3: MIC ranges of some quinolones on some strains of *S. aureus* (µg/ml)

CIP	OFX	PEF	SPAR
2.5-5.0	2.5-5.0	5.0-7.5	7.5-10.0

DISCUSSION

From the results, (Table 1), it was observed that ofloxacin (Travid) exhibited greater inhibitory effect against the organism than the rest of the quinolones studied. The drug inhibited 28 (93.3%) of the isolates showing that if this drug is not abused, it could provide succor to the prevalence of MRSA in hospitals and in the community. This fact was further established by the finding that the drug was able to achieve this nearly 100% inhibition rate at MIC of 2.5µg/ml (Table 2).

Even though, Tarivid was observed as the best of the quinolones from the results, the other members of the group studied were no less effective in exerting their antibacterial effect against isolates of the organism. Remotely following Tarivid and inhibiting 22 (73.3%) of the isolates at MIC 2.5 – 5.0µg/ml was ciprofloxacin while pefloxacin and sparfloxacin inhibited 21 (70.0%) at MIC 5.0-7.5 µg/ml and 19(63.3%) at MIC of 7.5-10.0 respectively. These results show that if properly used and the right does administered, these drugs could also be effectively utilized to treat infections due to *S.aureus* in addition to Tarivid. However, the last member of the group studied, norfloxacin, was not as effective as it was able to inhibit only 15 (50%) of the isolates. Since 50% of the isolates are still resistant, it follows that if abuse of this drug is continued through indiscriminate usage, efficacy of the drug could still reduce further, the organism could then develop complete resistant to it thereby rendering the drug ineffective in the treatment of *S.aureus* infections.

Over time, some bacteria, including *Staphylococcus aureus* have been known to develop ways to circumvent effects of antibiotics. This is especially possible due to the widespread use of antibiotics, which spurred evolutionary adaptation that enabled bacteria to survive these powerful drugs. Drug abuse is the bane of our society and an enabling factor to antibiotic resistance. To save the quinolones, and indeed other newly developed antibiotics, from suffering from ineffectiveness as some of those before them, we recommend that none of these quinolones must be taken without proper prescription from a qualified medical practitioner. Such medical practitioners on their part, should be guided by results of sensitivity tests from reputable medical laboratories. In localities where antibiotics can be purchased without prescription like buses, provision stores, street hawkers etc as is commonly

observed in some cities in Nigeria, laws could be enacted that will ban the sale of such drugs in those unauthorized and unapproved places.

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ONE POT METHOD FOR THE SYNTHESIS OF ARYLIDENE FLAVANONES AND SOME OF ITS ACTIVITIES

Joseph .L., George .M., Kassaye .G.

School of Pharmacy, Mekelle University,Ethiopia. E-mail: mathewlincg@yahoo.com

OBJECTIVE:

To synthesize E-3-arylidene flavanones by one pot method and screen their analgesic , anti-oxidant and antibacterial activities.

Method : A set of three E-3Arylidene flavanones were synthesized by simple base catalysed condensation of appropriate aryl aldehydes and 2'-hydroxy 4-methoxy acetophenone.

Analgesic activity was screened by hot plate method , anti-oxidant activity by spectrophotometric method and antibacterial activity by cup-plate method.

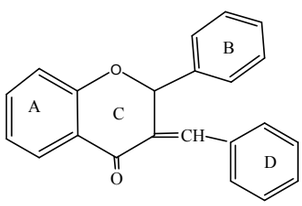
Results: A set of three E-3Arylidene flavanones were synthesized. Two were found to exhibit reliable degree of analgesic activity ,all produced anti-oxidant action and antibacterial activity .

Conclusion: Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, but only two were found to exhibit analgesic action. But all showed a reliable degree of anti-oxidant activity .In antibacterial activity studies,all were active against E.coli .

Key Words: E-3Arylidene flavanone , 2'-hydroxy 4-methoxy acetophenone , One Pot Method, Analgesic activity , Anti-oxidant activity, Natural flavanone.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds which are widely distributed through out the plant kingdom (1). Flavonoids can be classified into flavonols, flavones, flavanones and dihydroflavonols (2,3). Arylidene flavanones are also known as flavindogenides. Basic structure of E-3 Arylidene flavanones has four rings: Ring A, Ring B, Ring C and Ring D.



In addition to basic structure of flavanone, E-3 - arylidene flavanone have an extended conjugation at C-3 with carbonyl group. The special feature of long conjugation with the keto groups of flavanone moiety is expected to impart very significant biological activity or this type of cmpounds.

Krishna murthy (4) suggests E-3 arylidene flavanones and their heterocyclic analogues have poor solubility in aqueous medium starting either from 2-hydroxy chalcone or from o-hydroxy acetophenone. The reactivity of condensing aldehyde is an important factor in the synthesis. Presence of alcohol enhances the formation of products. This fact have been utilized for alkali catalyzed synthesis of many arylidene flavanones been reported by them. The special feature of long conjugation with 4-keto group of flavanone moiety is expected to impart very significant biological activity of this type of compounds.

Seiket *et al* (2) and Sha *et al* (3) reported the formation of 3-arylidene flavanone in alkaline medium. Chawla et al[06] reported the synthesis of

seven 3- arylidene flavanone by condensing 2 hydroxy acetophenones with aromatic aldehydes in aqueous alkaline medium, along with corresponding chalcones. According to these authors arylidene flavanones were accessible only by a low-yielding circuitous route. Knishnamurthy *et al* (3) concluded that 3-arylidene flavanones are obtained by acid catalyzed condensation between flavanone and aryl halide.

Their first representatives were synthesized by katshalowsky and kostanecky in 1904 (07). For a long time, E-3 arylidene flavanones (E-34) were synthesized solely by the acid catalyzed condensation of flavanones (08) and aromatic aldehydes (09-013. The reaction usually has been performed in alcoholic solution saturated with anhydrous hydrochloric acid at various temperatures and for different time. Albert levai et al[16] introduced a very simple base-catalyzed condensation for the synthesis of E3-arylidene flavanones. A mixture of equimolar amounts of flavanones and aromatic aldehyde and a few drops of piperidine was allowed to react at 150°c and E-3 arylidene flavanone was obtained in good yield without any purification. On the basis of ^{1}HNMR spectra Keane *et al* (17) explained the stereochemistry of synthetic E and Z- 3- arylidene flavanones.

So here is an attempt made to synthesise a few E-3 -arylidene flavanones by one pot method and to screen the synthesized compounds for the analgesic, anti-oxidant and antibacterial activities .

MATERIALS & METHO DS

For the synthesis of proposed compounds, 2'-hydroxy,4-methoxy acetophenone have been purchased from Sigma Aldrich chemical company Inc. U.S.A, Furfuraldehyde, P-Chloro benzal-dehyde, and p-methoxy benzaldehyde have been purchased from S.D fine chemicals, Mumbai.

INSTRUMENTS USED

U.V : Beckman 650 iu Spectrophotometer

I.R : Shimadzu – FTIR 8300

^{1H} NMR: Varian Gemini-200 MHz

SYNTHETIC PROTOCOL:

The proposed cmpounds were synthesized as per the following procedure;[18] One pot method: To a mixture of 2'-hydroxy,4-methoxy acetophenone (1 mM) and aromatic aldehyde (2.5 mM),a warm (45^{0}c) aqueous alcoholic solution of potassium hydroxide(15%) added and stirred the solution to get a uniform solution. The solution stand for four days in a stoppered condition. Methanol added dropwise to remove turbidity formed on cooling. The separated material washed with cold aqueous alcohol(50 % methanol). Then crystallized from aqueous alcohol. Each compound have been synthesized in the same manner.

Biological Experimental Protocol for Analgesic activity:

To study the analgesic activities of the synthesized compounds, albino mice of either sex were used. All mice were screened by exposure to thermal stimulus. Mice weighing between 20-25 g selected and made into six groups having six animals in each group. The first group served as control which received 2% gum acacia suspension. Second group served as standard,which received diclofenac sodium orally at a dose of 200mg/Kg body weight of animal (suspension of test compounds(20mg/ml)were prepared in 2% gum acacia). Animals were placed on perspax cylinder on heated surface and the time to exhibit discomfort reaction(licking paws or jumping) was considered as reaction time with the cut off time being 60 seconds. The first reading was taken immediately after administration of compounds and afterwards at the intervals of 30 minutes. The results were recorded.

EXPERIMENTAL PROTOCOL FOR ANTI-OXIDANT ACTIVITY:

Equimixture of 1,1-diphenyl-2-picryhydrazyl(3.9 mg in 10 ml ethanol) and test compounds (10 mg /10 ml ethanol) mixed and kept for 20 minutes at room temperature. Then absorbance measured at 517 n.m. Curcumin used as standard drug to compare the activity.

EXPERIMENTAL PROTOCOL FOR ANTI-BACTERIAL ACTIVITY:

The media used in present study, nutrient agar and nutrient broth, were prepared according to Indian pharmacopoeia .The pH of the solution was adjusted to 6.5-6.6 by using 1M sodium hydroxide and 1m hydrochloric acid. Then it was sterilized for 30 minutes at 15lbs pressure. 10mg of each test compound was dissolved in 10ml of DMF (dimethyl formamide) in serially and suitably labeled sterile test tubes, thus giving a final concentration of 100μg/0.1ml . Using sterile pipettes the standard and the sample solutions (0.1ml) of known concentrations were fed into the bored cups. As Cup-1: Standard (ciprofloxacin).Cup-2: solvent control (DMF).Cup3-: Test compound.

ASSESMENT OF SYNTHESIZED COMPOUNDS

Physical datas tested compounds are as follows. Ethanol had used as solvent to find out λ -max by U.V spectroscopy. KBr pellets used to measure I.R spectrum and $CDCl_3$ used for ^{1} HNMR spectrum .

Compond A:

λ-max- 244 n.m,350 n.m.

I.R(KBr): 1673.8c.m^{-1} (C=O), 1217.8 and 1189.6 c.m^{-1} (C-O-C), 854.1 and 812.2 c.m^{-1} (C-H def), 1474.8 and 1459.4 c.m^{-1} (C=C)

^{1}HMR ($CDCl_3$ ppm): 7.04-7.11(H-2), 6.19-6.22 (H-3', H-4'), 6.44-6.52 (H-6), 6.53-6.61 (H-4"), 7.24 ($CHCl_3$), 7.35-7.4 (H-5'), 7.52-7.59 (H-5"), 7.9-7.93 (H-5), 3.8-3.9 (OCH3-proton), 7.62-7.64 (H-β).

Compound B:

λ-max- 230 n.m,360 n.m.

I.R(KBr): 1668.12c.m^{-1} (C=O), 1361.5 and 1249.65c.m^{-1} (C-O-C), 943.02 & 844.669 c.m^{-1} (C-H def), 1637.27, 1523.49 & 1454.06(C=C)

^{1}HMR ($CDCl_3$ ppm): 6.59-6.625(H-2), 6.82-7.0 (H-6, H-8, H-3', H-5'), 7.2-7.43(H-7, H-2', H-6', H-2", H-6"), 3.7-3.9 (OCH_3 proton), 9.1-7.95 (H-5), 3.99 (OCH_3), 8.02-8.12 (H-β).

Compound C:

λ-max-258 n.m, 290 n.m.

