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LIPID PROFILE OF DRUG NAIVE HIV PATIENTS IN A TERTIARY HEALTH FACILITY IN LAGOS, NIGERIA

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ABSTRACT

Aim: To determine the effect of HIV syndrome on lipid profile in a cohort of Nigerians.

Objective: To determine the concentrations of total cholesterol (Tchol), triglyceride (TGL), high density lipoprotein (HDL), and low density lipoprotein (LDL) in HIV positive drug naive patients as against HIV negative people.

Methods: This study examined the lipid profiles of 50 HIV positive individuals (test group), and 50 HIV negative individuals (control group) at the Human Virology Laboratory of Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. Informed consent was obtained and the Institutional Review Board (IRB) of NIMR approved the study. A questionnaire based assessment was provided for the individuals to complete, before 5 ml of blood was taken by venopuncture. Blood collected in plain tubes was centrifuged at 3500 rpm for 10 minutes and the serum obtained, used for the various lipid profile tests mentioned above. Statistical analysis on data from the questionnaire was done using *Epi info 2000 (CDC).*

Results: The median lipid profile values for the control group were 168, 85, 99 and 58 mg/dl for Tchol, TGL, HDL and LDL respectively. Conversely, median lipid profile values for the HIV positive patients were 145, 98, 53, and 67 mg/dl for Tchol, TGL, HDL and LDL respectively. P values greater than 0.05 were taken to indicate an insignificant difference between the lipid profiles of the two groups.

Conclusion: Based on results obtained, there were significant differences in the Tchol and HDL values between the two groups, indicating a possible effect of HIV on lipid profile for drug naive patients.

Key words: lipid profile, HIV, TGL, HDL, LDL, Tchol.

INTRODUCTION

Lipids, a diverse group of compounds which serve as storage compounds, structural components, cofactors and pigments, are found almost universally as stored forms of energy in living organisms as derivatives of fatty acids. Phospholipids and sterols are components of cell membranes [1]. Some serve as hormones, or as intracellular messengers generated in response to an extracellular signal. Others function as enzyme cofactors in electron-transfer reactions in chloroplasts and mitochondria [1]. Fairly current knowledge regarding the etiology of altered lipid metabolism in HIV-positive patients does not afford a clear picture as to whether HIV disease itself or antiretroviral therapy (ART) is largely to blame; there are proponents on each side of the debate [2].

HIV-infected individuals, both those on ART and those who are untreated, appear to have higher rates of coronary heart disease (CHD) than HIV-uninfected individuals and higher rates of various risk factors for CHD, including dyslipidemia. HIV itself causes lipid perturbations, particularly in persons with more advanced immunosuppression; HIV-infected individuals who are not on antiretroviral (ARV) medications often have elevations in triglyceride (TG) levels and decreases in high-density lipoprotein (HDL) as well as in low-density lipoprotein (LDL) and total cholesterol (TC). Lipid abnormalities also may be caused by or compounded by ARVs [3].

Studies on lipid profile have focused on antiretroviral drug-induced lipid changes of HIV infected people.

Findings have been controversial, with some studies stating an increased lipid profile [4,5], and others reduced levels of some lipids. Few studies have documented drug naive lipid profile changes.

Cases of AIDS have been reported in every nation of the world, yet the disease affects some countries more than others. More than 95% of all HIV-infected people live in the developing world [6,7]. The use of effective ART has resulted in tremendous improvements in morbidity and mortality of HIV-infected individuals [7]. However there have been various studies linking HIV antiretroviral therapy with excess lipid depositions and abnormalities [8]. The widespread use of effective ART regimens has coincided with increasing reports of metabolic abnormalities such as impaired glucose metabolism and insulin resistance, lactic acidosis, osteopenia, and dyslipidemia [8].

Nutrition is of vital importance because it improves resistance and slows the progression of AIDS. Nutritional needs of HIV patients include increased intake of protein, vitamins, minerals and enough kilocalories to prevent weight loss and to meet increased metabolic demands.

Although lipid metabolism in HIV patients on ART and drug naive HIV patients (much less) has been widely studied across the globe, none to our knowledge has been done in our locality. Hence this study was undertaken to investigate the lipid profile of HIV patients, to assess if HIV infection is implicated in aggravating the normal lipid profile of Nigerians living with HIV.

METHODS

STUDY POPULATION

This was a case controlled study carried out between HIV positive drug naive and HIV negative persons. Recruitment period was from May to July, 2010. One hundred individuals were recruited, whose ages ranged between 20 to 55 years, with a mean of 34.2 years. Fifty HIV positive and 50 HIV negative, apparently healthy individuals were recruited from the Human Virology and the Clinical Diagnostic Laboratories of the Nigerian Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria. The individuals were randomly selected using a simple random sampling technique. The HIV negative persons were mostly Staff and Interns of the two laboratories. Inclusion criteria for the study were HIV seropositivity and antiretroviral naive status with clinical/laboratory stage of CD4 above 350 cells/µl and HIV viral load less than 400 RNA copies/ml.

ETHICAL CLEARANCE; INFORMED CONSENT

Ethical clearance was obtained from the Institutional Review Board of NIMR. Informed consent of patients was obtained before including them in the study. The study did not interfere with clinical management and the confidentiality of patients was maintained by de-identifying the specimens.

QUESTIONNAIRE DISTRIBUTION

Copies of a questionnaire were distributed to the 100 people, where data on physical parameters, (age, sex, etc) eating habits before and after HIV diagnosis were collected. The Control group (50) did not answer the 'After diagnosis' section of the questionnaire. The questionnaires were collected after filling, entered and analyzed by *Epi Info 2000 (CDC)*.

COLLECTION OF BLOOD SAMPLES

Five ml of blood was collected by clear venopuncture into plain vacutainers. The samples were centrifuged for 10 minutes at a speed of 3,500 rpm and the serum separated.

CHOLESTEROL ANALYSIS

Cholesterol was determined by enzymatic colorimetric method of *Randox (United Kingdom)* [9]. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase to give a pinkish coloration.

TRIGLYCERIDE ANALYSIS

Triglyceride was determined using *Randox* kits; a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide which reacts with

4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dye (Trinder endpoint reaction).

LOW DENSITY LIPOPROTEIN ANALYSIS

LDL was determined using a direct method (*Randox*). This method takes advantage of the selective micellary solubilisation of LDL by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons).

HIGH DENSITY LIPOPROTEIN ANALYSIS

HDL was determined by an enzymatic colorimetric method (*Randox*). This method uses polyethylene glycol-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order: LDL< VLDL ≈ chylomicrons < HDL.

RESULTS

Mean weight was 67.3 kg for the control group (HIV-) and 62.6 kg for the test group (HIV+), giving an insignificant difference ($p>0.05$) between them. Their mean heights were 1.61 m and 1.63 m for the control and test groups respectively. Sex distribution male: female ratio was 15:35, and 26:24 for the test and control groups respectively. Mean age was 34.2 years for both the control group and HIV positive individuals. The marital status showed 64% and 54% were married; 36% and 42% were single; while 0% and 4% were separated for the test and control groups respectively (Table 1).

From analysis of the questionnaires, eating habits before diagnosis and after, showed a slight improvement towards healthy eating in the test group. Figure 1 presents the lipid profile of the two groups of people with normal ranges (low and high) beside each parameter. Median total cholesterol, triglyceride, HDL and LDL was 168, 85, 99 and 58 mg/dl for the control group respectively; and 145, 98, 53 and 67 mg/dl for the test group respectively (Table 2).

DISCUSSION AND CONCLUSION

In this study, lipid profile was comparatively analyzed for 50 HIV positive and 50 HIV negative individuals in Lagos, South-western Nigeria. Both groups were closely matched in their mean ages (both 34.2 years). Heights and weights were also in general, fairly average. Body Mass Index (BMI) was 23.5 and 25.7 for the HIV positive and negative groups respectively. This ironically shows an apparently healthy HIV positive group and a slightly overweight HIV negative group. The observed differences in the values obtained after data analysis were statistically significant ($p<0.05$) for two of the parameters tested, Tchol and HDL, but insignificant for TGL and LDL. Significantly higher Tchol and HDL values were observed for the HIV negative group than the positive group.

These findings may be a factor pointing towards an initiation of body fat redistribution in the HIV infected people. Altered lipid metabolism is known to affect immune processes. Caraciolo *et al* [10] characterized the lipid profile of asymptomatic untreated HIV positive individuals compared with a control group. They investigated the relationship between serum lipid levels and CD4 lymphocytes (CD4) and viral load (VL) of asymptomatic untreated HIV positive men who have sex with men (MSM),

TABLE 1: PHYSICAL PARAMETERS OF THE 50 HIV POSITIVE (TEST) AND 50 HIV NEGATIVE (CONTROL) GROUPS

PARAMETER	HIV positive	HIV negative	P value
mean Weight (kg)	62.6	67.3	0.05
mean Height (m)	1.63	1.62	0.53
mean Age (years)	34.2	34.2	0.98
Sex (N): male Female	15 25	26 24	- -
Marital status (%) Married Single/Separated	 64 36	 54 42	 - -

TABLE 2: LIPID PROFILE OF THE 50 HIV POSITIVE PATIENTS AND 50 NEGATIVE CONTROL GROUPS (*- statistically significant)

PARAMETER	HIV Positive	HIV Negative	P value
median TChol (mg/dl)	145	168	0.001*
median TGL (mg/dl)	98	85	0.105
median HDL (mg/dl)	53	99	0.000*
median LDL (mg/dl)	67	58	0.614

comparing it with a control group, in Spain. They stated that the slightly lowered lipid variations observed, excluding antiretroviral therapy, hepatic and nutritional effects might be due to HIV action. This study however does not support this assumption.

Elevations in serum TChol and TGL levels, along with dyslipidemia that typically occur in patients with HIV infection, may predispose patients to complications such as premature atherosclerosis, particularly coronary heart disease, and pancreatitis [11]. It remains to be seen if lipid metabolism is altered at initiation of antiretrovirals (PI- or NNRTI-based) as reported in a study [8]. Adewole *et al,* [12] stated that abnormalities in serum lipid are common among treatment naive HIV patients. The results obtained here are in partial support to that of Adewole *et al* [12]. In conclusion, in this study the presence of HIV infection affected some of the lipid profile parameters of the patients tested (altered cholesterol and HDL).

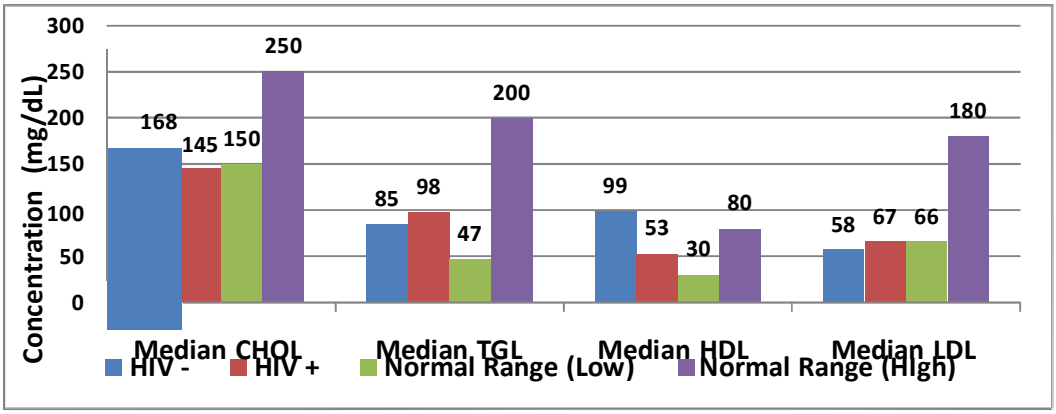

Figure 1: The median lipid profile of the HIV positive (test) and the HIV negative (control) groups

These findings however may be inconclusive due to the relatively small sample size utilized in this study. A broader study, involving the immunologic parameters (CD4, CD8), and viral load is recommended to further validate the findings of this study.