I.R(KBr):1668.2c.m $^{-1}$(C0=O), 1250.66 and1146.4c.m $^{-1}$(C-O-C), 854.4 and 824.2 c.m $^{-1}$(C-H def), 748.2 c.m $^{-1}$(monochloro), 1604.2and 1510.4 and1472 and 1458.6c.m $^{-1}$(C=C) .

^{1}HMR($CDCl_3$ppm):6.58-6.63(H-2),6.8-7(H-6,H-8,H-3',H-5',3",H-5"),7.2-7.42(H-7,H- 2',H-6',H-2',H-6"), 3.98 (OCH_3), 8.04-8.1(H- β).

RESULTS

As per the synthetic protocol three E-3 Arylidene flavanones have been synthesized and screened their analgesic activity by hot plate method. Anti-oxidant activity tested by spectrophotometric method.Antibacterial activity by cup-plate method. Observations for analgesic activity are shown in table1 , that of anti-oxidant activity in table 2 and antibacterial activity in table-3.

Table:1 Analgesic activity of synthesized compounds.

Compound I.D	Dose(orally) Mg/Kg	Average reaction time in seconds [a] 0	30	60	90
A	200	3.05	3.10	3.10	3.10
B	200	2.50	2.6	2.6	2.55
C	200	3.00	4.00	6.00	6.08
Std.	200	3.00	5.25	8.25	8.28
Control	-----	3.00	3.00	3.00	3.00

a=Average reaction time expressed as mean (±S.D)of a group.

Table2:Anti-oxidant activity.

Compound I.D	**Absorbance at 517 n.m**	**Relative % activity considering that of standard as 100%**
Curcumin(Std.)	2.596	100%
A	2.142	82.5%
B	2.012	77.5%
C	2.482	95.6%

Table:3 Antibacterial activity.

Compound I.D	Zone of inhibition in m.m E.Coli	Pseudomonas aerugenosa	Staphylococcus aureus	Bacillus subtilis
A	12	-	9	-
B	23	20	-	20
C	18	-	16	24
Std.(Ciprofloxacin)	28	26	24	32

DISCUSSIONS

Three E-3 Arylidene flavanones have been synthesized by one pot method which reduces the usual tedious multisteps involved in the synthesis of medicinal compounds. The results of the assessment of synthesized compounds have good agreement with the datas given in the literature. Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, as per the studies two were found to exhibit analgesic action. The results shows less analgesic activity for all tested compounds than the standard drug namely Diclofenac sodium. Among the three compounds compound C showed maximum analgesic activity. Compound B showed least analgesic activity. Among the three compounds exhibited analgesic activity ,the compound C showed more activity than others, probably due to the presence of halogen atom.

Considering anti-oxidant activity, compound C showed maximum activity and compound B showed least activity. More anti-oxidant activity of compound-C may be due to the presence of chlorine. In anti-bacterial studies all compounds exhibit activity against E.Coli. Only compound B showed activity against Pseudomonas.Compound C showed good activity against Staphylococcus aureus. Compound B as well as compound C showed good activity against Bacillus subtilis.

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BACTERIAL MENINGITIS AMONG CHILDREN IN FEDERAL MEDICAL CENTRE

Odedina, E.A. and Emumwen, EG

Medical microbiology department, federal medical centre p.m.b. 14, Bide, niger state

Niger Correspondence: Dr.E.A. Odedina, Federal Medical Centre P.M.B. 14, Bida, State

ABSTRACT

Cerebrospinal fluid (CSF) samples from one hundred and fifty children suspected of bacterial meningitis in the children's ward of the Federal Medical Centre, Bide, between January and December 2001 were studied. The children were aged twelve and below. Only twenty five (16.7%) of the samples were microbiologically proven. The commonest pathogens isolated were *Neisseria meningitides* (13), *Escherichia coli* (7) and *Streptococcus pneumonias* (4). The three bacteria constituted 92.3% (24 of 26) of the detected organisms from CSF either by culture, or by direct smear or both.

Antimicrobial susceptibility to Ofloxacin by *E. coli* and *Str. pneumonias* was 100% and 87% by *N. meningitides*. Susceptibility of *N. meningitides* and *Str. pnuemoniae* to penicillin was 0%. All the three main organisms showed poor susceptibility to Streptomycin. *N. meningitis* was 83.3% susceptible to Gentamicin while only one isolate each of the other organisms were tested on it and were found to be susceptible except *E. coli* that was resistant.

Key words: meningitis, children, bacterial pathogens, antimicrobial susceptibility

INTRODUCTION

Cerebrospinal meningitis is an acute medical emergency and is an important health problem in Nigeria. Large epidemics of meningitis occur periodically in Northern parts of Nigeria (1, 2, 3, 4, 5). Many researchers have reported sporadic outbreaks of meningitis in different parts of the country (1, 3, 4, 5). The aetiologic agents and the antimicrobial susceptibility patterns for Bida and its environs have not been documented.

Federal Medical Centre Bida is a young generation referral centre for Niger State, and caters for about three million people. The Centre is located in the Guinea Savannah, which is south of the meningitis belt of Africa.(6) High incidence of meningitis sometimes occurs during the hot, dry season, which is the usual period of epidemics in Northern Nigeria.(4,5,6)

MATERIALS AND METHODS

Cerebrospinal fluid samples collected from suspected meningitis children through lumbar puncture were received in the Microbiology main laboratory as soon as they were obtained in sterile Bijou bottles. The macroscopic appearances of the samples were noted. Well mixed CSF was charged into counting chamber using sterile Pasteur pipette for cell counting. Direct smears of specimens were made on clean glass slides and fixed. The smears were then stained by Gram's standard method. (7)

The remaining CSF samples were then centrifuged inside sterile tubes and the sediments were inoculated onto Chocolate, Mac Conkey and Blood agar plates.(8) The plates were then incubated anaerobically at 37^0C of 24hours, but chocolate agar plates were incubated in a candle jar system

for 24 hours. Cultures were then examined for growth. If there was no growth, cultures were re-incubated for another 24 hours before they were discarded as having no growth. The colonies were identified using standard methods. (7, 8)

RESULTS

One hundred and fifty samples of cerebrospinal fluid were received from children up to twelve years of age. Only three samples were reported turbid which were culture positive. Twenty five (25 of 150 or 16.7%) of these children had microbiology proven diagnosis of meningitis. Twenty one (21 of 25) was by culture, five by direct smear only and seven were positive for both culture and direct smear. The organisms isolated over the twelve-month period are shown in Table 1. A two-year-old girl had a mixed infection with N. *meningitides* and *E. coli.*

Only twelve case notes (12 of 25 or 48%) were retrieved out of the cases with proven bacterial meningitis. Four children survived, two died, two absconded and the outcome was not stated in two cases.

Table 1: Showing how organisms were detected

Microbiology Test	Number Positive
Direct Smear Only	5
Culture Only	21
Direct smear and Culture	7

Table II: Showing Isolates from CSF

Organisms	Frequency of Isolates	Percentage n = 26
N. meningitidis	13	50
E. coli	7	26
Str. pneumoniae	4	15
S. aureus	1	3.8
H. influenzae	1	3.8

Table III: Showing susceptibility patterns of isolates

	N. meningitidis **n = 8**	***E. coli*** **n = 7**	***Str. pneumoniae*** **n = 4**	***H. influenzae*** **n = 1**	***S. aureus*** **n = 1**
Oflocaxin	8 (100)	7 (100)	4 (100)	1 (100)	1 (100)
Chloramphenicol	7 (87.5)	5 (71.5)	4 (100)	1 (100)	0 (0)
Gentamicin	5 962.5)	6 (85.7)	N.T	0 (0)	1 (100)
Erythromycin	5 (62.5)	N.T	N.T	1 (100)	1 (100)
Streptomycin	4 950)	3 (42.8)	4 (100)	N.T	1 (100)
Penicillin	0 (0)	N.T	0 (0)	N.T	0 (0)
Cloxacillin	N.T	N.T	N.T	0 (0)	0 (0)
Unasyn	N.T	N.T	N.T	0 (0)	N. T
Tetracycline	N.T	N.T	N.T	N.T	1 (100)

n = Total number of isolated tested
( ) = Percentage of susceptible strains
N.T = Not tested

Table IV: Shows the age distribution of children with microbiology proven meningitis

Ages of Children	Freq.	Cumulative Frequency	Perc.
0 – 28 days	1	1	4
1 – 5 months	2	3	12
6 months – 2 years	14	17	68
3 – 5 years	3	20	80
16 – 12 years	5	25	100

The recovery rate of organisms among children suspected to have meningitis was16.7% (25 out of 15). As it the case with most medical emergencies, the disease had been over diagnosed. The rate is slightly higher than 12% obtained by Lehman *et al* in their study of bacterial meningitis in children, but lower than 25% obtained by Salih *et al* in their study of endemic meningitis among Sudanese children. (9,10). Both of these groups of researchers made diagnoses by culture, direct smear and or antigen detecting assays, as compared to this study where only direct smear and culture were employed. The use of antigen detecting assays has the added advantage of detecting non-viable organisms especially in samples not promptly sent to the laboratory after lumbar puncture. It will also detect organisms in patients who have been receiving anti-microbial chemotherapy up to 24 hours before lumbar puncture is performed. This reagent is however expensive and was not available for routine work in the centre of the present study.

The present study revealed that the three commonest organisms causing meningitis among children in Bida are *Neiseria meningitides* (13), *Escherichia coli* (7) and *Streptococcus pneumonias* (4). This together accounted for 24 out of 26 (or 92.3%) of the isolates. The study also shows that there was higher incidence of suspected cases of meningitis during the hot dry season before the start of the rains (November to April) (4, 5). This is the usual period of epidemic meningitis in Northern

DISCUSSION

Nigeria. Greenwood *et al* reported meningococcal epidemic in Zaire in 1977.(4) The report by Rebase *et al* in Maiduguri on epidemic meningococcal meningitis showed that the peak incidence of infection occurred in March, which was the peak of the dry season. Both reports from Zaire and Maiduguri support the fact that meningococcal epidemics in Northern Nigeria usually occur during the hot dry season. The low humidity promotes breaches in the nasal mucosa for the entry of *Neisseria* into the bloodstream. The present study does not represent the occurrence of an epidemic but it shows high incidence in the month of November. Immunization against meningococcal meningitis was commenced in December (year 2001) in Bida Local Government Area. Maximum antibody response to the infection takes about four weeks to develop and so immunization should be given not later than one month to the onset of outbreaks(5). In fact, vaccination should commence when the nasal carriage rate of *Neisseiria* is 6% in the populations. (11, 12)

The recovery rate (16.7%) of bacteria found in this study shows that there were various clinical manifestations that can easily be confused with meningitis especially in children below the age of five who may give very little specific complaint. The mortality rate is highest in this age group especially below the age of one year (10, 13, 14). Fever is a common presenting problem in all the children irrespective of their definite diagnoses in this study. Early diagnosis and institution of appropriate treatment is difficult in young children below the age of five (2, 3). This depends on the alertness of their parents to seek medical care and high index of suspicion by the attending caregivers (15, 16) Headache is reported only in children above five years (16, 17). Other diagnoses made by

the clinicians that could be confused with meningitis as were found in the case files were: severe anemia (8), septicemia (5), severe malaria (4), bronchopneumonia (3), chronic osteomyelitis (2), and otitis media (2). Meningitis can complicate any of these diseases if poorly managed.

The standard regimen with chloramphenicol advocated by Tefurani and Vince for children with bacterial meningitis remains valid for this community as shown by the study (14). Further improvements in outcome are likely to be achieved not by changes in antibiotic policy, but by improving early diagnosis and basic supportive care and by preventing convulsion (13, 14).

Aminoglycosides should be added to empiric treatment when Gram negative infection is suspected (18). Meningitis causes an increase in permeability of the blood brain barrier and thus increases in the cerebrospinal fluid protein. The duration of the main complaint of vomiting, irritability, cyanosis, petechiae hemorrhages and disturbed consciousness were independent predictors or bacterial meningitis. The only independent predictors from subsequent laboratory tests were the serum C-reactive protein (CRP) concentration. Estimation of CRP in CSF samples may be made to give a preliminary or additional diagnosis of meningitis regardless of its aetiology (20). Without missing a single case, this model identifies 35% of patients without bacterial meningitis i.e. patients with meningeal signs in whom a lumbar puncture can be withheld (16).

From the foregoing, it cannot be overemphasized that future prospective study to estimate C-reactive protein for Bida's community will be invaluable in provisional diagnosis of bacterial meningitis.

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MICROBIAL SPECTRUM OF PELVIC INFLAMATORY DISEASES IN NGURU, NIGERIA

Okon, K.O., Ayilara R., Bello K., Uba A., Aniesona T. A.

Department of Medical Microbiology, University of Maiduguri Teaching Hospital Maiduguri.
Pathology/Obstetritic & Gynecology department, Federal Medical Centre Nguru, Yobe State.
Department of Biological Sciences, Abubakar Rafawa Balewa University, Bauchi
Department of Veterinary Microbiology and Parasitology, University of Maiduguri

Corresponding Address: Okon, K.O. Department of Medical Microbiology University of Maiduguri Teaching Hospital P.M.B. 1414 Maiduguri. E-mail: okon_kenneth@yahoo.com

ABSTRACT

Pelvic inflammatory diseases, a leading gynecological problem worldwide, are associated with socio-economic and psychological costs. A retrospective study of 1350 high vaginal swabs analyzed between Jan-Dec. 2005, showed that 845 (62.8%) were positive for 9 microorganisms by culture/or wet preparation. Microbial growth was found in 645 (76.3%) cases. Polymicrobial growth was found in 90 (10.7%), fungal growth in 110 (13.0%) cases, and 3(0.4%) yielded anaerobic growth. *Staphylococcus aureus* accounted for 355 (42.0%) cases, followed by *Escherichia coli* 190 (22.5%), *Trichomonas vaginalis* 100 (11.8%) *Candida spp* and *Neisseria gonorrhoeae* 70 (8.3) and the least, *Pseudomonas spp* 5 (0.6%) Microbial-associated infection was prominent in the group 21-30 years old (46.6%) and 31-40 (23.9%) years respectively. Antibiotic susceptibility pattern showed that mean susceptibility greater than 50% were recorded with ofloxacin 80%, ceftazidime 80%, rifampicin 81.9% compared to mean susceptibility less that 50% recorded with trimethoprim-sulthamethoxazole 34.7%, and ampicillin 26.1%.

In conclusion, the reported microbial-associated infection in PID with a prevalence of 62.8% is of public health importance. Early diagnosis of causative agents and prompt institution of chemotherapeutic agents will help to prevent clinical complications that are expensive to treat.

Keywords: pelvic inflammatory diseases, microorganisms, antibiotic susceptibility.

INTRODUCTION

Pelvic inflammatory disease (PID), is an infection of the upper genital tract in women that include endometritis, parametritis, salpingitis, oophoritis, tubo-ovarian abscess and peritonitis (1,2). It accounts for 5-20% of hospital admissions for gynecological problems in general/gynecological clinics worldwide (3). In USA, infertility that affects approximately 10-15% of all couples attribute tubal damage due to pelvic infection (4,5). Clinical presentation varies in severity, and ranges from sub clinical, asymptomatic infections exerting medical and psychological cost that include chronic pelvic pain, ectopic pregnancy and infertility (1). It has been associated with increase risk of ovarian cancer6-8. The pathogenesis is complex interaction of genetic, immunological and bacterial virulence factors (9).

The prevalence and incidence of PID varies greatly, because of significant misdiagnosed/or unreported cases. In developed countries, annual incidence of PID increased in women aged 15-45 years, with peak of infection in 20-24 years (10). Polymicrobial agents are associated and initiated pathogenesis of PID, particularly in presence of facultative aerobic and anaerobic bacterial isolates (11-14), with *Niesseria. Gonorrhoea* and *Chlamudia tracomatis* as leading pathogens, accounted for 60-80% in women of aged less than 25 years12-14. Other less pathogenic mycoplasma, and endogenous aerobic and anaerobic bacteria have also been implicated (15). Co-existence of sexually transmitted diseases (STD) etiological agent in genital tract predispose the women to acquisition of PID (1,16). Korn *et al (*17) reported that clinical presentation and course of PID in women with sy,ptomatic HIV disease and/or severe immune suppression may be more aggressive than in HIV negative women.

Clinical diagnosis is rather difficult, as no single clinical and laboratory test in definite as gold standard, thus comibination of test seems to impove sensitivity and specificity (18,19). Epidemiological and microbiological indices associated with PID are important source of preventable reproductive infertility in women, and other clinical squealed. Little information is available on PID epidemiology in this environment, this there is no baseline in assessment of its relationship in case of infertility and HIV infection.

Early diagnosis/treatment of PID could stemmed down the effect on the fallopian tubes; and in case of microbe-related inflammation and tubal necrosis can similarly precedes manifestation of symptoms, especially in aetiological agent due to chlamydial3. Prompt diagnosis and institution of appropriate antibiotic therapy would prevent possible sequelae of PID. The retrospective study examined the aetiological spectrum in high vaginal swabs of pelvic inflammatory diseases in this environment.

MATERIALS AND METHODS

Study Site

The retrospective study was conducted in Federal Medical Center, Nguru, between Jan-Dec 2005, which involved the Pathology and Obsterictic/Gynecology departments. The patients folder presented at the general out-patients/gynecology clinic, with clinical complaint suggestive of pelvic inflammatory diseases, ranged from pelvic viginal discharge to lower abdominal pain, with high vaginal swabs collected and sent for bacteriological analysis. Criteria of inclusion are consecutive non-duplicate high vaginal swabs, repeated swab analysis and mixed growth of doubtful significance were excluded. Information retrieved from the patients folder included age, sex, and clinical complaint.

Processing of the Specimens

The high vaginal swab was processed, with inoculation on Blood, Chocolate and Sabouraud agar plates, incubated at 37^0C for 24hours. Bacterial/yeast were identified by standard bacteriogical and mycological techniques (20-22). Yeats were further identified by germ tube

test. Direct smear was prepared stained by Gram methods, and wet preparation of the specimen for parasitic examination. Antibiotic susceptibility testing was determined by disc diffussion23, using the following antibiotic discs, ofloxacin)OFX), ciprofloxacin (CPX), perfloxacin (PEF), ceftazidime (CAZ), cefuroxime (CXM), rifampicin(RF), strepromycin(S), tetracycline (TET), trimethoprim sulthamethoxazole (SXT), anpicillin (AMP), gentamycin (CN), erythromycin (E), and augmentime(AU). The zone of inhibition of the disc was measured to determined whether resistant or sensitive in accordance to NCCLS guidelines (23). The mean susceptibility percentage of each antibiotic was calculated as the number of bacterial isolates susceptible divided by total number of bacterial isolates tested multiply by 100.

Data Analysis

Data and information retrieved from patients flders were entered into study datavasem using SPSS version13.0. The value were expressed mean and percentage, and appropriate statistical package where necessary.

RESULTS

Of the 1350 high vaginal swabs results analyzed, 846 (62.8%) were positive for 9 microorganisms by culture/or wet preparation examination (7 bacterial pathogens, 1 fungi and 1 protozoan) as in table 1. The means age of the patient was 22.4 + 2.7 years. The ratio of gram-negative bacteria ratio was 1:2:5 Monomicrobic growth was recorded in 645 (76.3%), polymicrobic growth in 90 (10.7%) and bacterofungal in 110 (12.0%) cases. Three (0.4%) cases yielded anaerobic growth.

Staphylococcus aureus was the accounted for 355 (41.9%) cases, followed by *Escherichia coli* 190 (22.4%), *Trichomonas vaginalis* 100 (11.8%), *Neisseria gonorrhoeae* and *Candida spp* 70 (8.3%) repetitively. Microbial-associate infection distribution, in accordance with the age group of the patients studied (tableII), frequency of occurrence was predominant with the age group 21-30 (46.7%) and 31-40(23.9%) years and least in 10-20(8.1%) and >51(6.4%) years respectively. There was statistically significant difference between microbial infection and the age-group ($p<0.05$). Similarly, there was a decreasing trend pattern in frequency of occurrence of microorganism and age-group.

Antibiotic susceptibility pattern of bacterial isolates as shown in table III, showed that mean susceptibility percentage greater than 50 was observed with ofloxacin, gentamycin, ciprofloxacin, pefloxacin, rifampicin, cefuroxime, ceftazidime, erythromycin and streptomycin, and less than 50 in trimethoprim-sulthamethoxazole, tetracycline, ampicillin, and augmentime.

Table I: Frequency of occurrence of Microorganisms Isolated

Microorganisms	Frequency of Occurrence (%)
Gram-positive bacteria (n=370)	
Staphylococcus aureus	355 (42.0)
Streptococcus spp.	15 (1.8)
Gram-negative bacteria (n=305)	
Escherichia coli	190(22.5)
Neisseria gonorrohea	70(8.3)
Klebsiella spp	30(3.6)
Proteus spp.	10(1.2)
Pseudomonas spp.	5(0.6)
Anaerobic bacteria	3(0.4)
Fungi (n=70)	
Candida spp	70(8.3)
Parasites (n=100)	
Trichomonas vaginalis	100(11.8)
Total	**848**

Table II: Distribution of bacterial isolates according to age-group of patients studied

Age-group	S. aureus	Strep. Spp	Kleb spp	E.coli	Proteus spp	Pseud. spp	N.gonorrhea	T. Vaginalis	Candida spp	Anae. bact	Total
10-20	30	-	-	10	-	1	5	16	7		**69**
21-30	115	10	15	95	8	-	40	74	37	2	**396**
31-40	100	3	8	45	2	-	15	8	21	1	**205**
41-50	75	2	5	25	-	1	10	2	5	-	**125**
>51	35	-	2	15	-	3	-	-	-	-	**55**
Total	**355**	**15**	**30**	**190**	**10**	**5**	**70**	**100**	**70**	**3**	**848**

Table III: Antibiotic susceptibility pattern of the bacterial isolates (% susceptibility)

BACTERIA ISOLATES	OFX	CN	CIP	PEF	SXT	AMP	RD	E	AU	S	CXM	CAZ	TET
S.aureus	**90**	**65**	**80**	**89**	**25**	**21**	**89**	**75**	**60**	**70**	**85**	**72**	**45**
E.coli	**82**	**70**	**75**	**83**	**43**	**26**	**78**	**65**	**72**	**73**	**78**	**80**	**54**
Kleb spp	**78**	**56**	**78**	**78**	**45**	**35**	**85**	**67**	**56**	**67**	**76**	**82**	**42**
Strep spp	**90**	**82**	**89**	**79**	**50**	**42**	**90**	**90**	**80**	**80**	**84**	**79**	**35**
Proteus spp	**75**	**67**	**75**	**74**	**35**	**22**	**76**	**69**	**67**	**67**	**73**	**80**	**45**
Pseudo spp	**60**	**42**	**73**	**67**	**20**	**12**	**65**	**52**	**45**	**45**	**75**	**82**	**38**
N.gonorroheae	**85**	**65**	**73**	**74**	**25**	**25**	**90**	**68**	**50**	**78**	**80**	**85**	**38**
Mean Susceptibility(%)	**80**	**63.7**	**77.6**	**77.7**	**34.7**	**26.1**	**81.9**	**69.4**	**69.4**	**68.6.**	**78.7**	**80**	**42.2**

DISCUSSION

Clinical significance of PID becomes pronounced because its association with sexually transmitted diseases/HIV/AIDS infections. Particularly in asymptomatic individuals who may laer present with various complications irrespective of the social and psychological cost (19,24). Consequently, a dramatic increase in the

the incidence of PID has led to a parallel increase in infertility (25).