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STUDY OF ORAL AND GINGIVAL MICROBIAL FLORA IN INSTITUTIONALIZED MENTALLY RETARDED PATIENTS OF SARI- 2011

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ABSTRACT

Introduction and Objectives: Mental retardation (MR) is a generalized disorder appearing before adulthood, characterized by significantly impaired cognitive function and deficits in two or more adaptive behaviors. The prevalence and severity of dental caries,gingivitis and periodontitis is high in patients with mental retardation. This shift to a diseased state may lead to the experience of a high mortality from septicemia,sepsis,pneumonia and endocarditis.bOur purpose was to study oral and gingival microbial flora in institutionalized mentally retarded patients of Sari and to estimate D% (percentage with untreated decayed teeth) and DMFT% (percentage of population affected with dental caries)

Materials and Methods: This study was a descriptive cross-sectional type in which Plaque samples were collected from the mouth and gingiva of 138 institutionalized mentally retarded patients of Sari to culture in specific media to identify the microorganisms. In this study anaerobic bacteria were not isolated because the instrument was not available in the laboratory. The information has been analyzed by X2 T-test methods by SPSS 17 software.

Results: The isolated microorganisms were: pnuemococci S(37.7%); Streptococci sp(18.8%); E.coli (16.7%); Staphylococcus(1.4%); Neisseria sp(45/6%); Salmonella(8.7%); Proteus(3.6%); Diphteroid (4.2%); Pseudomonas(0.7%). The percentage of resistant strains was found to be highest with penicillin(67.9%) and lowest with vancomycin(11%).

Conclusion: D% between all the patients were (66.66%) . Bacterial flora in mentally retarded patients were significanty higher in frequency than in normal persons. With improvement in oral health care, we can decrease these undesirable changes.

Key words: Oral and gingival microbial flora, Mental retardation, D%, Sari

INTRODUCTION

Mental retardation (MR) is a generalized disorder, which appears before adulthood and is characterized by impairment in cognitive ability and deficits in two or more adaptive behaviors (1). The prevalence and severity of dental caries, gingivitis and periodontal infection are high in patients with mental retardation. Surfaces of teeth and gum are colonized by a dense population of microbes, called plaques, which is the main factor for dental caries. Considering that dental caries and gingival diseases occur earlier in mentally retarded patients than in normal people, it is more prevalent in these patients due to their mental and physical disabilities and inability to effectively brush their teeth and properly and regularly use mouthwashes (2-3-4). In addition to these factors, mental disabilities and motor skill disorders such as oral breathing, lip seal, the diet causing dental caries, difficulty in chewing and swallowing, abnormal stretch of face muscles, reduction of saliva flow and effects of medicines should be mentioned (2). Washing these people's teeth by nurses also involves many problems and patients have little collaboration in this regard (5). Thus, conducting this study seems necessary for physically disabled people.

Normal oral flora (the flora with proper hygiene in the mouth) can be considerably changed. Weak hygiene of the mouth leads to increased microorganisms and their pathological types in oral flora. This shift toward sickness increases mortality due to septicemia, pneumonia and endocarditis (5). In addition to the mentioned problems, it has been reported for mentally retarded patients that their bacterial resistance of mouth to the conventional antibiotics used in dentistry is different from that of normal people (6).

Different studies have been conducted to study orodental hygienic condition and prevalence of dental caries, gingivitis and periodontal infection in mentally retarded people (7&8). Different studies have been conducted to investigate their microbial flora of gingival and dental plaques and its difference from that of healthy people along with its relationship with respiratory infections. Some other studies have been done to compare antibiotic sensitivity in highly prevalent microorganisms in dental caries of mentally retarded people with that in healthy people. In the present research, for the first time, orodental hygienic condition and prevalence rate of dental caries were studied in mentally retarded people in rehabilitation centers in the city of Sari and oral bacterial flora of these people was investigated.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in 2011 on mentally retarded patients in rehabilitation centers in the city of Sari. Sampling of mentally retarded people in these centers was random. Considering the fact that the present study was conducted for the first time, around 50% (138 people) were statistically sampled. The data were recorded in the related questionnaire, which included questions on their demographics such as gender, age and education and those for their parents and some questions about their orodental hygiene condition such as daily tooth brushing, dental caries, tooth extraction, mouth ulcers and history of oral surgery, all of which were nominal qualitative variables. Questionnaires were filled by research team members while examining the patients, by the assistance of the center authorities using the data recorded in the centers, and by the cooperation of the dentist of the related center.

The samples were directly taken from the mouth (and gum) of the participants using a sterile swab, placed in liquid transport medium (Amies) and transferred to the laboratory of Sari Faculty of Medicine. After the samples were transferred to the laboratory, they were cultured in EMB and blood Agar media. Then colonies were gram stained after 24 h of incubation at 37^{o}C; in case the isolated bacteria were gram positive cocci, catalase test was performed on them. The positive catalase colonies were identified as staphylococcus and coagulase test was performed on them. In case of positive coagulase test, the related colony was identified as staphylococcus aureus and, in case of negative test, novobiocin disc was placed. The colonies sensitive to novobiocin were recognized as staphylococcus epidermidis and resistant colonies were considered staphylococcus saprophyticus. For negative catalase colonies which indicated their streptococcus property, hemolytic test was performed. For the colonies with alpha type hemolysis, optochin disc was used and the colonies sensitive to optochin were identified as pnuemococcus while resistant ones were recognized as viridans streptococcus. For beta type hemolytic colonies, bacitracin disc and SXT were used. The colonies sensitive to bacitracin and SXT resistant colonies were identified as streptococcus pyogenes while SXT and bacitracin resistant colonies were identified as streptococcus sp. In case the isolated bacteria were gram negative baccilus, they were cultured on special enterobacteriaceae media and species of the considered bacteria was identified after comparison with the enterobacteriaceae chart. One of the limitations of this project was failure to study obligate anaerobes.

Resistance and sensitivity of the isolated bacteria to ampicillin, amoxicillin, garamycin, penicillin, vancomycin and trimethoprim sulfamethoxazole were investigated for gram positive bacteria. The same was examined using cifixim, ceftriaxon, tetracycline, jentamycin, trimethoprim-sulfamethoxazole, ciprofloxacin and cefazolin for gram negative bacteria. Antibiogram was performed in Moller Hinton medium using Kirby-Bauer method according to 0.05 McFarland standards. The available data were analyzed in SPSS software (version 17) using Chi square test and ANOVA.

RESULTS

In this study, out of 138 mentally retarded patients (48 patients) in the city of Sari, 31.4% were in Pardis Center and 21.7% (30 patients)

were in each of Mehregan, Sina and Ferdowsi Centers. The studied people were classified into three age groups of below 20, 20 to 40 and above 40. In this study, P<0.05 was considered significant. The studied people included 68.8 % (95 patients) females and 31.2% (43 patients) males. Mean and standard deviation of the studied people's age were 28.28 and 12.05, respectively. 4.3% (6 patients) were literate and 95.7% (132 patients) were illiterate.

36.2% (50 patients) used toothbrush and 63.8% (88 patients) did not use it. 65.9% (135 patients) had dental caries and 34.1% (3 patients) did not. 50.97% (70 patients) had a history of tooth extraction but 49.3% (68 patients) had no record. 10.979% (15 patients) had mouth ulcers but 89.1% (123 patients) did not. 5.1 % (7 patients) had record of oral surgery while 94.9% (131 patients) had no such records. 13.18% (19 patients) used antibiotics every day but 86.2% (119 patients) did not. D% index was estimated in three age groups in all of the studied patients, which was as follows: below 20 (54%), 20 to 40 (76.2%), above 40 (73.3%), total (69.5%). Based on Chi square statistical test, there was a significant relationship between dental caries and age ($x^2 - 6.394$ $df - 2$ P_{value} =). **0.041).**

Frequency distribution of isolated bacteria was calculated from the mouth samples of the mentally retarded patients in this study as follows:

Pnuemococcus 37.7% (52 patients), E.coli 16.7% (23 patients), niseriasp 45.6% (63 patients), streptococci sp 18.8% (26 patients), staphylococci sp 1.4% (2 patients), pseudomonas 0.7% (1 patient), salmonella 8.69% (12 patients), proteus 3.6% (5 patients), diphtheroid 4.2% (6 patients).

In the performed study based on Chi-square test, there was a significant relationship between frequency of isolated bacteria from the mouth samples of the studied mentally retarded patients and the following cases were found: rehabilitation centers (p=0.000, df=45), gender of patients (p=0.000 and df=15), using toothbrush (p=0.25 and df=15), dental caries (p=0.03 and df=15), tooth extraction (p=0.009 and df=15), mouth ulcers (p=0.05 and df=15) and daily antibiotic use (p=0.01 and df=15); however, it had no significant relationship with oral surgery (p=5, df=15).

Antibiotic resistance to the tested antibiotics on gram positive bacteria was as follows: ampicillin 18.5% (15 patients), amoxicillin 50.6% (41 patients), jentamycin 12.3% (10 patients), penicillin 67.9% (55 patients), vancomycin 11% (9 patients), trimethoprim-sulfamethoxazole 65.4% (53 patients).

The antibiotic profile for gram negative bacteria was as follows: gentamycin 0%, tetracyclin 2.6% (1 patient), ceftazidime 13.1% (5 patients), ciprofloxacin 0%, ceftriaxone 5.3% (2 patients), cephotaxin 42.1% (16 patients), cefixim 36.8% (14 patients), trimethoprim-sulfamethoxazole 5.3% (2 patients).

Based on Chi-square test (p=0.00 and df=60), there was a significant relationship between resistance to the tested antibiotics on gram positive and negative bacteria and the rehabilitation centers. Based on ANOVA statistical test, there was a significant relationship between age groups and frequency of isolated bacteria (p=0.009) and antibiotic resistance (p=0.001).

DISCUSSION

The present research showed that the prevalence of dental caries among 138 mentally retarded patients in Sari rehabilitation centers (D %) was 69.5%. The estimated D % was higher than the value reported by Marcia H. Tanaka et al. (2009) in Brazil [2], in which D % was equal to 0%. In the performed statistical study in this research, D% was about 63% in the age group of 7-12 years old, which was lower than the one reported by Alghahtani and Vien (2004) in Saudi Arabia [3], reported as 100%. In this study, D % was about 70% in the age group of 3-30 years old, which was in line with the one reported by Rao et al. (2001) in India (18) as 71.5%.

In this study, the prevalence of pnuemococcus among mentally retarded people was 37.7%, which was higher than its prevalence in oral flora of normal people (25%) according to the International Reference (15).

In this study, the prevalence of streptococcus (sp) among mentally retarded people was 18.8%, which was higher than its prevalence reported by W. J. Losche et al. (1972) (8) as 37.8% and the rate reported by Salaco (2007) in Kuwait (6) as 39%. The estimated prevalence of streptococcus mutans in oral flora of the mentally retarded patients in this research was not significantly different from its prevalence in oral flora of the normal people in the study by Salaco (6).

FIGURE 1: FREQUENCY OF ISOLATION OF DIFFERENT BACTERIA FROM ORAL SAMPLES

BACTERIAL ISOLATE	USE OF ANTIBIOTICS		TOTAL (%)
	YES (%)	NO (%)	
Pneumococci	6 (11.5%)	46 (88.5%)	52 (100%)
E.coli	16 (69.6%)	7 (30.4%)	23 (100%)
Neisseria spp.	13 (30.6%)	50 (79.4%)	63 (100%)
Streptococci	7 (27%)	19 (73%)	26 (100%)
Staphylococci	0 (0%)	2 (100%)	2 (100%)
Pseudomonas	1 (100%)	0 (0%)	1 (100%)
Fungi	3 (37.5%)	5 (62.5%)	8 (100%)
Diphtheria	3 (50%)	3 (50%)	6 (100%)
Salmonella spp.	0 (0%)	12 (100%)	12 (100%)
Proteus spp.	0 (0%	5 (100%)	5 (100%)

For the difference to be significant, p=0.01 Df=15

According to the International Reference, prevalence rate of this microorganism in normal floral of the normal people was 100% (15).