The reported prevalence of microbial-associated infection in PID of 62.8% of our patients is high. Our report is similar to the rates reported in similar studies conducted in Sokoto (26) and Gombe (27) of same geographical zone. However, comparison of PID prevalence in studies conducted at different geographical location/countries might be rather difficult, because of certain inherent biases involved, particularly presence of PID-related infections (10,28-30). Similarly, PID prevalence are influenced by variation in case definition (particularly between different clinical settings), changes in disease chronicity associated with clinically mild Chlamydia infection, variation in health seeking behaviour and increase management of PID in outpatient setting (31,32).

The frequency of occurrence of microbial-associated infection was high in the age group of 21-30(46.7%) and 31-40(23.9%) years. This finding simply confirms reported findings that highest PID prevalence and highest rate of increase are associated and seen in the 16-24 years age groups, and substantial numbers of bacterial sexually transmitted infection are high these age group (16-193,6,19,33). Also PID accounts for approximately 60% of gynecological problems in women aged less than 25 years34. High prevalence of PID episodes in sexually active age group, re-emphases the correlation that co-existence of aetiological agent in the genital tract of the females predisposes to acquisition of PID (3,19,33-35). Some studies found demographic risk factors associated with PID, like sexual activity at young age, racial, and both pre-delivery history and post-partum diagnosis of chlamdyial and gonococci infections (36,37). However implication of these factors in this environment need further evaluation.

From the present study, 10 microorganism (8 bacterial pathogens, 1 fungal and 1 protozoan) were recorded. S. *aureus* was the commonest and accounted for 42%, polymicrobial infection wasfound in 10.7% of cases and fungal infection in 13.0% of cases. This pattern simply confirms polymicrobial spectrum aetiology associated with pathogenesis of PID (11-14). S. *aureus* (42.0%), and *E.coli* (22.5%) were predominant bacterial isolates in the study, thesepathogens are most isolated in lower genital tract infections; and are responsible for a significant proportion of sexually transmitted diseases in Nigeria (26,38-40). The dominance of these bacterial pathogens as STI pathogens and their existence in the female genital tract clearly reaffirmed it as a predisposing factor in acquisition of PID (1,16). Polymicrobial infection with other organisms such as anaerobes or faculitative aerobes may be initiated by gonorrhea, chlamydia or both (4,5,15,35). The low frequency of occurrence of *N.gonorrhoeae* as evident in this study, might probably be due to variation in the studied population, method of microbial investigation, variation in severity of the diseases, sampling technology and site of sampling (40). Technically, *N. gonorrhoeae* is highly fastidious fragile organisms, isolation is dependent on viability of the organism in the specimen, prompt delivery to specimen, and suitability of isolation medium.

Trichomonas vaginalis with a prevalence of 11.6% in a center posed public health problem, because of close association of trichonomasis with HIV infection (42-45). *T.vaginalis*, is an irritating protozoan and is a common parasitic sexually transmitted disease reported worldwide (45). It is associated with inflammation of the cervix that may mimic cervical tenderness associated with PID (42). Buve et al (43) reported that trichomonasis incidence is higher in cities where there are higher number of HIV-positive individuals. The high prevalence of trichomonasis and candiasis observed in this study basically revealed close association of poor personal hygienic conditions especially among the low socio-economic class and transmitted sexually, particularly in cases of multiple sex partners (10), with high probability of PID infection.

The in-vitro antimicrobial susceptibility pattern of bacterial isolates revealed that mean percentage susceptibility of greater than 50% was observed with the fluoroquinolones (ofloxacin, ciprofloxacin, perfloxacin), cephalosporins (ceftfazidime, cefuroxime) and rifampicin ; and those less than 50% observed with gentamycin, erythromycin, augmentine and streptomycin, and least susceptibility observed in tetracycline (42.2%) trimethoprim-sulphamethoxazole (34.7%) and ampicillin (2.1%). These antibiotic susceptibility patterns are similar to reports by other workers (26,27). The reduced susceptibility of antibiotics like ampicillin, tetracyclines anmd trimthoprim-sulphamethoxazole, clearly revealed the abuse of these agents by self-medication practice, a common norm in most towns/cities in many developing countries.

The fluoroquinolones showed favourable in-vitro susceptibility pattern that could serve as drugs of choice in PID treatment/management. However, documented studies have reported emergence of fluoroquinolones-resistant *N.gonorrheae* (3). With considerable numbers of antibiotics resistants strains, particularly of *N. gonorrhoeae* to penicillin and cephalosporins (particularly 1st generation), the used of second-generation cephalosporins that posses extended spectrum activity over wide ranged of microorganisms associated with PID3. from the in-vitro antibiotic susceptibility pattern of the study, gentamycin, erythromycin and streptomycin, could served the antibiotic of choice considering the relative cost and also posses extended-specturm activity. One of the greater threat to the efficacy of antibiotics is the presence/or emergence of resistant strain, thus, cautious approach is required in prescription/administration, as safeguard policy against possible emergence of muliresistant strain in a remote city, like Nguru.

In conclusion, the prevalence of microbes-associated PID of 62.8%, is high. It is important that microorganisms associated with PID are diagnosed early and appropriate chemotherauptic treatment/management commenced, as clinical complications are always very expensive to treat.

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IN VITRO EFFECT OF SOME QUINOLONE ANTIBIOTICS ON STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM A HOSPITAL ENVIRONMENT.

***Obi , R.K[1] and Nwanebu, F.C[1]**

Dept of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

***Author for correspondence: robertobi_2003@yahoo.ca**

ABSTRACT

A total of 30 different strains of *Staphylococcus aureus* were isolated from some selected wards of Madonna University Teaching Hospital (MUTH), Elele, Nigeria, using blood agar and nutrient agar. All the isolates were subjected to some selected quinolones (ciprofloxacin, pefloxacin, ofloxacin, norfloxcin and sparfloxacin) to determine their antibiotic susceptibility pattern using the disk diffusion method. Ofloxacin had the highest percentage susceptibility of 93.3%, followed by ciprofloxacin with 73.3%; pefloxacin was next with 70%, sparfloxacin 63.3%, while norfloxacin recorded the lowest percentage of 50%. The minimum inhibitory concentration (MIC) of the quinolones to the isolates was also determined. The results show that all the tested quinolones had an MIC ranging from 2.5-10μg/ml.

Key words: Staphylococcus aureus, quinolones, hospital environment

INTRODUCTION

Staphylococcus aureus, the most common cause of staphylococcal infections, is a Gram positive, coagulase and catalase positive, spherical bacterium frequently living on the skin or in the nose of a person, that can cause a range of illnesses from minor skin infections (such as folliculitis, impetigo, cellulitis and abscesses), to life threatening diseases such as pneumonia, meningitis endocarditis, toxic shock syndrome (TSS) and septicemia [1]. In addition, 40% of all cases of urinary tract infections (UTI) are due to *Staphylococcus aureus* [2], while in infants the organism causes a severe disease known as staphylococcal scalded skin syndrome (SSSS) [3].

Staphylococcus aureus is one of the few organisms associated with nosocomial infections in health care institutions. In actual fact, they account for as much as one-third of all cases of nosocomial infections in many hospitals. The infections are mostly asymptomatic with human carriers presenting a much more stable problem, being a frequent source of confusion to infection controllers and healthcare providers. This is because most patients serve as formidable reservoirs of antibiotic – resistant pathogens that are responsible for cross-infections in health care institutions [4, 5, 6].

Staphylococcus aureus infection can be spread through contact with discharge from an infected wound, skin-to-skin contact with an infected person, and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person [7].

Treatment of *Staphylococcus aureus* infection is normally carried out with antimicrobial agents. However, the organism has been known to develop resistance to many of the commonly used antibiotics. This resistance, especially to penicillin, is mediated by penicillinase (β – lactamase) production, which is an enzyme that breaks down the β -lactam ring of the penicillin molecule. To overcome this problem, penicillinase resistant penicillins such as methicillin, oxacillin,

cloxacillin, dicloxacillin and flucloxacillin were developed to treat penicillin resistant *Staphylococcus aureus* infections. Methicillin was the first antibiotic in this class to be used, having been introduced in 1959; but two years later, the first case of methicillin–resistant *Staphylococcus aureus* (MRSA) was reported, reaching its peak in the 1980s when there was an explosion in MRSA prevalence in hospitals where it is now endemic [8, 7, 9].

After the destruction of the efficacy of methicillin by the microorganism, vancomycin became the drug of choice for treating MRSA infections. However, treatment failures, adverse side effects and emergence of vancomycin-resistant MRSA led to urgent requirements for alternative anti-MRSA therapies. In view of this, linezolid (a new agent) was recently developed for Gram-positive bacterial infections, including MRSA. However, resistance to this drug is already developing, thus necessitating the need for the development of more superior anti-MRSA drugs [10, 11, 12].

It is important in this fight to overcome the menace of MRSA, to develop/test for drugs that will target specifically, and inhibit the more aggressive virulent factors of the organism. This need has led to the manufacture of a variety of antimicrobial agents and antibiotics, one of which is a group of new drugs known as quinolones.

Quinolones are antimicrobial agents effective in the treatment of selected community-acquired and nosocomial infections. They are usually administered orally, but some can be given intravenously for treatment of serious infections. They are bactericidal and exhibit concentration-dependent killing. The mode of action of all quinolones involves inhibition of bacterial DNA synthesis by blocking of the DNA gyrase and topoisomerase iv enzymes.

Early quinolones such as nalidixic acid, oxolinic acid and cinoxacin had poor systemic distribution and limited antibacterial activity and were only used primarily for treatment of Gram-negative urinary tract infections. The fluorinated derivatives (e.g. ciprofloxacin, ofloxacin, norfloxacin, enofloxacin, pefloxacin, lomefloxacin etc) have greater antibacterial activity with low toxicity and achieve clinically useful levels in blood and tissues [13, 14].

Due to the ability of *Staphylococcus aureus* to resist treatment with the more common antibiotics, search for more efficacious alternatives by medical and allied scientist has continued unabated. In this study therefore, five (5) quinolones, namely, ciprofloxacin, pefloxacin, ofloxacin norfloxacin and sparfloxacin were tested for antibacterial activity against *S. aureus* isolated from a hospital environment, with a view to making appropriate recommendations to infection controllers and healthcare providers.

MATERIALS AND METHODS

Sources and collection of specimen: A total of 30 swab samples were collected at different times from floors, sink taps, tables and toilets in selected wards, at Madonna University Teaching Hospital, Elele, Rivers State, Nigeria using sterile swab sticks (Evapon sterile swab stick). Each collected sample was immediately taken to the laboratory for culture on blood agar and nutrient agar. After 18-24hrs incubation period at 37^0C, *Staphylococcus aureus* isolates were initially identified based on their cultural characteristics on blood agar and nutrient agar. Confirmation of the isolates was carried out through microscopy, catalase, coagulase and motility tests. The confirmed isolates were sub-cultured using peptone water and after another 18hrs incubation at 37^0C, preserved in the refrigerator at 4^0C as a stock culture that will be used for assay of antibacterial activity.

Antibacterial assay: pure cultures of bacterial isolates were subjected to antimicrobial susceptibility using the disk diffusion (or Kirby Bauer) method as applied by [15] Bruner *et al*., (1995). A volume of 0.1ml of purified stock culture of *S. aureus* was transferred to each nutrient agar plates and spread over the surface of the medium using a bent glass rod (or Hockey stick) in duplicates. The surface of the agar plate was allowed to dry. Single discs, each impregnated with standard concentrations of one of the five test quinolones, (ciprofloxacin (CIP), ofloxacin (OFX), pefloxacin (PEF), sparfloxacin (SPAR) and norfloxacin (NORF)) were carefully and asceptically placed on the inoculated agar medium. The plates were then inverted and incubated at 37^0C for 24hrs after which observations were made for emergence of zones of inhibition. Zones measuring 18mm diameter and above were regarded as indicative of susceptibility while those between 13-17mm were regarded as intermediate and the ones less than 12mm resistant.

Determination of Minimum inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the antimicrobial agents was determined by the agar dilution method as adopted by [15] Brauner *et al*., (1995). Serial dilutions of standard concentrations of each of the test drug were carried out and incorporated into 9ml volumes of Diagnostic Sensitivity Test agar broth (oxoid) in test tubes to give final concentrations of 2.5µg/ml, 5.0µg/ml, 7.5µg/ml, 10µg/ml, 20µg/ml and 30µg/ml. As reported by Jawetz et al., (2001), 1ml of standard concentrations of *S. aureus* was added into each tube and incubated for 24hrs at 37^oC. The MIC for each drug was recorded as the lowest concentration of the drug that inhibited visible growth. Microbial growth however was indicated by turbidity presence while clearance indicated 'no growth' or bactericidal activity.

RESULTS

Results of the susceptibility test show that Ofloxacin (OFX) exhibited a greater antibacterial activity than the rest of the drugs. As shown in Table 1, Ofloxacin inhibited 28 of the isolates while only 2 were resistant. It is followed by ciprofloxacin (CIP) which inhibited 22 of the isolates with only 8 showing resistance.

The least susceptibility was achieved with norfloxacin (NORF) which inhibited 15 of the isolates, while the rest of the 15 isolates were resistant.

Table 1: Antibiotic susceptibility pattern of *S. aureus* isolated from MUTH

Isolate	OFX		CIP		PEF		SPAR		NORF	
	S	R	S.	R.	S.	R.	S.	R.	S.	R
S. aureus	28	02	22	08	21	07	19	11	15	15

Key

CIP	**Ciprofloxacin**
OFX	Ofloxacin
PEF	Pefloxacin
SPAR	Sparfloxacin
NORF	Norfloxacin
S.	Susceptible

RESISTANT

Table 2 shows the percentage susceptibility pattern of *Staphylococcus aureus* to the quinolones tested. With ofloxacin 93.3% of the isolates were susceptible, followed by ciprofloxacin, which inhibited 73.3% of the isolates. Pefloxacin was next with 70% susceptible, while sparfloxacin achieved 63.3% susceptibility with the organism. Norfloxacin achieved the least susceptibility of 50% with the isolates.

Table 2: Percentage (%) susceptibility of *Staphylococcus aureus* strains to some selected quinolones

Number of *S. aureus*	OFX	CIP	PEF	SPAR	NORF
	S 28	S 22	S 21	S 19	S 15
30	93.3	73.3	70	63.3	50

Key

S. Sensitive

The results of the tube dilution (Table 3) show that all the strains of Staphylococcus aureus were killed by these drugs at MIC ranging from 2.5-10µg/ml. At MIC of 2.5µglml OFX was active against most Staphylococcus aureus isolated from the sites. At the same concentration, PEF was bactericidal to a lower number of the isolates but showed greater inhibition at MIC 5µg/ml-7.5µg/ml, while SPAR was able to exhibit its antibacterial activity against the isolates at MIC 7.5µg/ml-10µg/ml. The ability to inhibit most of the strains of this organism at MIC of 2.5µg/ml still shows that ofloxacin (Tarivid) is the drug of choice in the treatment of hospital acquired Staphylococcus aureus infections.

Table 3: MIC ranges of some quinolones on some strains of *S. aureus* (µg/ml)

CIP	OFX	PEF	SPAR
2.5-5.0	2.5-5.0	5.0-7.5	7.5-10.0

DISCUSSION

From the results, (Table 1), it was observed that ofloxacin (Travid) exhibited greater inhibitory effect against the organism than the rest of the quinolones studied. The drug inhibited 28 (93.3%) of the isolates showing that if this drug is not abused, it could provide succor to the prevalence of MRSA in hospitals and in the community. This fact was further established by the finding that the drug was able to achieve this nearly 100% inhibition rate at MIC of 2.5µg/ml (Table 2).

Even though, Tarivid was observed as the best of the quinolones from the results, the other members of the group studied were no less effective in exerting their antibacterial effect against isolates of the organism. Remotely following Tarivid and inhibiting 22 (73.3%) of the isolates at MIC 2.5 – 5.0µg/ml was ciprofloxacin while pefloxacin and sparfloxacin inhibited 21 (70.0%) at MIC 5.0-7.5 µg/ml and 19(63.3%) at MIC of 7.5-10.0 respectively. These results show that if properly used and the right does administered, these drugs could also be effectively utilized to treat infections due to *S.aureus* in addition to Tarivid. However, the last member of the group studied, norfloxacin, was not as effective as it was able to inhibit only 15 (50%) of the isolates. Since 50% of the isolates are still resistant, it follows that if abuse of this drug is continued through indiscriminate usage, efficacy of the drug could still reduce further, the organism could then develop complete resistant to it thereby rendering the drug ineffective in the treatment of *S.aureus* infections.

Over time, some bacteria, including *Staphylococcus aureus* have been known to develop ways to circumvent effects of antibiotics. This is especially possible due to the widespread use of antibiotics, which spurred evolutionary adaptation that enabled bacteria to survive these powerful drugs. Drug abuse is the bane of our society and an enabling factor to antibiotic resistance. To save the quinolones, and indeed other newly developed antibiotics, from suffering from ineffectiveness as some of those before them, we recommend that none of these quinolones must be taken without proper prescription from a qualified medical practitioner. Such medical practitioners on their part, should be guided by results of sensitivity tests from reputable medical laboratories. In localities where antibiotics can be purchased without prescription like buses, provision stores, street hawkers etc as is commonly

observed in some cities in Nigeria, laws could be enacted that will ban the sale of such drugs in those unauthorized and unapproved places.

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BACTERIAL MENINGITIS AMONG CHILDREN IN FEDERAL MEDICAL CENTRE

Odedina, E.A. and Emumwen, EG

Medical microbiology department, federal medical centre p.m.b. 14, Bide, niger state

Niger Correspondence: Dr.E.A. Odedina, Federal Medical Centre P.M.B. 14, Bida, State

ABSTRACT

Cerebrospinal fluid (CSF) samples from one hundred and fifty children suspected of bacterial meningitis in the children's ward of the Federal Medical Centre, Bide, between January and December 2001 were studied. The children were aged twelve and below. Only twenty five (16.7%) of the samples were microbiologically proven. The commonest pathogens isolated were *Neisseria meningitides* (13), *Escherichia coli* (7) and *Streptococcus pneumonias* (4). The three bacteria constituted 92.3% (24 of 26) of the detected organisms from CSF either by culture, or by direct smear or both.

Antimicrobial susceptibility to Ofloxacin by *E. coli* and *Str. pneumonias* was 100% and 87% by *N. meningitides*. Susceptibility of *N. meningitides* and *Str. pnuemoniae* to penicillin was 0%. All the three main organisms showed poor susceptibility to Streptomycin. *N. meningitis* was 83.3% susceptible to Gentamicin while only one isolate each of the other organisms were tested on it and were found to be susceptible except *E. coli* that was resistant.

Key words: meningitis, children, bacterial pathogens, antimicrobial susceptibility

INTRODUCTION

Cerebrospinal meningitis is an acute medical emergency and is an important health problem in Nigeria. Large epidemics of meningitis occur periodically in Northern parts of Nigeria (1, 2, 3, 4, 5). Many researchers have reported sporadic outbreaks of meningitis in different parts of the country (1, 3, 4, 5). The aetiologic agents and the antimicrobial susceptibility patterns for Bida and its environs have not been documented.

Federal Medical Centre Bida is a young generation referral centre for Niger State, and caters for about three million people. The Centre is located in the Guinea Savannah, which is south of the meningitis belt of Africa.(6) High incidence of meningitis sometimes occurs during the hot, dry season, which is the usual period of epidemics in Northern Nigeria.(4,5,6)

MATERIALS AND METHODS

Cerebrospinal fluid samples collected from suspected meningitis children through lumbar puncture were received in the Microbiology main laboratory as soon as they were obtained in sterile Bijou bottles. The macroscopic appearances of the samples were noted. Well mixed CSF was charged into counting chamber using sterile Pasteur pipette for cell counting. Direct smears of specimens were made on clean glass slides and fixed. The smears were then stained by Gram's standard method. (7)

The remaining CSF samples were then centrifuged inside sterile tubes and the sediments were inoculated onto Chocolate, Mac Conkey and Blood agar plates.(8) The plates were then incubated anaerobically at 37^0C of 24hours, but chocolate agar plates were incubated in a candle jar system

for 24 hours. Cultures were then examined for growth. If there was no growth, cultures were re-incubated for another 24 hours before they were discarded as having no growth. The colonies were identified using standard methods. (7, 8)

RESULTS

One hundred and fifty samples of cerebrospinal fluid were received from children up to twelve years of age. Only three samples were reported turbid which were culture positive. Twenty five (25 of 150 or 16.7%) of these children had microbiology proven diagnosis of meningitis. Twenty one (21 of 25) was by culture, five by direct smear only and seven were positive for both culture and direct smear. The organisms isolated over the twelve-month period are shown in Table 1. A two-year-old girl had a mixed infection with N. *meningitides* and *E. coli.*

Only twelve case notes (12 of 25 or 48%) were retrieved out of the cases with proven bacterial meningitis. Four children survived, two died, two absconded and the outcome was not stated in two cases.