In this research, prevalence rate of E.coli was 16.7% and that of proteus was 3.6% while studying enterobacteriaceae family and the prevalence rate of these bacteria was higher in normal flora of the normal people, each of whom had 25% of normal flora. Prevalence rate of salmonella was 8.7% which did not exist in normal people's mouth flora and this important fact indicated fecal-oral contamination due to low hygienic level in these people.

Prevalence rate of Neisseria was 44% in this study. In normal people's flora, the prevalence of Neisseria sp and Neisseria meningitides was 25% (15). This increased rate which was found in the present study was due to lack of separation of its different species.

Prevalence of pseudomonas aeruginosa in the present research was 0.7% which was equal to its rate in normal oral flora of normal people (<%5) (15).

Prevalence of staphylococci sp in this study was 1.4%; this rate was about 100% in normal oral floral of normal people in terms of staphylococcus epidermidis (15). Also, it was 25% for staphylococcus aureus (15).

The prevalence rate of diphtheroids in this study was 3.5%, which was less than its prevalence rate in normal oral flora of normal people as 25 % (15).

During statistical studies in this research, antibiotic sensitivity and resistance of gram positive cocci to six antibiotics of amoxicillin, ampicillin, jentamycin, penicillin, vancomycin and trimethoprim-sulfamethoxazole were studied which were 50.6%, 18.5%, 12.3%, 67.9%, 10% and 65.4%, respectively. The highest and lowest resistance was to penicillin (67.9%) and vancomycin (11%), respectively. In addition, in the study by Salaco et al. in Kuwait, the highest

resistance was to amoxicillin (43%), which was lower than that obtained in this study in two groups of healthy and mentally retarded people and the lowest resistance was to vancomycin (12%), which was in line with the rate obtained in the present work.

The present research showed that oral and gingival bacterial flora of the studied mentally retarded people was significantly difference from oral bacterial flora in the normal people (according to the recorded scale in International References), which is justified considering their clinical differences and their inability to maintain their oral hygiene.

Higher prevalence of pneumococcus in these people than that in normal people increases risk of bacterial respiratory infections and dental plaques such as salmonella, which are never found in oral flora of normal people, were found in these people due to their oral–fecal contamination, both of the mentioned plaques could cause severe infections, especially in case of immunodeficiency. In this study, prevalence of bacterial oral flora had a significant relationship with oral hygiene of these people.

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ANTIBACTERIAL PROPERTIES OF *MANGIFERA INDICA* ON *STAPHYLOCOCCUS AUREUS.*

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ABSTRACT

Antibacterial activity of *Mangifera indica* stem bark extracts was determined using disk diffusion, agar and broth dilution methods. In disk diffusion method, inhibition zone sizes were used to determine the susceptibility of *S. aureus* to the extracts. The results showed that the stem-bark extracts *of M. indica* have antimicrobial activity against *S. aureus*. Methanol extracts showed the highest inhibition zone diameter of 25 mm, followed by ethyl acetate, water and hexane extracts with inhibition zone diameter of 22 mm, 14 mm and 10 mm, respectively. The antibacterial activities of different extracts were found to be concentration dependent, in agar and broth dilution methods. The plant extracts were shown to have a MIC range of 0.62 mg/ml to 4.17 mg/ml, in agar dilution method. Results from the broth dilution method had a MIC range of 0.16 mg/ml to 1.25 mg/ml. The control (ampicillin) was however, more effective than plant extracts since only a concentration of 0.03 mg/ml in agar dilution and 0.001 mg/ml in broth dilution method were effective to inhibit the growth of *S. aureus*. The extracts were shown to be bacteriostatic at low concentrations. Phytochemical screening of the extracts revealed the presence of phyto-compounds such as alkaloids and tannins which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs. These phyto-constituents may be responsible for the *M. indica* antibacterial activity.

Keywords: *Staphylococcus aureus*, antimicrobial activity, MIC, Phytochemical screening, MBC.

INTRODUCTION

Plant derived products like gums, oils and extracts have been used for therapeutic purpose before the introduction of modern drugs (1; 2) and continues to provide health coverage for over eighty percent of the world's population (3). Serious attention is being given to medicinal plants as evidenced by the recommendation given by the World Health Organization in 1970 (4). WHO gave emphasis on the need to include traditional remedies within national drug policies as these plants serve as the best sources of a variety of drugs. It is important to study plants so that a better understanding of their properties, safety and efficacy is derived for improved benefit. The first plant compound with antimicrobial activity was reported in the 1930s (5) and now a multitude of plant compounds are readily available from herbal suppliers and natural-food stores. In Africa, self-medication with these substances is common and growing in popularity (6). The reasons can be attributed to easy accessibility and affordability of plants compared to commercial drugs. Contrary to the belief that natural medicine has no ill effects (2) several people have been hospitalised by consuming plants of unknown properties. To address such challenges, plants must be investigated to validate and standardise their dosages. An estimated 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use. More than 25% of modern medicines are thought to have descended from plants whilst others are synthetic analogues built on prototype compounds, isolated from plants (7). Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities for synthetic drugs.

There are many drugs in clinical use today that were discovered from the way plants were used in traditional communities. Such discoveries include quinine which has been used to manage malaria for many years (8), digitoxin an indispensable cardiac drug from Foxglove *(Digitalis purpurea*). Other examples are Strychne from *Strychnox nuxvomica* which was isolated as a central nervous system (CNS) stimulant and ephedrine from *Ephedra sinica* which was discovered for asthma (9) and taxol which is used as modern therapy for ovarian cancer (10;11). There is little or no doubt that ethnographic research can provide important clues leading to new drugs for the modern pharmacies.

Pathogens develop natural resistance to antimicrobial agents. Most gram-negative bacteria are impermeable to the antibiotic penicillin G and platensimycin (12). Development of virulent factors among infectious agents varies. Some bacteria can resist phagocytosis, for instance, *Streptococcus pneumonia* and *Haemophilus influenza* produce a

slippery mucoid capsule that prevents the phagocyte from effectively contacting the bacterium. *Staphylococcus aureus* produce leukocidins that destroys phagocytes before phagocytosis. The bacteria produce coagulase, which coagulates fibrinogen in plasma thus protecting the pathogen from phagocytosis and isolates it from other host defences (13). *Pseudomonas aeruginosa* cleaves laminin associated with basement membranes, *E. coli* lyses erythrocytes and weakened host defences. The emergence of multi-resistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (14). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agent (15). The new compound may actually be more effective than the parent compound. Since resistance is based on structural recognition, the new compound may not be recognised by resistance factors.

Modern drugs are associated with several side effects like nausea and headaches. Man has resorted to plants for treatment due to high prices of synthetic drugs. Plants are regarded as cheaper and safe alternative source of drugs. However, cases of overdose or self poisoning through use of medicinal plants have been increasing. An investigation of the antibacterial activity of stem-bark extracts of *Mangifera indica* on *Staphylococcus aureus* was carried out with a view to screen for phyto-chemical compounds and determine susceptibility of the bacterium. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different plant extracts on *S. Aureus* was also determined so was the bacterial inhibition time course in water extracts.

MATERIALS AND METHODS

Study area

The bark of *Mangifera indica* Linn Variety Zill was collected in January 2012 from Bindura along Shamva road. The area lies in the following 3D GPS location; Northing 0327972, Southing 80842212, altitude 1002 m. The area has savannah grassland with loam soil, the mean annual rainfall of the area is about 750 mm to 1000mm. Study area is shown in figure 2 below.

Materials used in the research were autoclaved. Glass rods and loops were disinfected by dipping in alcohol and then flaming on a Bunsen burner. The working bench was swabbed with 70% alcohol before and after each experiment.

Preparation of plant material

Fresh stem-bark were collected from a *Mangifera indica tree*, variety Zill. The stem-barks were washed thoroughly with water and then air dried at room temperature for five days. After drying, the pieces were ground into powder and then sieved using a sieve. Two kilograms of powdered plant extracts were transferred into airtight containers and stored at room temperature.

Extraction of the crude extracts from stem-bark powder.

Plant active components were extracted using the cold extraction method (10). Four different extraction solvents namely methanol, ethyl acetate, hexane and distilled water were used.

To 500ml each of pure methanol, ethyl acetate, hexane and sterile distilled water were added 50g portions of the stem-bark powder in sterile conical flasks and allowed to soak at room temperature for 48 hours. A Khan shaker set at 120 rpm was used to improve extraction of phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump through a 127c-1 filter funnel aided by a Whatman® filter paper. Filtering was repeated three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40°C. Drying was done to allow the calculation of the yield of the extraction process. The extraction efficiency was quantified by determining the weight of each of the extracts and the percentage yield was calculated as (weight of dry extracts in grams /initial dry plant extracts) × 100. The procedure was done separately for the four solvents used. A small proportion of dry extracts was stored for phyto-chemical analysis. For the preparation of dilutions of dry extracts for antibacterial assay, dry extracts were reconstituted by re-dissolving in their respective extracting solvent. The final filtrates were filter-sterilized by using cellulose nitrate filter with a pore size of 0.45 µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles, in a refrigerator at 4°C before use during the antibacterial sensitivity tests.

Preparation of culture media

Nutrient agar media

Nutrient agar powder weighing 15.5 g was suspended in 500 ml of cold distilled water, the mixture was stirred and boiled to dissolve.

For plates

The media was sterilized by autoclaving at 121°C (15psi) for 15 minutes. After autoclaving the liquid agar was cooled to a temperature range of 44°C to 47°C and poured aseptically into sterile plates (90mm diameter). The agar was allowed to cool and solidify at room temperature. Labeled plates with nutrient agar were stored at 4°C. A single plate was examined for sterility by incubating at 35°C for 24 hours.

For agar slants
The media was dispensed in 10ml aliquots into universal bottles and then autoclaved. Molten agar was slanted and allowed to cool in the universal bottles in a sloping position.

Mannitol Salt Agar

60g of powder was suspended in 500ml of distilled water. A suspension was dissolved by stirring and boiling, and then sterilised in an autoclave. Labelled plates with Mannitol Salt agar were stored at 4°C. Plates were wrapped in plastic to prevent contamination.

Mueller Hinton Agar
Dehydrated medium weighing 9.5g was suspended in 250ml of distilled water. The mixture was brought to boil, with constant stirring until complete dissolution. Sterilization was done using an autoclave. Cooled Mueller Hinton agar was poured into sterile Petri dishes on a level, horizontal surface to give uniform depth. Prepared media was allowed to cool and solidify at room temperature. The final pH of the prepared Mueller Hinton agar was checked using a pH metre and it was 7.3 at 25°C. Labelled plates with Mueller Hinton agar were stored at 4°C. Sterility was checked by incubating a plate at 35°C for 24 hours.

Nutrient broth
Nutrient broth powder weighing 6.5g was dispensed in 500ml of distilled water. After mixing the solution, it was heated gently to dissolve. The broth was distributed into capped tubes in 9 ml aliquots and autoclaved. The broth was kept in a sterile dark room at a temperature range of 15-20°C.

Sterility proofing of extracts
After membrane filtration, extracts were tested for sterility, by streaking on freshly prepared sterile nutrient agar which was incubated for 24 hours at 37°C.

***S. aureus* as a test organism**
Using a sterile cotton swab, the bacterial culture was swabbed on the surface of pre-poured nutrient agar plates. Inoculated plates were inverted and incubated for 24 hours at 35°C. After incubation, colonies formed were used for confirmatory tests. Identification of *Staphylococcus aureus* was based on cultural, morphological and biochemical characterization.

Growth on mannitol salt agar
Bacterial cells were streaked on pre-poured MSA media, a selective medium with 7-9 % sodium chloride. After incubation, colonies of *S. aureus* were confirmed by a yellow colour and by turning the medium around the colony yellow due to the drop in pH around the colony.

Colony morphology on nutrient agar media
Nutrient agar was inoculated with a small amount of test culture using sterile loops. Inoculated plates were incubated at 37°C for 48 hours. Spherical, pinhead colonies which are convex with entire margins were observed on nutrient agar, after incubation period.

Gram stain
A few drops of distilled water were added onto a sterile glass slide. A loopfull of the bacterium was transferred and spread in circular motion over a small area of the slide. The smear was allowed to air dry. The microorganism was heat fixed by placing the bottom of the slide to heat for approximately 30 seconds without exposing. Forceps were used to hold the slide above the sink, the surface of the slide was flooded with crystal violet for 1 minute. Rinsing of the slide with distilled water was done for 5 seconds. Gram iodine solution was applied and allowed to act for 1 minute as a mordant. The slide was thoroughly rinsed with distilled water. Excess water was drained from the slide and the slide was blotted so that alcohol used for decolourization is not diluted. A few drops of 95 % ethanol were applied onto the slide for 10 seconds and washed off with tap water. The slide was drained to remove excess water. A few drops of safranin solution were used to counter stain the slide for 30 seconds, rinsed off with tap water, drained blotted out to dry with bibulous paper. The slide was read with oil immersion lens of the slide of the microscope at high power (x 1000).

Catalase test
A small amount of growth from the culture was placed onto a sterile microscope slide. A few drops of hydrogen peroxide were added onto the smear and mixed with a sterile toothpick. A negative result would be no bubbles or a few scattered.

Maintenance of pure cultures
Colonies confirmed to be *S. aureus* were sub-cultured on MSA media, three times to obtain pure cultures. Pure cultures were sub-cultured onto new agar slants, incubated at 37°C for 24 hours and stored in the refrigerator at 4°C. These cultures were frequently sub-cultured on new agar slants to maintain their viability.

Preparation of Turbidity standard for inoculum preparation
0.5 Mcfarland standard was prepared by adding 0.5ml of 0.048M $BaCl_2$ (1.17 % w/v $BaCl_2.2H_2O$) to 99.5 ml of 0.18M sulphuric acid (H_2SO_4) (1 % w/v) with constant stirring. A barium sulphate precipitate was checked for optical density using matched curvettes with 1 cm path and distilled water as a blank standard. A UV-Vis spectrophotometer was used to measure the absorbance at 625 nm. An absorbance of 0.1 was obtained which was in the accepted range of 0.08-

0.13. The suspension was distributed into tubes of the same size as those used for test inoculum's adjustment. Sealed tubes were stored in the dark at room temperature. The approximate cell density corresponding to 0.5 McFarland is 1×10^8 cfu/ml.

Physiological sterile saline preparation
Sodium chloride of 4.25g was dissolved completely in 1 litre of distilled water by heating. It was autoclaved and stored at ambient temperature in sterile containers with caps tightened to prevent evaporation.

Phytochemical analysis of plant extracts
The stembark extracts were evaluated for the presence of phytochemical compounds using standard methods (16). Phytochemical examination was carried out separately for all the extracts, and the procedure was done three times for confirmatory purpose.

Detection of steroids (Salwoski's test)
100mg of dry extracts were dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were added to form a lower layer. A reddish brown colour at the interface was indicative of the presence of steroidal ring.

Detection of Cardiac glycosides (Keller Killian' test)

100mg of dry extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

Detection of Saponins (Froth Test)
Extracts were diluted with distilled water to 20ml which was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

Detection of Resins
100mg of dry extracts were dissolved in ethanol and 5 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of concentrated sulphuric acid was added. Bright purple colour produced indicated the presence of resins.

Detection of Phenols (Ferric chloride test)
100mg of dry extracts were dissolved in ethanol and 2 ml of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. Ten percent aqueous ferric chloride solution was prepared by mixing 5ml of ferric chloride to 45 ml of distilled water. Formation of a blue-green colour indicated the presence of phenols.

Detection of Tannins (Ferric chloride test)
50mg of dry extracts were dissolved in 2ml of distilled water and mixed with 2 ml of ferric chloride. A blue-black precipitate indicated the presence of tannins.

Detection of Terpenoid (Liebermann-Burchard test)
2ml of chloroform and 1 ml of concentrated sulphuric acid were added to 1mg of the dry extract. A reddish-brown colour indicated the presence of terpenoid.

Detection of Glycosides
50 mg of dry extract dissolved in 1ml ethanol was mixed with 1 ml of water and then aqueous sodium hydroxide was added. A yellow colour observed indicated the presence of glycosides.

Detection of Flavonoids
200mg of dry extracts were dissolved in 10ml of ethanol and filtered. A few drops of HCl and magnesium ribbon were added to 2 ml of the filtrate. Pink tomato red colour indicated the presence of flavonoids.

Detection of Phlobatannins
100mg of dry extract dissolved in 2ml distilled water was boiled with 1 % aqueous HCl (1ml HCl mixed with 9ml of distilled water). Deposition of a red precipitation was taken as evidence for the presence of phlobatannins.

Acidic compound
50mg of dry extract was dissolved in 2ml of ethanol and Sodium bicarbonate solution added. A positive result is indicated by the production of effervescence. For a negative result, there was no effervescence.

Anthraquinone (Borntrager's Test)
About 200mg of the extract was placed in a dry test tube and 2 ml of chloroform added for 5 minutes. The extract was filtered and the filtrate was shaken with 2 ml of 10% ammonia solution. A pink violet or red colour shows the presence of anthraquinone.

Determination of antibacterial activity
Antimicrobial activity was performed by standard methods like the disk diffusion method on Mueller Hinton agar and MIC was calculated using dilution methods. Cells used for antibacterial assays are harvested at log phase while they are most active.

Preparation of inoculums

Direct colony suspension method was used to make a suspension of *S. aureus,* approximately corresponding to 1×10^8 cfu/ml for *S. aureus*. Three to four colonies from overnight grown (18 hours) were suspended in saline using a sterile loop. The turbidity standard was shaken vigorously before use, and used to make a visual comparison with the

density of the suspension against a white background with black lines. Density of the suspension was adjusted to 0.5 Mcfarland either by adding sterile saline. The standardised culture was used within 15 minutes of preparation for sensitivity tests.

Disk diffusion test

Antibacterial activities of the extracts were tested on Mueller-Hinton agar by disk diffusion method. Six Mueller Hinton agar plates were warmed to room temperature while inverted. Moisture was drained from the plates by inverting. To maintain sterile conditions this procedure was done in a laminar flow cabinet. A sterile cotton swab was added to the inoculum adjusted to the standard opacity, and excess fluid drained by pressing the swab on the walls of the tube. The inoculum was spread evenly over the entire surface by swabbing in three directions (15). Inoculated plates were allowed to dry for ten minutes before depositing the disks (17).

Sterile paper disc having a diameter of 10 mm, were impregnated with 1ml of each extract at a concentration of 200mg/ml and were allowed to dry for 15 minutes. After drying, paper disks were placed on the agar plate using sterile forceps. Five filter paper disks were placed on each plate and were placed at the same distance from each other and the edge, to prevent overlapping of inhibition zones. Sensitivity discs were pressed with forceps to make complete contacts with the surface of the medium. Plates were kept at room temperature for 30 minutes (pre-diffusion time), inverted and incubated at 37°C for 24 hours, in an aerobic atmosphere. A pair of divider was used to mark the diameter of the zone and a transparent ruler was used to measure the distance in mm. The experiment was repeated three times for each extract and the mean diameter was taken. The mean diameter zones produced were compared to the expected reference chart (18).

TABLE 1: ZONE SIZE INTERPRETIVE CHART.

	Zone size produced (mm)			
Test organism	**Disk content**	**Resistance**	**Intermediate**	**susceptible**
S. aureus	**1ml**	**≤ 20**	**21-28**	**>29**

Controls

Negative controls were set as follows; different absorbent paper discs were saturated each with a solvent only and another disk with 1 ml sterile saline. They were allowed to dry for 15 minutes at room temperature. The paper disks were later placed on the surface of an inoculated plate with standard density of bacteria and incubated at 37°C for 24 hours.

A positive control was set as follows; absorbent paper discs were saturated with an antibiotic (Ampicillin) at 500µg/ml. The paper disks were placed on the surface of inoculated petri plates. Plates were incubated at 37°C for 24 hours.

Dilution methods

Dilution susceptibility testing methods were used to determine the minimum concentration of plant extracts to inhibit growth of *S. aureus*. This was achieved by two fold dilution of plant extracts in either agar or broth media.

Determination of MIC using agar dilution

The reconstituted sterile plant extracts were serially diluted to obtain a concentration of 10^{-6} (3,25 mg/ml) to (200 mg/ml). Then 1ml of the extract was incorporated in 19 ml molten nutrient agar to give a final concentration range of 10 mg/ml to 0,16 mg/ml. Solidification was allowed in a laminar airflow. A standard loop was used to transfer 100µl (10^8 cfu/ml) of the inoculum. The test organisms were streaked in radial patterns on the agar plate and incubated at 37 °C for 48 hrs. A negative control was set by incorporating 1ml of solvent in molten media, and inoculated with the test organism. Plates with medium only were set as controls for sterility of the medium. A positive control was set with antibiotic (Ampicillin). The antibiotic was serially diluted from a concentration of (0.05mg/ml). Plates were evaluated for the presence or absence of bacterial growth, after incubation period. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of the test organism in the agar plate (7). The presence of one or two colonies was disregarded. All tests were carried out in triplicates. The procedure was repeated three times for each extract. Ampicillin was set as the positive control.

Determination of MIC using broth dilution method

The MIC values were determined by broth dilution assay. Sterile plant extracts were serially diluted (two-fold) to obtain a concentration range of 200mg/ml to 1.625mg/ml. Then, 0.1 ml of each concentration was added to 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. Negative controls were equally set

up by using solvents and test organisms without extracts. Tubes with medium only were set as controls for sterility of the medium. Test tubes were evaluated for the presence or absence of visible turbidity in the broth after the incubation period. The lowest concentration (highest dilution) of the extract preventing appearance of turbidity (growth) was considered and recorded as the MIC (19). Ampicillin was used as a positive control.

Determination of MBC

From the tubes showing no visible sign of growth/turbidity in MIC, 0.1 ml of the sample was inoculated onto sterile nutrient agar using the streak plate method. The plates were then incubated at 37°C for 48 hours. The least concentration that did not show growth of the test organism was considered as the MBC (20). A plate with media only was set as a negative control to check the sterility of the media. The MBC for ampicillin were also determined.

Determination of the death rate of the bacterial isolate in the extract

Assay for the rate of killing of bacterial isolates by water extracts was determined using the Kelsey and Maurer method (21). This was done by mixing 0,5ml of (10^4 cfu/ml) test isolates to 4.5 ml of MBC (5 mg/ml) of the water extracts. These were held at in a water bath at 37 °C and the killing rate was determined over a period of three hours. Exactly, 0.1ml volume was withdrawn at 30 minutes intervals using a micropipette, and plated out on the surface of solidified nutrient agar containing 3 % tween 80, for viable count. The number of colonies formed on each plate at the time intervals, were counted using a digital colony counter. Incubation was done at 37°C for 48 hours. A control was set containing nutrient media and the test organism but without the extract. The test results were compared with that of the control. Emergent colonies were counted and compared with the count of a culture control. All the counts obtained, were the mean of triplicate tests.

Statistical analysis

Results were expressed as mean ± standard errors and the comparison of the antibacterial activity of the samples with standard antibiotics was evaluated by applying one way analysis of variants.