Table 1: Showing how organisms were detected

Microbiology Test	Number Positive
Direct Smear Only	5
Culture Only	21
Direct smear and Culture	7

Table II: Showing Isolates from CSF

Organisms	Frequency of Isolates	Percentage n = 26
N. meningitidis	13	50
E. coli	7	26
Str. pneumoniae	4	15
S. aureus	1	3.8
H. influenzae	1	3.8

Table III: Showing susceptibility patterns of isolates

	N. meningitidis **n = 8**	***E. coli*** **n = 7**	***Str. pneumoniae*** **n = 4**	***H. influenzae*** **n = 1**	***S. aureus*** **n = 1**
Oflocaxin	8 (100)	7 (100)	4 (100)	1 (100)	1 (100)
Chloramphenicol	7 (87.5)	5 (71.5)	4 (100)	1 (100)	0 (0)
Gentamicin	5 962.5)	6 (85.7)	N.T	0 (0)	1 (100)
Erythromycin	5 (62.5)	N.T	N.T	1 (100)	1 (100)
Streptomycin	4 950)	3 (42.8)	4 (100)	N.T	1 (100)
Penicillin	0 (0)	N.T	0 (0)	N.T	0 (0)
Cloxacillin	N.T	N.T	N.T	0 (0)	0 (0)
Unasyn	N.T	N.T	N.T	0 (0)	N. T
Tetracycline	N.T	N.T	N.T	N.T	1 (100)

n = Total number of isolated tested
( ) = Percentage of susceptible strains
N.T = Not tested

Table IV: Shows the age distribution of children with microbiology proven meningitis

Ages of Children	Freq.	Cumulative Frequency	Perc.
0 – 28 days	1	1	4
1 – 5 months	2	3	12
6 months – 2 years	14	17	68
3 – 5 years	3	20	80
16 – 12 years	5	25	100

The recovery rate of organisms among children suspected to have meningitis was16.7% (25 out of 15). As it the case with most medical emergencies, the disease had been over diagnosed. The rate is slightly higher than 12% obtained by Lehman *et al* in their study of bacterial meningitis in children, but lower than 25% obtained by Salih *et al* in their study of endemic meningitis among Sudanese children. (9,10). Both of these groups of researchers made diagnoses by culture, direct smear and or antigen detecting assays, as compared to this study where only direct smear and culture were employed. The use of antigen detecting assays has the added advantage of detecting non-viable organisms especially in samples not promptly sent to the laboratory after lumbar puncture. It will also detect organisms in patients who have been receiving anti-microbial chemotherapy up to 24 hours before lumbar puncture is performed. This reagent is however expensive and was not available for routine work in the centre of the present study.

The present study revealed that the three commonest organisms causing meningitis among children in Bida are *Neiseria meningitides* (13), *Escherichia coli* (7) and *Streptococcus pneumonias* (4). This together accounted for 24 out of 26 (or 92.3%) of the isolates. The study also shows that there was higher incidence of suspected cases of meningitis during the hot dry season before the start of the rains (November to April) (4, 5). This is the usual period of epidemic meningitis in Northern

DISCUSSION

Nigeria. Greenwood *et al* reported meningococcal epidemic in Zaire in 1977.(4) The report by Rebase *et al* in Maiduguri on epidemic meningococcal meningitis showed that the peak incidence of infection occurred in March, which was the peak of the dry season. Both reports from Zaire and Maiduguri support the fact that meningococcal epidemics in Northern Nigeria usually occur during the hot dry season. The low humidity promotes breaches in the nasal mucosa for the entry of *Neisseria* into the bloodstream. The present study does not represent the occurrence of an epidemic but it shows high incidence in the month of November. Immunization against meningococcal meningitis was commenced in December (year 2001) in Bida Local Government Area. Maximum antibody response to the infection takes about four weeks to develop and so immunization should be given not later than one month to the onset of outbreaks(5). In fact, vaccination should commence when the nasal carriage rate of *Neisseiria* is 6% in the populations. (11, 12)

The recovery rate (16.7%) of bacteria found in this study shows that there were various clinical manifestations that can easily be confused with meningitis especially in children below the age of five who may give very little specific complaint. The mortality rate is highest in this age group especially below the age of one year (10, 13, 14). Fever is a common presenting problem in all the children irrespective of their definite diagnoses in this study. Early diagnosis and institution of appropriate treatment is difficult in young children below the age of five (2, 3). This depends on the alertness of their parents to seek medical care and high index of suspicion by the attending caregivers (15, 16) Headache is reported only in children above five years (16, 17). Other diagnoses made by

the clinicians that could be confused with meningitis as were found in the case files were: severe anemia (8), septicemia (5), severe malaria (4), bronchopneumonia (3), chronic osteomyelitis (2), and otitis media (2). Meningitis can complicate any of these diseases if poorly managed.

The standard regimen with chloramphenicol advocated by Tefurani and Vince for children with bacterial meningitis remains valid for this community as shown by the study (14). Further improvements in outcome are likely to be achieved not by changes in antibiotic policy, but by improving early diagnosis and basic supportive care and by preventing convulsion (13, 14).

Aminoglycosides should be added to empiric treatment when Gram negative infection is suspected (18). Meningitis causes an increase in permeability of the blood brain barrier and thus increases in the cerebrospinal fluid protein. The duration of the main complaint of vomiting, irritability, cyanosis, petechiae hemorrhages and disturbed consciousness were independent predictors or bacterial meningitis. The only independent predictors from subsequent laboratory tests were the serum C-reactive protein (CRP) concentration. Estimation of CRP in CSF samples may be made to give a preliminary or additional diagnosis of meningitis regardless of its aetiology (20). Without missing a single case, this model identifies 35% of patients without bacterial meningitis i.e. patients with meningeal signs in whom a lumbar puncture can be withheld (16).

From the foregoing, it cannot be overemphasized that future prospective study to estimate C-reactive protein for Bida's community will be invaluable in provisional diagnosis of bacterial meningitis.

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ONE POT METHOD FOR THE SYNTHESIS OF ARYLIDENE FLAVANONES AND SOME OF ITS ACTIVITIES

Joseph .L., George .M., Kassaye .G.

School of Pharmacy, Mekelle University,Ethiopia. E-mail: mathewlincg@yahoo.com

OBJECTIVE:

To synthesize E-3-arylidene flavanones by one pot method and screen their analgesic , anti-oxidant and antibacterial activities.

Method : A set of three E-3Arylidene flavanones were synthesized by simple base catalysed condensation of appropriate aryl aldehydes and 2'-hydroxy 4-methoxy acetophenone.

Analgesic activity was screened by hot plate method , anti-oxidant activity by spectrophotometric method and antibacterial activity by cup-plate method.

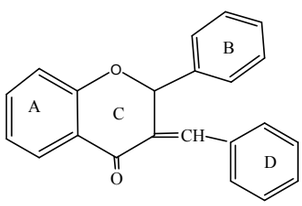
Results: A set of three E-3Arylidene flavanones were synthesized. Two were found to exhibit reliable degree of analgesic activity ,all produced anti-oxidant action and antibacterial activity .

Conclusion: Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, but only two were found to exhibit analgesic action. But all showed a reliable degree of anti-oxidant activity .In antibacterial activity studies,all were active against E.coli .

Key Words: E-3Arylidene flavanone , 2'-hydroxy 4-methoxy acetophenone , One Pot Method, Analgesic activity , Anti-oxidant activity, Natural flavanone.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds which are widely distributed through out the plant kingdom (1). Flavonoids can be classified into flavonols, flavones, flavanones and dihydroflavonols (2,3). Arylidene flavanones are also known as flavindogenides. Basic structure of E-3 Arylidene flavanones has four rings: Ring A, Ring B, Ring C and Ring D.



In addition to basic structure of flavanone, E-3 - arylidene flavanone have an extended conjugation at C-3 with carbonyl group. The special feature of long conjugation with the keto groups of flavanone moiety is expected to impart very significant biological activity or this type of cmpounds.

Krishna murthy (4) suggests E-3 arylidene flavanones and their heterocyclic analogues have poor solubility in aqueous medium starting either from 2-hydroxy chalcone or from o-hydroxy acetophenone. The reactivity of condensing aldehyde is an important factor in the synthesis. Presence of alcohol enhances the formation of products. This fact have been utilized for alkali catalyzed synthesis of many arylidene flavanones been reported by them. The special feature of long conjugation with 4-keto group of flavanone moiety is expected to impart very significant biological activity of this type of compounds.

Seiket *et al* (2) and Sha *et al* (3) reported the formation of 3-arylidene flavanone in alkaline medium. Chawla et al[06] reported the synthesis of

seven 3- arylidene flavanone by condensing 2 hydroxy acetophenones with aromatic aldehydes in aqueous alkaline medium, along with corresponding chalcones. According to these authors arylidene flavanones were accessible only by a low-yielding circuitous route. Knishnamurthy *et al* (3) concluded that 3-arylidene flavanones are obtained by acid catalyzed condensation between flavanone and aryl halide.

Their first representatives were synthesized by katshalowsky and kostanecky in 1904 (07). For a long time, E-3 arylidene flavanones (E-34) were synthesized solely by the acid catalyzed condensation of flavanones (08) and aromatic aldehydes (09-013. The reaction usually has been performed in alcoholic solution saturated with anhydrous hydrochloric acid at various temperatures and for different time. Albert levai et al[16] introduced a very simple base-catalyzed condensation for the synthesis of E3-arylidene flavanones. A mixture of equimolar amounts of flavanones and aromatic aldehyde and a few drops of piperidine was allowed to react at 150°c and E-3 arylidene flavanone was obtained in good yield without any purification. On the basis of ^{1}HNMR spectra Keane *et al* (17) explained the stereochemistry of synthetic E and Z- 3- arylidene flavanones.

So here is an attempt made to synthesise a few E-3 -arylidene flavanones by one pot method and to screen the synthesized compounds for the analgesic, anti-oxidant and antibacterial activities .

MATERIALS & METHO DS

For the synthesis of proposed compounds, 2'-hydroxy,4-methoxy acetophenone have been purchased from Sigma Aldrich chemical company Inc. U.S.A, Furfuraldehyde, P-Chloro benzal-dehyde, and p-methoxy benzaldehyde have been purchased from S.D fine chemicals, Mumbai.

INSTRUMENTS USED

U.V : Beckman 650 iu Spectrophotometer

I.R : Shimadzu – FTIR 8300

^{1H} NMR: Varian Gemini-200 MHz

SYNTHETIC PROTOCOL:

The proposed cmpounds were synthesized as per the following procedure;[18] One pot method: To a mixture of 2'-hydroxy,4-methoxy acetophenone (1 mM) and aromatic aldehyde (2.5 mM),a warm (45^{0}c) aqueous alcoholic solution of potassium hydroxide(15%) added and stirred the solution to get a uniform solution. The solution stand for four days in a stoppered condition. Methanol added dropwise to remove turbidity formed on cooling. The separated material washed with cold aqueous alcohol(50 % methanol). Then crystallized from aqueous alcohol. Each compound have been synthesized in the same manner.

Biological Experimental Protocol for Analgesic activity:

To study the analgesic activities of the synthesized compounds, albino mice of either sex were used. All mice were screened by exposure to thermal stimulus. Mice weighing between 20-25 g selected and made into six groups having six animals in each group. The first group served as control which received 2% gum acacia suspension. Second group served as standard,which received diclofenac sodium orally at a dose of 200mg/Kg body weight of animal (suspension of test compounds(20mg/ml)were prepared in 2% gum acacia). Animals were placed on perspax cylinder on heated surface and the time to exhibit discomfort reaction(licking paws or jumping) was considered as reaction time with the cut off time being 60 seconds. The first reading was taken immediately after administration of compounds and afterwards at the intervals of 30 minutes. The results were recorded.