RESULTS

The extraction efficient ranged from 14.5 % to 0.92 % for the most efficient solvent, water and the least efficient extract, hexane. The colour and state of the final extracts were also observed, refer to fig 1. Results are recorded in table 1.

TABLE 2: MEAN PERCENTAGES OBTAINED AFTER USING DIFFERENT SOLVENTS IN EXTRACTION OF COMPOUNDS FROM THE STEM BARK OF *MANGIFERA INDICA* .

Solvent used in	Weight of starting material	Mean percentage of extracts (w/w)	Standard errors
Water	50	13.67	± 0.33
Methanol	50	10.33	± 0.33
Ethylacetate	50	12,33	± 0.33
Hexane	50	0,009	± 0.00

There was a significant different between the mean percentages obtained after using different solvents. The highest mean percentage was obtained using water extracts. The least percentage was obtained in hexane extracts.

FIGURE 1: COLOURS OF DIFFERENT EXTRACTS OBSERVED AFTER 72 HOURS OF SOAKING PLANT MATERIAL IN DIFFERENT SOLVENTS.

Ethyl acetate extracts showed a black colour, after vaporisation of solvent, a black-solid extracts was obtained. Methanol extracts had a brownish- blackish colour, the extract was solid after vaporisation of solvent. Water extracts were black in colour; a solid extract was obtained after vaporisation. Hexane extracts were yellowish in colour and a gummy extracts was obtained.

A range of phytochemicals were confirmed to be present in stembark extracts of *M. indica*. Results from ethyl acetate and methanol extracts confirmed the presence of all the phytochemicals tested. Phytochemical compounds were almost common in stem-bark extracts. However acidic compounds and anthraquinone were not present in water extracts. Results from phytochecimal analysis are shown in table 2.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF *MANGIFERA INDICA* STEM-BARK EXTRACTS.

Phytochemical Constituency	Water extract	Methanol extract	Ethylacetate extract	Heaxane extracts
Steroids	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	+	+	+
Resins	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Terpenoid	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	+	+	+	+
Acidic compound	-	+	+	+
Anthraquinone	-	+	+	-

Key : + = present - = absent

The result revealed that the extracts of *M. indica* possess good antibacterial activity against *S. aureus*. Stem-bark extracts inhibited the growth of *S. aureus* and the inhibition zones ranged from 10mm to 25mm. The extracts were less potent than the antibiotic. Negative Controls showed that solvents without extracts had no inhibitory effect on bacterial growth. The mean inhibition zone

diameter of the different extracts to *S. aureus* are shown in table 4.

TABLE 4: ACTIVITY OF CRUDE EXTRACTS FROM THE STEM BARK EXTRACTS OF *MANGIFERA INDICA* ON *S. AUREUS* AT A CONCENTRATION OF 200MG/ML.

	Disk Content				
					Positive Control
(Plant extracts)	H_2O	Meth	Ethyl	Hex	Amp
Mean zone of inhibition	14	25	22	10	29
Standard errors	± 1.00	± 1.15	± 1.0	± 1.52	± 0.577

Key :

H_2O = water Meth = methanol Ethyl = ethyl acetate Hex = Hexane Amp = Ampicillin (Amp)

There was a significant difference in mean zone of inhibition between ampicillin (control) and plant extracts ($P < 0.05$). There was no significant difference between methanol and ethyl acetate extracts ($P = 0.82$). Negative controls: There was no zone of inhibition around disks submerged in solvents only. There was no zone of inhibition around disks soaked in sterile saline solution only.To determine the extent of antibacterial of antibacterial activity, the extracts were subjected to MIC assay by serial two-fold dilution method of extracts and then dilution methods for inhibitory concentration assays.

TABLE 5 : RESULTS FROM AGAR DILUTION METHOD ON MINIMUM INHIBITORY CONCENTRATION (MIC) OF STEM-BARK EXTRACTS ON *S.AUREUS*. VALUES GIVEN ARE BASED ON FINAL CONCENTRATION OF EXTRACT IN MEDIA.

	Control				
Plant extracts	H_2O	Meth	Ethyl	Hex	Amp
Mean conc	2.08	0.62	1.04	4.17	0.03
Standard Errors	± 0.417	± 0.00	± 0.20	± 0.833	± 0.00

Key; H_2O - water , Meth – methanol, Ethyl – ethylacetate, Hex – Hexane, Mean conc - mean concentration,

Amp – ampicilin.

There was no significant difference between ampicillin , methanol and ethylacete extracts ($P > 0.05$). There was a significant difference between ampicillin and water, hexane extracts ($P > 0.05$). There was no significant difference between water and ethyl acetate extracts ($P = 0.115$).

TABLE 6: MINIMUM INHIBITORY CONCENTRATION (MIC) OF *M. INDICA* STEM-BARK EXTRACTS USING BROTH DILUTION METHOD.

Control					
Plant extracts	**H_2O**	**Meth**	**Ethyl**	**Hex**	**Amp**
Mean Conce	**1.25**	**0.16**	**0.27**	**1.04**	**0.001**
Standard Errors	**± 0.00**	**± 0.00**	**± 0.54**	**± 0.208**	**± 0.001**

TABLE 7: MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF CRUDE EXTRACTS OF *M. INDICA* ON *S. AUREUS*.

Plant extracts	**H_2O**	**Meth**	**Ethyl**	**Hex**	**Amp**
Concentration (mg/ml)	**5**	**0.3125**	**0.625**	**10**	**0.0125**
Standard errors	**±0.417**	**± 0.000**	**± 0.208**	**± 0.83**	**± 0.00**

Key : H_2O = water Meth = methanol Ethyl = ethyl acetate Hex = hexane

Negative Control : There was no growth in a plate which was set for sterility test.

TABLE 8. RESULTS SHOWING THE NUMBER OF COLONIES OBTAINED ON PLATE COUNT.

Time interval (minutes)		**0**	**30**	**60**	**90**	**120**	**150**	**180**
Average No of colonies	**Test**	**96**	**100**	**17**	**13**	**4**	**2**	**0**
	Control	**98**	**157**	**179**	**182**	**180**	**183**	**185**

Key :

Test = experimental tube with water extracts Control = experimental tube with bacteria only.

DISCUSSION

The basic parameters influencing the quality of an extract are plant parts used as a starting material, the solvent used for extraction, the extraction technology and sterilisation method (7). These findings on extraction potential of the different solvents are consistant with previous investigation, in which the percentage yield of water extract was higher than that of hexane extracts (22). The observed differences in the extract yields of different solvents might be ascribed to the fact that the extract has different solubility or to the polarity of the solvent. Different extractable components were present in different quantities within the extract. The difference in ethyl acetate and methanol solvents compared with hexane extracts may be due to the fact that plant compounds such as phenolics are often extracted in higher amounts in more polar solvents than in non-polar.

Phytochemical analysis conducted on *M. indica* extracts revealed the presence of tannins, flavonoids, steroids, saponins, glycosides and resins among others. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, *M. indica* containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases such as pneumonia. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (23). They also serve as health promoting compounds as a result of their anion radicals (24). These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds. Alkaloids were also detected and their common biological property is cytotoxicity (25).

The stem-bark extracts of *M. indica* had significant antibacterial potency against the test organism. This result may suggest that all extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs, for therapy of infectious diseases in human. Ethyl acetate and methanol extracts had an inhibition zone diameter of 22 mm and 25 mm respectively, which is close to a standard antibiotic, hence we suggest their effectiveness as antimicrobials.

The active components in the crude extract may be acting synergistically to produce antimicrobial effects (22), the disparity between the activities of the extracts and the standard antimicrobial drug , may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic (26). Thus a standard drug had the highest zone of inhibition of 29mm. Methanol and ethyl acetate are polar solvents, since they showed the highest antibacterial activities. We could suggest that some of the principal antibacterial components of this plant were polar compounds. Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds which are more soluble in polar solvents such as water and methanol. However water extracts were less potent. This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents (7). The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases.

There are also reports in literature that methanol is a better solvent for consistent extraction of antimicrobial substances for medicinal plants (22). This may be attributed to two reason, firstly, the nature and potentiality of biologically active components (alkaloids, flavonoids, essential oils biterpenoids), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (27). This is also proved in our study in which methanol extracts exhibited the highest antibacterial activity against *S. aureus* compared to other extracts.

In another study, (27) found out that methanolic extracts had the least antibacterial activity against *S. aureus* using *N. oleander* plant materials. Thus besides choice of a good solvent for extraction of active compounds, antibacterial activity also depends on phyto-constituents present in the plant. The contents of active ingredients in plant materials have been shown to fluctuate constantly with the genetic heterogeneity of a

plant species, differences in soil condition, variation in seasonal cycle, climatic influences, age of plant, alteration in weather, sun and shade fluctuations. Hexane is a non-polar solvent and since the n-hexane extracts showed the least antibacterial activity, this could suggest that a small quantity of the antibacterial component of this plant was lipid soluble. Hexane is the most widely used solvent to extract edible oils from medicinal plants, however its use is decreasing due to its toxicity.

The MIC results showed that the activities of different extracts were concentration dependent. MIC values obtained from agar dilution were higher than broth values. Major differences between the agar dilution and broth dilution was seen for methanol, ethyl acetate and hexane extracts , where MIC values from broth dilution were almost four times lower than MIC values in agar dilution.

Similar differences were reported (28) when comparing the broth dilution with the standard agar dilution method on *Mycobacterium avium* complex strains. Some studies (29) found that the MIC determined by the broth dilution was four to eight times lower than those found by the agar plate method. This trend has been observed by several researchers using antibiotics (29). The differences may be caused as suggested by factors such as higher absorption and degradation of drug in solid, agar medium. Furthermore on solid medium bacteria grow on the surface and a concentration gradient could develop during incubation and growth of bacteria leading to altered or pseudo resistance. In contrast in liquid, broth medium, there are more cell to antimicrobial compound contact as the bacteria is sub-merged in the plant extracts-containing medium.

MBC was determined using tubes showing no turbidity. Results obtained showed that MIC values were lower than MBC values, suggesting that the extracts were bacteriostatic at lower concentration. At higher concentration the extracts were bactericidal as shown in table 4.6. As shown clearly in table 6 and 7 the MBC results varied considerably from the MIC. Low MIC and MBC values obtained of methanol and ethylacetate extracts, is an indication that *M. indica* stem-bark extracts are effective as antimicrobials. However, a high MIC and MBC values for hexane and water extracts, is an indication that they are less effective against *S. aureus* as antibiotics and the bacteria has the potential of developing resistance against the extracts.

From the study of the kinetics of disinfection, it was clear that the number of colony forming units were reduced to zero within the first 180 minutes of exposure to plant extracts. The reduction in bacterial cell number to zero shown in table 8 confirms that water extracts are bactericidal at this concentration. In the first 30 minutes the extracts cell numbers were almost constant, a significant decrease in cell number occurred during the first hour. The mechanism by which plant extracts inhibit bacterial cells is not well understood. The extracts were shown to have effect on bacterial growth after thirty minutes, thus we suggest that the extracts were being absorbed and possibly inhibit cell growth by acting inside bacterial cells. The findings suggest the possibility of using water extracts at bactericidal concentration as a disinfectant, and the time to disinfect bacteria cells (10^4) was determined to be 180 minutes at a concentration of 5 mg/ml.

CONCLUSIONS

The stem-bark extracts were found to have antibacterial activity against *S. aureus*. The stem bark extracts of *M .indica* contains several phyto-compounds. There is need for lead compounds from the plant extracts to be isolated so that they can serve as templates for the production of new antibiotics.

RECOMMENTATIONS

Further research on the antibacterial activities of stembark extracts of *Mangifera indica* should be done using different strains of bacteria. This is important to establish whether the extracts have a broad spectrum activity. Research should also be done on isolation and identification of the main active compounds in the stem-bark of *M. indica*. Moreover, further studies should be done on the bactericidal effect of the plant extracts.

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THE USE OF MORPHOLOGICAL AND CELL WALL CHEMICAL MARKERS IN THE IDENTIFICATION OF STREPTOMYCES SPECIES ASSOCIATED WITH ACTINOMYCETOMA

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ABSTRACT
Most aerobic, filamentous, spore-forming *Actinomycetes* are saprophytes but some are considered pathogens of humans and animals, notable examples are the causal agents of mycetoma. The present study aimed to identify *Streptomyces* spp. isolated from actinomycetoma cases in Sudan by examining some morphological traits and analyzing the cell wall composition. Nineteen *Streptomyces* strains isolated from purulent materials of patients with mycetoma (human) or fistulous withers (donkeys) were included in the study. Isolates were tentatively identified as *Streptomyces* species based on morphological and cultural characteristics. Cell wall analysis of isolates yielded LL-diaminopimelic acid (LL-DAP) which authenticates that the isolates are members of genus *Streptomyces*. The isolates, though they are *Streptomyces*, but are variable phenotypes. The study concluded that using few selected criteria, as above, would allow identification of unknown actinomycetoma agent to the genus level. The study also assumes that apparently limitless, numbers of saprophytic *Streptomyces* enter human or animal skin tissue causing actinomycetoma and perhaps other complications in man and animals.

KEYWORDS: Actinomycetoma, *Streptomyces* species, Madura foot, Sudan

INTRODUCTION

Mycetoma is a slow destructive infection of cutaneous and subcutaneous tissues, fascia and bone, caused by fungi (eumycetoma) and actinomycetes (actinomycetoma). It is mainly prevalent in tropical rural areas in a belt that matches the *Acacia* belt in Africa, India, Central and South America (1, 2, 3). Mycetoma is a major health problem in Sudan notably among rural workers, particularly male farmers, peasant and shepherds. Thorns from *Acacia nilotica* and other tropical trees, which grow in most parts of tropical Africa, poses serious threat to health by predisposing to mycetoma through direct inoculation of contaminated soil and plant debris to skin.

Actinomycetoma is reportedly caused by *Actinomadura madurae, A. pelletieri, Nocardia brasiliensis, N. otitidiscaviarum, N. transvalensis, Streptomyces sudanesnsis* and *S. somaliensis* (4, 5). Most mycetoma cases in Sudan are attributed to *S. somaliensis* (1, 6) and *S. sudanesnsis* (5). Currently, the genus *Streptomyces* includes over 500 validly described species (7, www.ncbi.nlm.nih.gov/Taxonomy/). They form an integral part of soil microbial communities and making up approximately 10% of total soil microbial flora (8). The majority of research focused on the classification of these saprophytic strains (9, 10), albeit the genus contains few human and plant pathogens (4, 11). *Streptomyces* species are causal agents of diseases in man (*S. somaliensis* and *S. sudanesnsis);* animals (*Streptomyces* species) and plants *(Streptomyces scabies)* (6, 12, 13). The cultural and microscopic features of genus *Streptomyces,* which are commonly used for routine identification, include aerobic growth, gram-positive, non-acid-alcohol-fast, non-motile *Actinomycete* which forms extensively branched, light yellow substrate mycelia on a variety of media with or without aerial hyphae, with or without diffusible pigments on medium surface (7, 14). Cell wall components of *Actinomycetes* enable rapid qualitative identification of certain *Actinomycetes*. Such outcome has been believed as "completely satisfactory" (15, 16).

The present study was aimed to investigate some growth and morphological features and chemical markers for the identification of *Streptomyces* species isolated from patients with mycetoma and fistulous withers in Sudan.

MATERIALS AND METHODS

Clinical specimens

Purulent material (0.5 mL) was collected by needle aspiration from unopened parts of lesions from donkeys with fistulous withers. In case of human mycetoma, grains were taken from deep excision biopsy material of patients, stored in sterile containers and transported to the laboratory where they were either kept on ice for up to 24 hours or used immediately.

Isolation of *Streptomyces* species

Clinical specimens (needle aspirates, grains) were used to inoculate Tryptic Soy agar (TSA; Difco) plates which had been incubated at 37°C for up to two weeks. Plates were examined daily until *Streptomycete*-like colonies were seen, the latter were subcultured onto fresh TSA agar plates which were incubated at 30°C for up to 14 days to allow better morphological observation.

Nineteen (n = 19) *Streptomyces* strains have been isolated between 1998 and 2003 from various parts of Sudan from cases of actinomycetoma in human (madura foot) and actinomycetoma in donkeys (fistulous withers). In this study bacteriological and chemotaxonomic characterization was completed on the isolated *Streptomyces* strains as part of a project that had completed some parts **(**5, 17, 18**)** and other part are underway.

Strains

The 19 *Streptomyces* strains are labeled as *S. somaliensis* DSM 40738^T, *S. sudanensis* DSM 41923^T (SD504), D501, SD509, DSM41607, *Streptomyces* spp.: SD511, SD524, SD528, SD534 and DSM40760 (human isolates); SD551, SD552, SD559, SD572, SD573, SD574, SD575, SD576, SD579 (donkey isolates) and *S. somaliensis* DSM 40738T, *S. sudanensis* DSM 41923^T, SD509, DSM41607, DSM41608, DSM41609, *Streptomyces* spp.: SD511, SD524, SD528, SD534 and DSM40760 (human isolates). *S. somaliensis* DSM 40738T and . *sudanensis* DSM 41923^T served as controls.

Morphological characterization

Isolates were tentatively identified as member of genus *Streptomyces* based on selected morphologic criteria (7, 14). The clusters of the isolates were recognized based on colony color, substrate and aerial mycelia and the presence of diffusible pigments on TSA media.

Cell wall analysis

Biomass for chemotaxonomic studies was prepared by growing each strain for 2 weeks at 30°C in a 100 ml shake flask containing 25 ml of trypticase soy broth (Difco). The isolates were examined for the presence of the isomers of diaminopimelic acid (DAP) in whole-organism hydrolysates by thin-layer chromatography (TLC) of whole-organism hydrolysates following the procedure described by Staneck and Roberts (19). A standard solution (10 mM) of A_2pm (Sigma) containing a mixture of LL- and *meso*-DAP isomers was used as a reference. The following markers were also used to control the TLC analysis: *S. sudanesnsis* (DSM 41923^T (SD504) as it reveals LL-DAP; *Nocardia farcinica* ATCC 3318 which reveals *meso*-DAP and *Dermatophilus congolensis* DSM 44180 which reveals neither LL-DAP nor *meso*-DAP (19).

RESULTS

The isolates recovered from human and donkey's actinomycetoma cases exhibited different phenotypic features. The initial identification of isolates to cluster and phenotypic groups was done according to growth and colony features characteristics and microscopic appearance (Table 1). The isolates revealed colony morphology of various forms and colors that ranged from grey to blue to grey brown or grey white colonies (Fig. 1).

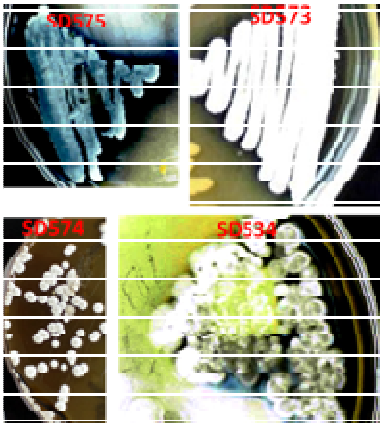

FIGURE 1. Growth of *Streptomyces* spp. isolated from actinomycetoma cases showing variations in colony morphology which ranged from grey to blue to grey brown or grey white in color.

These different phenotypic features triggered further studies so as to recognize new species among them. Overall, these isolates had common shared properties of *Streptomyces* i.e. these were aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycete that formed extensively branched substrate mycelium on standard media (Fig. 2). The resultant analyzed data revealed that most of the isolates were distinct from both *S. sudanensis* and *S. somaliensis*. These results are in line with the known description of *Streptomyces* spp. (4, 7).

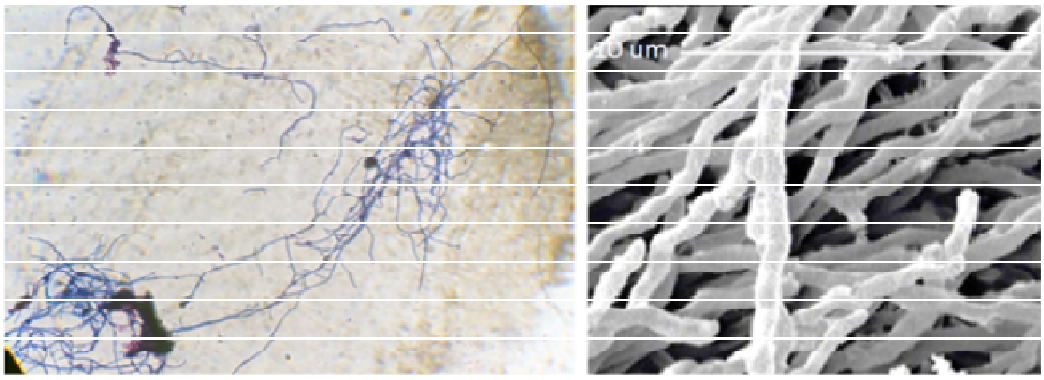

FIGURE 2. Microscopic features of isolated *Streptomyces* sp. (SD574) (left) and scanning electron micrograph of *Streptomyces* sp. (SD509) (right). The organism are gram-positive, non-acid-alcohol-fast, forms extensively branched mycelia that are none fragmenting.

In TLC analysis, all the strains were found to contain LL-DAP similar in chromatographic behavior to that produced by the marker species *S. sudanesnsis* (Fig. 3). Such chemical markers strongly support the identification of the isolates as members of the genus *Streptomyces* and in accordance with standard descriptions of the genus (7).

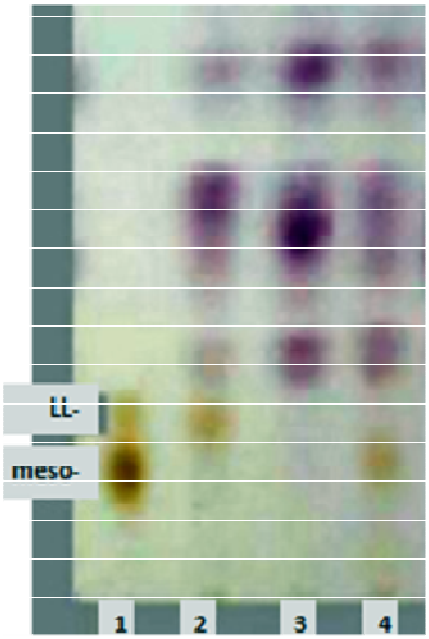

FIGURE 3. TLC analysis of whole cell hydrolysate of Streptomyces isolates. All test strains contain LL- A2pm (lane 2) similar in chromatographic behavior to that produced by the marker strain (lane 1) but distinct from Nocardia farcinica (lane 4) and the negative control (Dermatophilus congolensis; lane 3)

DISCUSSION

The isolated organisms were tentatively identified as *Streptomyces* specie on the basis of culture-morphological characteristics (Fig. 1 and 2). Nevertheless, a good level of support to this initial identification was achieved with the analysis of cell wall diaminopimelic acids, namely LL- and *meso*-DAP. This

chemotaxonomic feature is a robust technique in differentiating *Streptomyces* from *Nocardia* species and from many other species within the order *Actinomycetales* of the phylum *Actinobacteria* (7, 14).