EXPERIMENTAL PROTOCOL FOR ANTI-OXIDANT ACTIVITY:

Equimixture of 1,1-diphenyl-2-picryhydrazyl(3.9 mg in 10 ml ethanol) and test compounds (10 mg /10 ml ethanol) mixed and kept for 20 minutes at room temperature. Then absorbance measured at 517 n.m. Curcumin used as standard drug to compare the activity.

EXPERIMENTAL PROTOCOL FOR ANTI-BACTERIAL ACTIVITY:

The media used in present study, nutrient agar and nutrient broth, were prepared according to Indian pharmacopoeia .The pH of the solution was adjusted to 6.5-6.6 by using 1M sodium hydroxide and 1m hydrochloric acid. Then it was sterilized for 30 minutes at 15lbs pressure. 10mg of each test compound was dissolved in 10ml of DMF (dimethyl formamide) in serially and suitably labeled sterile test tubes, thus giving a final concentration of 100μg/0.1ml . Using sterile pipettes the standard and the sample solutions (0.1ml) of known concentrations were fed into the bored cups. As Cup-1: Standard (ciprofloxacin).Cup-2: solvent control (DMF).Cup3-: Test compound.

ASSESMENT OF SYNTHESIZED COMPOUNDS

Physical datas tested compounds are as follows. Ethanol had used as solvent to find out λ -max by U.V spectroscopy. KBr pellets used to measure I.R spectrum and $CDCl_3$ used for ^{1} HNMR spectrum .

Compond A:

λ-max- 244 n.m,350 n.m.

I.R(KBr): 1673.8c.m^{-1} (C=O), 1217.8 and 1189.6 c.m^{-1} (C-O-C), 854.1 and 812.2 c.m^{-1} (C-H def), 1474.8 and 1459.4 c.m^{-1} (C=C)

^{1}HMR ($CDCl_3$ ppm): 7.04-7.11(H-2), 6.19-6.22 (H-3', H-4'), 6.44-6.52 (H-6), 6.53-6.61 (H-4"), 7.24 ($CHCl_3$), 7.35-7.4 (H-5'), 7.52-7.59 (H-5"), 7.9-7.93 (H-5), 3.8-3.9 (OCH3-proton), 7.62-7.64 (H-β).

Compound B:

λ-max- 230 n.m,360 n.m.

I.R(KBr): 1668.12c.m^{-1} (C=O), 1361.5 and 1249.65c.m^{-1} (C-O-C), 943.02 & 844.669 c.m^{-1} (C-H def), 1637.27, 1523.49 & 1454.06(C=C)

^{1}HMR ($CDCl_3$ ppm): 6.59-6.625(H-2), 6.82-7.0 (H-6, H-8, H-3', H-5'), 7.2-7.43(H-7, H-2', H-6', H-2", H-6"), 3.7-3.9 (OCH_3 proton), 9.1-7.95 (H-5), 3.99 (OCH_3), 8.02-8.12 (H-β).

Compound C:

λ-max-258 n.m, 290 n.m.

I.R(KBr):1668.2c.m $^{-1}$(C0=O), 1250.66 and1146.4c.m $^{-1}$(C-O-C), 854.4 and 824.2 c.m $^{-1}$(C-H def), 748.2 c.m $^{-1}$(monochloro), 1604.2and 1510.4 and1472 and 1458.6c.m $^{-1}$(C=C) .

^{1}HMR($CDCl_3$ppm):6.58-6.63(H-2),6.8-7(H-6,H-8,H-3',H-5',3",H-5"),7.2-7.42(H-7,H- 2',H-6',H-2',H-6"), 3.98 (OCH_3), 8.04-8.1(H- β).

RESULTS

As per the synthetic protocol three E-3 Arylidene flavanones have been synthesized and screened their analgesic activity by hot plate method. Anti-oxidant activity tested by spectrophotometric method.Antibacterial activity by cup-plate method. Observations for analgesic activity are shown in table1 , that of anti-oxidant activity in table 2 and antibacterial activity in table-3.

Table:1 Analgesic activity of synthesized compounds.

Compound I.D	Dose(orally) Mg/Kg	Average reaction time in seconds [a] 0	30	60	90
A	200	3.05	3.10	3.10	3.10
B	200	2.50	2.6	2.6	2.55
C	200	3.00	4.00	6.00	6.08
Std.	200	3.00	5.25	8.25	8.28
Control	-----	3.00	3.00	3.00	3.00

a=Average reaction time expressed as mean (±S.D)of a group.

Table2:Anti-oxidant activity.

Compound I.D	**Absorbance at 517 n.m**	**Relative % activity considering that of standard as 100%**
Curcumin(Std.)	2.596	100%
A	2.142	82.5%
B	2.012	77.5%
C	2.482	95.6%

Table:3 Antibacterial activity.

Compound I.D	Zone of inhibition in m.m E.Coli	Pseudomonas aerugenosa	Staphylococcus aureus	Bacillus subtilis
A	12	-	9	-
B	23	20	-	20
C	18	-	16	24
Std.(Ciprofloxacin)	28	26	24	32

DISCUSSIONS

Three E-3 Arylidene flavanones have been synthesized by one pot method which reduces the usual tedious multisteps involved in the synthesis of medicinal compounds. The results of the assessment of synthesized compounds have good agreement with the datas given in the literature. Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, as per the studies two were found to exhibit analgesic action. The results shows less analgesic activity for all tested compounds than the standard drug namely Diclofenac sodium. Among the three compounds compound C showed maximum analgesic activity. Compound B showed least analgesic activity. Among the three compounds exhibited analgesic activity ,the compound C showed more activity than others, probably due to the presence of halogen atom.

Considering anti-oxidant activity, compound C showed maximum activity and compound B showed least activity. More anti-oxidant activity of compound-C may be due to the presence of chlorine. In anti-bacterial studies all compounds exhibit activity against E.Coli. Only compound B showed activity against Pseudomonas.Compound C showed good activity against Staphylococcus aureus. Compound B as well as compound C showed good activity against Bacillus subtilis.

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MICROBIAL SPECTRUM OF PELVIC INFLAMATORY DISEASES IN NGURU, NIGERIA

Okon, K.O., Ayilara R., Bello K., Uba A., Aniesona T. A.

Department of Medical Microbiology, University of Maiduguri Teaching Hospital Maiduguri.
Pathology/Obstetritic & Gynecology department, Federal Medical Centre Nguru, Yobe State.
Department of Biological Sciences, Abubakar Rafawa Balewa University, Bauchi
Department of Veterinary Microbiology and Parasitology, University of Maiduguri

Corresponding Address: Okon, K.O. Department of Medical Microbiology University of Maiduguri Teaching Hospital P.M.B. 1414 Maiduguri. E-mail: okon_kenneth@yahoo.com

ABSTRACT

Pelvic inflammatory diseases, a leading gynecological problem worldwide, are associated with socio-economic and psychological costs. A retrospective study of 1350 high vaginal swabs analyzed between Jan-Dec. 2005, showed that 845 (62.8%) were positive for 9 microorganisms by culture/or wet preparation. Microbial growth was found in 645 (76.3%) cases. Polymicrobial growth was found in 90 (10.7%), fungal growth in 110 (13.0%) cases, and 3(0.4%) yielded anaerobic growth. *Staphylococcus aureus* accounted for 355 (42.0%) cases, followed by *Escherichia coli* 190 (22.5%), *Trichomonas vaginalis* 100 (11.8%) *Candida spp* and *Neisseria gonorrhoeae* 70 (8.3) and the least, *Pseudomonas spp* 5 (0.6%) Microbial-associated infection was prominent in the group 21-30 years old (46.6%) and 31-40 (23.9%) years respectively. Antibiotic susceptibility pattern showed that mean susceptibility greater than 50% were recorded with ofloxacin 80%, ceftazidime 80%, rifampicin 81.9% compared to mean susceptibility less that 50% recorded with trimethoprim-sulthamethoxazole 34.7%, and ampicillin 26.1%.

In conclusion, the reported microbial-associated infection in PID with a prevalence of 62.8% is of public health importance. Early diagnosis of causative agents and prompt institution of chemotherapeutic agents will help to prevent clinical complications that are expensive to treat.

Keywords: pelvic inflammatory diseases, microorganisms, antibiotic susceptibility.

INTRODUCTION

Pelvic inflammatory disease (PID), is an infection of the upper genital tract in women that include endometritis, parametritis, salpingitis, oophoritis, tubo-ovarian abscess and peritonitis (1,2). It accounts for 5-20% of hospital admissions for gynecological problems in general/gynecological clinics worldwide (3). In USA, infertility that affects approximately 10-15% of all couples attribute tubal damage due to pelvic infection (4,5). Clinical presentation varies in severity, and ranges from sub clinical, asymptomatic infections exerting medical and psychological cost that include chronic pelvic pain, ectopic pregnancy and infertility (1). It has been associated with increase risk of ovarian cancer6-8. The pathogenesis is complex interaction of genetic, immunological and bacterial virulence factors (9).

The prevalence and incidence of PID varies greatly, because of significant misdiagnosed/or unreported cases. In developed countries, annual incidence of PID increased in women aged 15-45 years, with peak of infection in 20-24 years (10). Polymicrobial agents are associated and initiated pathogenesis of PID, particularly in presence of facultative aerobic and anaerobic bacterial isolates (11-14), with *Niesseria. Gonorrhoea* and *Chlamudia tracomatis* as leading pathogens, accounted for 60-80% in women of aged less than 25 years12-14. Other less pathogenic mycoplasma, and endogenous aerobic and anaerobic bacteria have also been implicated (15). Co-existence of sexually transmitted diseases (STD) etiological agent in genital tract predispose the women to acquisition of PID (1,16). Korn *et al (*17) reported that clinical presentation and course of PID in women with sy,ptomatic HIV disease and/or severe immune suppression may be more aggressive than in HIV negative women.

Clinical diagnosis is rather difficult, as no single clinical and laboratory test in definite as gold standard, thus comibination of test seems to impove sensitivity and specificity (18,19). Epidemiological and microbiological indices associated with PID are important source of preventable reproductive infertility in women, and other clinical squealed. Little information is available on PID epidemiology in this environment, this there is no baseline in assessment of its relationship in case of infertility and HIV infection.

Early diagnosis/treatment of PID could stemmed down the effect on the fallopian tubes; and in case of microbe-related inflammation and tubal necrosis can similarly precedes manifestation of symptoms, especially in aetiological agent due to chlamydial3. Prompt diagnosis and institution of appropriate antibiotic therapy would prevent possible sequelae of PID. The retrospective study examined the aetiological spectrum in high vaginal swabs of pelvic inflammatory diseases in this environment.

MATERIALS AND METHODS

Study Site

The retrospective study was conducted in Federal Medical Center, Nguru, between Jan-Dec 2005, which involved the Pathology and Obsterictic/Gynecology departments. The patients folder presented at the general out-patients/gynecology clinic, with clinical complaint suggestive of pelvic inflammatory diseases, ranged from pelvic viginal discharge to lower abdominal pain, with high vaginal swabs collected and sent for bacteriological analysis. Criteria of inclusion are consecutive non-duplicate high vaginal swabs, repeated swab analysis and mixed growth of doubtful significance were excluded. Information retrieved from the patients folder included age, sex, and clinical complaint.