TABLE 1. MORPHOLOGICAL CHARACTERISTICS OF *STREPTOMYCES* SPP. (N= 19) ISOLATED FROM HUMAN AND DONKEY

Species	Strain code	Colony colour	Reverse colony colour	Aerial hyphae
Streptomyces sudanensis (n = 4)	DSM 41923^T (SD504), D501, SD509, DSM41607	Light gray	Light yellow	No aerial hyphae
Streptomyces somaliensis (n = 1)	DSM 40738^T	Light gray	Light yellow	No aerial hyphae
Streptomyces isolates (n = 14)	SD 511	Light gray	Light yellow	No aerial hyphae
	SD 534	White	Yellow	White aerial hyphae
	SD 528	Light gray	Colorless	No aerial hyphae
	SD524	Light gray	Medium red brown	Light gray aerial hyphae
	DSM 40760	White	Light yellow brown	White aerial hyphae
	SD 551	Medium gray	Light brown gray	Medium gray aerial hyphae
	SD 552	Light gray	Light gray yellow brown	Light gray aerial hyphae
	SD 559	Light gray brown	Brown gray	Light gray brown aerial hyphae
	SD 572	White	Buff	White aerial hyphae
	SD 573	White	Medium yellow brown	White aerial hyphae
	SD 574	White	Medium yellow brown	White aerial hyphae
	SD 575	Light green gray	Gray green	Light green gray aerial hyphae
	SD 576	White	Buff	White aerial hyphae
	SD 579	White	Medium yellow brown	White aerial hyphae

Abbreviations: T, type strain; DSM, Deutsche Sammlung von ikroorganismen; Inhoffenstraße 7B, 38124 Braunschweig, Germany

Donkey's fistulous withers and human mycetoma share some pathological and ecological attributes. However, a question remained to be answered: why the infection mainly affects man and donkeys? Some isolates from these lesions have been previously identified as *Streptomyces* (5, 17, 19).

The 16S rDNA gene sequence analysis of some strains analyzed so far confirmed that the isolates falls within the phylogenetic clade, which encompasses the genus *Streptomyces* (data not shown). Studies are underway to further describe these bacteria and assign names to them. This report represents a good evidence to further implicate *Streptomyces* in the etiology of fistulous withers in donkeys and increases the rate of *Streptomyces* spp. as causal agents of actinomycetoma in Sudan (6).

Soil saprophytes cause considerable health hazard as demonstrated by a significant, apparently limitless, number of saprophytic phenotypes of *Streptomyces*. These *Streptomyces* spp. enter human or animal skin tissue through traumatic injuries, cause actinomycetoma and perhaps other complications in man and animals. DNA-DNA pairing and further phenotypic characterization of these isolates may enable descriptions of new species. This paper has achieved the view of seeking and endorsing the development of simple diagnostic appraoches especially in low income coutries or in laboratory with limited resources.

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KNOWLEDGE, ATTITUDES AND PRACTICES OF PREVENTION OF MOTHER TO CHILD TRANSMISION OF HIV AMONG WOMEN IN LAROO DIVISION G ULU MUNICIPALITY, UGANDA.

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ABSTRACT

This study is exploring the knowledge, attitudes and practices of Prevention of Mother to Child Transmission (PMTCT) of Human Immunodeficiency Virus (HIV) among child bearing women aged between 15- 45 years old in Laroo division in Gulu municipality. The goal of the study was to determine the level of attitude as well as of knowledge and belief about PMTCT and proportion of women practicing it among the child bearing women of Laroo Division. The Cross-sectional and Descriptive study design was applied in multistage sampling method with random selection of a parish followed by random selection of a village within the parish from which a sample of homesteads was randomly selected. From each homesteads, a single respondent was randomly selected and registered in the study. Questionnaires were given to respondents that had consented to take part in the study. A total of 208 respondents were registered and interviewed in a period of February to march 2011. There were 165 (79.3%) of the mothers who had knowledge about various PMTCT methods. Of these 86(52%) heard about PMTCT first from hospital. 50 (30%) knew about exclusive breast feeding, while another 50(30%) use of ART, 45(27%) knew about replacement feedings and only 21(13%) knew of elective caesarean section as method of PMTCT. However the majority of the mothers 159(96.5%) thought that PMTCT was beneficial. Nevertheless some mothers thought that PMTCT causes various counter indications including infertility 17 (10.5%) and abnormalities in children at birth 27 (16.7). Their HIV serostatus also varied. Of the 135(81.7%) screened for HIV 42(31.2%) tested HIV positive and only 27 (64.2%) used contraceptives to prevent pregnancy while they were HIV positive. However of the correspondents who tested positive in their last HIV tests, the majority (83%) had had at least a pregnancy after testing HIV positive of which 6.8% did not practice PMTCT. Our study suggests evidence that Knowledge, attitude and practice of PMTCT among women of reproductive age in Laroo Division in Gulu municipality in Northern Uganda is adequate. Nevertheless with the infection rate of 31.2% among them points out that there is a substantial burden of HIV in the women community.

INTRODUCTION

Prevention of Mother to Child Transmission (PMCT), services traditionally follows four-pronged strategies. These include primary prevention of HIV, family planning, reduction of vertical transmission from infected mothers to their babies including antiretroviral drugs (ART), and support for HIV-positive pregnant women and their families (1, 2). Without interventions, there is a 20-45% chance that a baby born to an HIV-infected mother will become infected with HIV. In recent years, promising developments have been made in preventing mother to child transmission of HIV in the developing world (3). The risk of mother-to-child transmission of HIV can be reduced to less than 5 percent through a combination of prevention measures (PMTCT) , including antiretroviral therapy (ART) for the expectant mother and her new-born child, hygienic delivery conditions and safe infant feeding (4). According to a study, *HIV/AIDS TRaC,a Study Examining the Use of Prevention of Mother-to-Child Transmission Services among Women of Reproductive Age* done in 2005 in Uganda, showed that MTCT is high (95%)(5) .

But only 58% knew that MTCT occurs during delivery and pregnancy, while 68% were aware of where they could access PMTCT services and only 35% of the respondents knew two or more benefits of PMTCT(5). In 2011, there were approximately 96,700 HIV-infected pregnant women in Uganda and in 2010, 7% of under-5 mortality was due to HIV (6). Between 2009 and 2011, Uganda experienced a 25% decline in the number of new pediatric HIV infections from 27,300 to 20,600 (7) .In 2010, PMTCT services were available in 81% of Antenatal care (ANC) facilities in Uganda (8). Although HIV testing coverage among pregnant women increased from 18% in 2005 to 63% in 2010(9), it is still low. In 2011, only 50% of pregnant women living with HIV in Uganda received efficacious ARV regimens for PMTCT (7), and only 22% children born to pregnant women living with HIV received ARVs for PMTCT in 2010 (9). In 2009, an estimated 860,000 pregnant women were found to be living with HIV in Eastern and Southern Africa, more than in any other region of the world. The region is also home to 47 percent of the global total of children living with HIV, of which over 90 percent were infected through vertical transmission from the mother to the baby during pregnancy, delivery or breastfeeding (4). The majority (92.5%) of health facilities in Kenya offer PMTCT services (10) and 67% of pregnant women living with HIV received efficacious ARVs for PMTCT IN 2011(7). In 2009, UNICEF endorsed the UNAIDS call for a virtual elimination of mother-to-child transmission of HIV by 2015(4).

We in this study investigated gaps of information about the knowledge, attitude and practice of PMTCT among females in Laroo Division in anticipation for future interventional measures.

METHODOLOGY

A cross sectional (analytical) and descriptive study design was employed. The descriptive part described the level of knowledge, attitude and practice of PMTCT among women of child bearing age in Laroo Division. The cross sectional part compared the effects of knowledge and attitude on practice of PMTCT among women of child bearing age in Laroo division.

Study area

The study was carried out in Laroo Division in Gulu municipality which is situated approximately 330 kilometers North of Uganda's Kampala city. Laroo Division has both peri-urban and urban settlements with a good tropical climate. It covers approximately 5km square of land. Both the District and the municipal headquarters are located in this division. Administratively Laroo Division is at local council three (LC3) levels. It is divided into four parishes namely; Queens, Pece prison, Agwee and Iriaga all of which are at LC2 level. It has 14 sub wards at LC1.
According to Uganda population and housing census conducted in September to November 2002 by the Uganda bureau of statistics the population of Laroo division stood at 21,214 out of which 10,380 were male and 10,834 were female[12]. Due to the past northern war, the population in Laroo increased due to influx of people from rural areas into town which resulted into very unstable population level.

The family structure in Laroo division is of extended family system with mean family size of 5 to 6 persons per house hold but the number may be as large as 12 persons per house hold. The population at Laroo is multi ethnic consisting of mainly of Acholi (85%). Others include the Langi, Madi, and Alur (15%). The main languages spoken in this community are Luo, English, Swahili, Madi, Lugbara, Luganda and Kinubi. Occupationally the people of Laroo Division are small scale business men and women who operate small bars and shops. There are few civil servants, but yet with other involve in small scale industries of bricks laying, metal work fabrication. Only about 25% of the population lives in good permanent homes, 15% have semi permanent homes and 60% live in much crowded poor grass thatched and mud huts. These occupational activities and the environmental settlements predispose the inhabitants to HIV transmission.

Study population

The study population was all females in Laroo division and the target population was females of child bearing age 15-49 years .Only one participant was chosen randomly from a house hold after consenting.

Sampling Procedure

The study was carried out using a multistage sampling method. The Division is made up of

four parishes namely: Queens, Agwee, Pece and Iriaga. By simple random sampling, Queens Parish was chosen for the study out of the four parishes. By further simple randomization again a village was selected out of Queens. From the selected village we randomly select 209 homesteads from which 209 respondents were selected (one respondent from each home stead by random selection in homes with more than one female eligible member). In cases of homestead with no eligible women with

inclusion criterion, a respondent was selected randomly from another neighboring homestead that had not been selected before.

Ethical considerations

The intent and the benefits of the research were explained to the local leaders and the respondents in the study so that their cooperation and consent to participate was sought. They were assured of risk free, humane process and no penalty of any kind for those who would refuse to participate in the study and lastly they were assured of confidentiality of any information they would give. The ethical committee of Faculty of Medicine and the Uganda National Council of Science and Technology granted the permission for the study.

Data management and analysis

The data was entered and analyzed using Graph Pad Prism computer software.

Results

The result for knowledge, attitudes and practices of prevention of mother to child transmission of HIV among 208 women who were registered and interviewed in Laroo Division in Gulu municipality between February and March 2011is reported. Figure 1 shows the distribution of respondents registered and interviewed according to their age group. Out of the 208 mothers interviewed 165 (79.3%) had various

knowledge about PMTCT. These included 86(52%) respondents who heard about PMTCT first from hospital, 50 (30%) knew about exclusive breast feeding method for prevention of PMTCT, while another 50(30%) are using ART, 45(27%) knew about replacement feedings and only 21(13%) knew of elective caesarean section as method of PMTCT (Fig.2). In spite the majority of the mothers 159(96.5%) thought that PMTCT was beneficial, some mothers thought that PMTCT causes various health related complications including 17 (10.5%) thought that PMTCT causes infertility, 27 (16.7) abnormalities in children at birth. The tribal distribution showed that the indigenous 184(88.5%) Acholi are the main inhabitants of Laroo division although 24 (11.5%) others tribes also live in Laroo division. The majority of the respondents were married (fig3). Their HIV sera status also varied. Of the 135(81.7%) screened for HIV 42(31.2%) tested HIV positive and only 27 (64.2%) used contraceptive. All though a few of the respondents expressed knowledge of PMTCT induce health problems, the majority of them had very positive attitude about PMTCT (fig.4) and that the same respondents practice the various PMTCT methods (figs 5 and 6). interviewed in Laroo division Gulu municipality.