Processing of the Specimens

The high vaginal swab was processed, with inoculation on Blood, Chocolate and Sabouraud agar plates, incubated at 37^0C for 24hours. Bacterial/yeast were identified by standard bacteriogical and mycological techniques (20-22). Yeats were further identified by germ tube

test. Direct smear was prepared stained by Gram methods, and wet preparation of the specimen for parasitic examination. Antibiotic susceptibility testing was determined by disc diffussion23, using the following antibiotic discs, ofloxacin)OFX), ciprofloxacin (CPX), perfloxacin (PEF), ceftazidime (CAZ), cefuroxime (CXM), rifampicin(RF), strepromycin(S), tetracycline (TET), trimethoprim sulthamethoxazole (SXT), anpicillin (AMP), gentamycin (CN), erythromycin (E), and augmentime(AU). The zone of inhibition of the disc was measured to determined whether resistant or sensitive in accordance to NCCLS guidelines (23). The mean susceptibility percentage of each antibiotic was calculated as the number of bacterial isolates susceptible divided by total number of bacterial isolates tested multiply by 100.

Data Analysis

Data and information retrieved from patients flders were entered into study datavasem using SPSS version13.0. The value were expressed mean and percentage, and appropriate statistical package where necessary.

RESULTS

Of the 1350 high vaginal swabs results analyzed, 846 (62.8%) were positive for 9 microorganisms by culture/or wet preparation examination (7 bacterial pathogens, 1 fungi and 1 protozoan) as in table 1. The means age of the patient was 22.4 + 2.7 years. The ratio of gram-negative bacteria ratio was 1:2:5 Monomicrobic growth was recorded in 645 (76.3%), polymicrobic growth in 90 (10.7%) and bacterofungal in 110 (12.0%) cases. Three (0.4%) cases yielded anaerobic growth.

Staphylococcus aureus was the accounted for 355 (41.9%) cases, followed by *Escherichia coli* 190 (22.4%), *Trichomonas vaginalis* 100 (11.8%), *Neisseria gonorrhoeae* and *Candida spp* 70 (8.3%) repetitively. Microbial-associate infection distribution, in accordance with the age group of the patients studied (tableII), frequency of occurrence was predominant with the age group 21-30 (46.7%) and 31-40(23.9%) years and least in 10-20(8.1%) and >51(6.4%) years respectively. There was statistically significant difference between microbial infection and the age-group ($p<0.05$). Similarly, there was a decreasing trend pattern in frequency of occurrence of microorganism and age-group.

Antibiotic susceptibility pattern of bacterial isolates as shown in table III, showed that mean susceptibility percentage greater than 50 was observed with ofloxacin, gentamycin, ciprofloxacin, pefloxacin, rifampicin, cefuroxime, ceftazidime, erythromycin and streptomycin, and less than 50 in trimethoprim-sulthamethoxazole, tetracycline, ampicillin, and augmentime.

Table I: Frequency of occurrence of Microorganisms Isolated

Microorganisms	Frequency of Occurrence (%)
Gram-positive bacteria (n=370)	
Staphylococcus aureus	355 (42.0)
Streptococcus spp.	15 (1.8)
Gram-negative bacteria (n=305)	
Escherichia coli	190(22.5)
Neisseria gonorrohea	70(8.3)
Klebsiella spp	30(3.6)
Proteus spp.	10(1.2)
Pseudomonas spp.	5(0.6)
Anaerobic bacteria	3(0.4)
Fungi (n=70)	
Candida spp	70(8.3)
Parasites (n=100)	
Trichomonas vaginalis	100(11.8)
Total	**848**

Table II: Distribution of bacterial isolates according to age-group of patients studied

Age-group	S. aureus	Strep. Spp	Kleb spp	E.coli	Proteus spp	Pseud. spp	N.gonorrhea	T. Vaginalis	Candida spp	Anae. bact	Total
10-20	30	-	-	10	-	1	5	16	7		**69**
21-30	115	10	15	95	8	-	40	74	37	2	**396**
31-40	100	3	8	45	2	-	15	8	21	1	**205**
41-50	75	2	5	25	-	1	10	2	5	-	**125**
>51	35	-	2	15	-	3	-	-	-	-	**55**
Total	**355**	**15**	**30**	**190**	**10**	**5**	**70**	**100**	**70**	**3**	**848**

Table III: Antibiotic susceptibility pattern of the bacterial isolates (% susceptibility)

BACTERIA ISOLATES	OFX	CN	CIP	PEF	SXT	AMP	RD	E	AU	S	CXM	CAZ	TET
S.aureus	**90**	**65**	**80**	**89**	**25**	**21**	**89**	**75**	**60**	**70**	**85**	**72**	**45**
E.coli	**82**	**70**	**75**	**83**	**43**	**26**	**78**	**65**	**72**	**73**	**78**	**80**	**54**
Kleb spp	**78**	**56**	**78**	**78**	**45**	**35**	**85**	**67**	**56**	**67**	**76**	**82**	**42**
Strep spp	**90**	**82**	**89**	**79**	**50**	**42**	**90**	**90**	**80**	**80**	**84**	**79**	**35**
Proteus spp	**75**	**67**	**75**	**74**	**35**	**22**	**76**	**69**	**67**	**67**	**73**	**80**	**45**
Pseudo spp	**60**	**42**	**73**	**67**	**20**	**12**	**65**	**52**	**45**	**45**	**75**	**82**	**38**
N.gonorroheae	**85**	**65**	**73**	**74**	**25**	**25**	**90**	**68**	**50**	**78**	**80**	**85**	**38**
Mean Susceptibility(%)	**80**	**63.7**	**77.6**	**77.7**	**34.7**	**26.1**	**81.9**	**69.4**	**69.4**	**68.6.**	**78.7**	**80**	**42.2**

DISCUSSION

Clinical significance of PID becomes pronounced because its association with sexually transmitted diseases/HIV/AIDS infections. Particularly in asymptomatic individuals who may laer present with various complications irrespective of the social and psychological cost (19,24). Consequently, a dramatic increase in the

the incidence of PID has led to a parallel increase in infertility (25).

The reported prevalence of microbial-associated infection in PID of 62.8% of our patients is high. Our report is similar to the rates reported in similar studies conducted in Sokoto (26) and Gombe (27) of same geographical zone. However, comparison of PID prevalence in studies conducted at different geographical location/countries might be rather difficult, because of certain inherent biases involved, particularly presence of PID-related infections (10,28-30). Similarly, PID prevalence are influenced by variation in case definition (particularly between different clinical settings), changes in disease chronicity associated with clinically mild Chlamydia infection, variation in health seeking behaviour and increase management of PID in outpatient setting (31,32).

The frequency of occurrence of microbial-associated infection was high in the age group of 21-30(46.7%) and 31-40(23.9%) years. This finding simply confirms reported findings that highest PID prevalence and highest rate of increase are associated and seen in the 16-24 years age groups, and substantial numbers of bacterial sexually transmitted infection are high these age group (16-193,6,19,33). Also PID accounts for approximately 60% of gynecological problems in women aged less than 25 years34. High prevalence of PID episodes in sexually active age group, re-emphases the correlation that co-existence of aetiological agent in the genital tract of the females predisposes to acquisition of PID (3,19,33-35). Some studies found demographic risk factors associated with PID, like sexual activity at young age, racial, and both pre-delivery history and post-partum diagnosis of chlamdyial and gonococci infections (36,37). However implication of these factors in this environment need further evaluation.

From the present study, 10 microorganism (8 bacterial pathogens, 1 fungal and 1 protozoan) were recorded. S. *aureus* was the commonest and accounted for 42%, polymicrobial infection wasfound in 10.7% of cases and fungal infection in 13.0% of cases. This pattern simply confirms polymicrobial spectrum aetiology associated with pathogenesis of PID (11-14). S. *aureus* (42.0%), and *E.coli* (22.5%) were predominant bacterial isolates in the study, thesepathogens are most isolated in lower genital tract infections; and are responsible for a significant proportion of sexually transmitted diseases in Nigeria (26,38-40). The dominance of these bacterial pathogens as STI pathogens and their existence in the female genital tract clearly reaffirmed it as a predisposing factor in acquisition of PID (1,16). Polymicrobial infection with other organisms such as anaerobes or faculitative aerobes may be initiated by gonorrhea, chlamydia or both (4,5,15,35). The low frequency of occurrence of *N.gonorrhoeae* as evident in this study, might probably be due to variation in the studied population, method of microbial investigation, variation in severity of the diseases, sampling technology and site of sampling (40). Technically, *N. gonorrhoeae* is highly fastidious fragile organisms, isolation is dependent on viability of the organism in the specimen, prompt delivery to specimen, and suitability of isolation medium.

Trichomonas vaginalis with a prevalence of 11.6% in a center posed public health problem, because of close association of trichonomasis with HIV infection (42-45). *T.vaginalis*, is an irritating protozoan and is a common parasitic sexually transmitted disease reported worldwide (45). It is associated with inflammation of the cervix that may mimic cervical tenderness associated with PID (42). Buve et al (43) reported that trichomonasis incidence is higher in cities where there are higher number of HIV-positive individuals. The high prevalence of trichomonasis and candiasis observed in this study basically revealed close association of poor personal hygienic conditions especially among the low socio-economic class and transmitted sexually, particularly in cases of multiple sex partners (10), with high probability of PID infection.

The in-vitro antimicrobial susceptibility pattern of bacterial isolates revealed that mean percentage susceptibility of greater than 50% was observed with the fluoroquinolones (ofloxacin, ciprofloxacin, perfloxacin), cephalosporins (ceftfazidime, cefuroxime) and rifampicin ; and those less than 50% observed with gentamycin, erythromycin, augmentine and streptomycin, and least susceptibility observed in tetracycline (42.2%) trimethoprim-sulphamethoxazole (34.7%) and ampicillin (2.1%). These antibiotic susceptibility patterns are similar to reports by other workers (26,27). The reduced susceptibility of antibiotics like ampicillin, tetracyclines anmd trimthoprim-sulphamethoxazole, clearly revealed the abuse of these agents by self-medication practice, a common norm in most towns/cities in many developing countries.

The fluoroquinolones showed favourable in-vitro susceptibility pattern that could serve as drugs of choice in PID treatment/management. However, documented studies have reported emergence of fluoroquinolones-resistant *N.gonorrheae* (3). With considerable numbers of antibiotics resistants strains, particularly of *N. gonorrhoeae* to penicillin and cephalosporins (particularly 1st generation), the used of second-generation cephalosporins that posses extended spectrum activity over wide ranged of microorganisms associated with PID3. from the in-vitro antibiotic susceptibility pattern of the study, gentamycin, erythromycin and streptomycin, could served the antibiotic of choice considering the relative cost and also posses extended-specturm activity. One of the greater threat to the efficacy of antibiotics is the presence/or emergence of resistant strain, thus, cautious approach is required in prescription/administration, as safeguard policy against possible emergence of muliresistant strain in a remote city, like Nguru.

In conclusion, the prevalence of microbes-associated PID of 62.8%, is high. It is important that microorganisms associated with PID are diagnosed early and appropriate chemotherauptic treatment/management commenced, as clinical complications are always very expensive to treat.

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