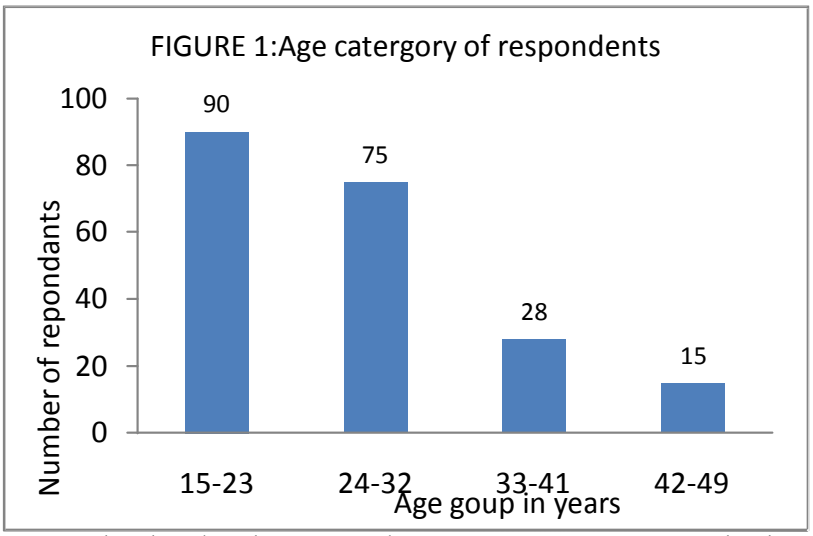
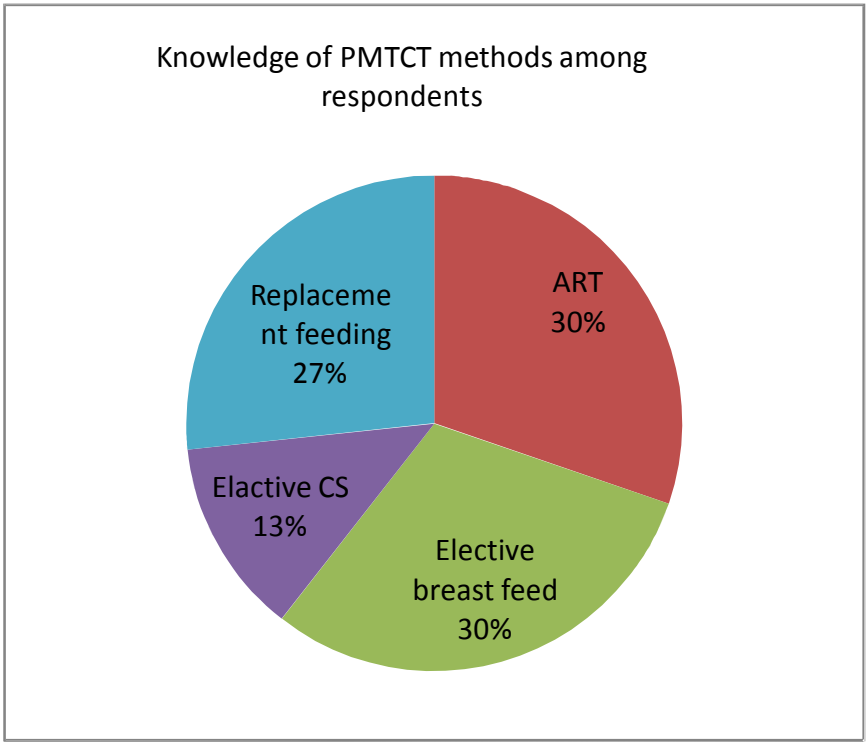


Fig.2: Showing knowledge of respondents to various methods of PMTCT among the 165 mothers



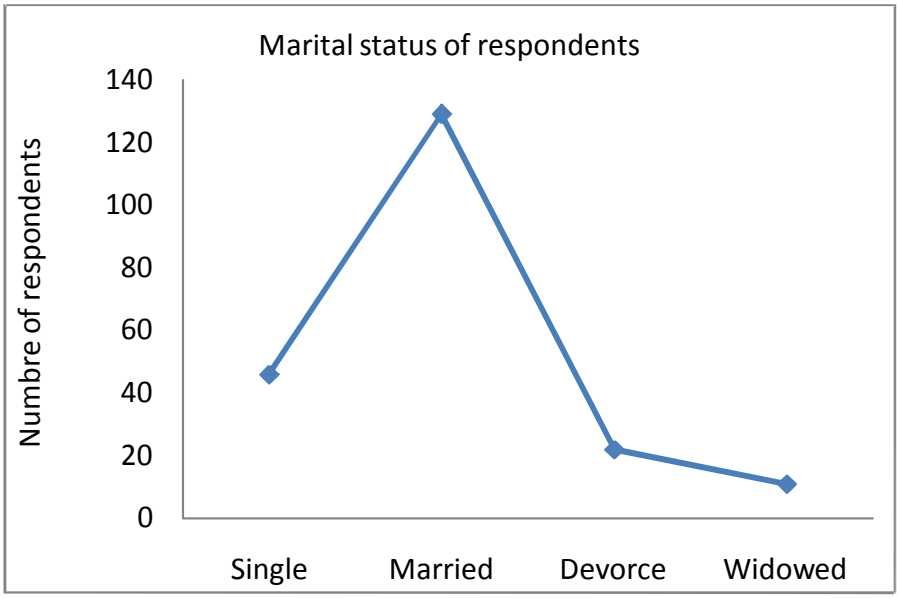


Fig 3: Showing the marital status of the 165 respondents interviewed in Laroo division

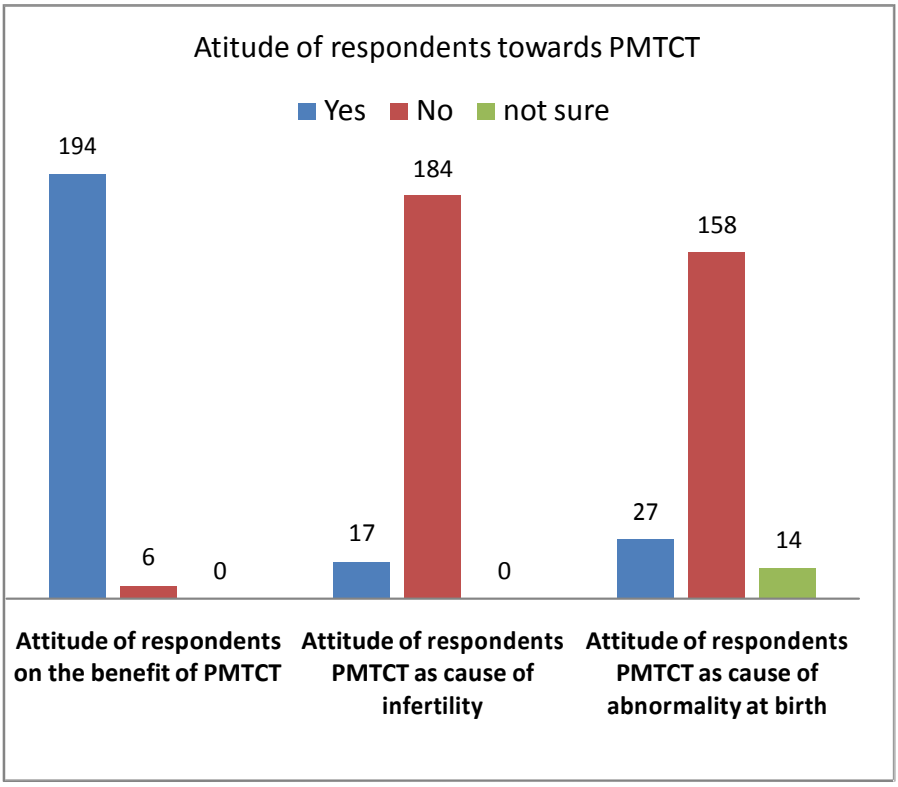


Fig.4: Showing attitude of respondents towards PMTCT

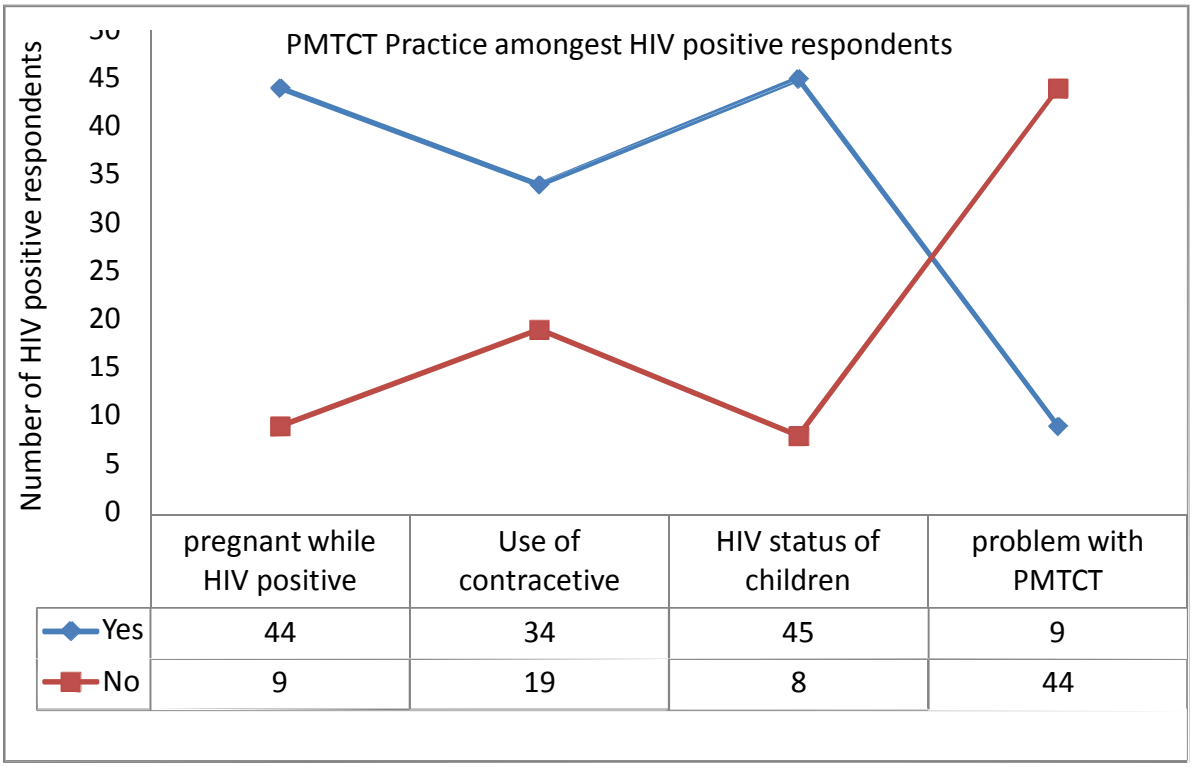


Fig. 5: Shows the majority of respondents in Laroo division practice different methods of PMTCT.

Table 1: showing pregnancies in which PMTCT was practiced among respondents who got pregnant after testing HIV positive.

Number of pregnancies	Number of respondents
All	30
Some	11
None	3

DISCUSSION:

A total of 208 respondents were interviewed in Laroo division in a period of one month. The respondents were randomly drawn from queens parish one of the four parishes in Laroo division. The respondents were stratified according to Age, Tribe, Occupation, Religion, and Level of education and marital status. According to the distribution of respondents by Age group (figure 1), the majority of respondents (43.3%) were in the age group of 15-23 years. Most of the respondents (88.8%) were Acholi by tribe. 54% of the respondents had only attained primary level of education and most of the respondents(34.6%) were house wives(figure 3). 62% of the respondents were Catholics and the majority(62%) were married.

The majority of the respondents as noted above in the age range of 15-23, most of which were married, had attained a low level of education and were unemployed(house wives). With these early marriages, low level of education and unemployment, the women are prone to early and frequent pregnancies, putting them at risk of contracting HIV and passing it on to their babies.

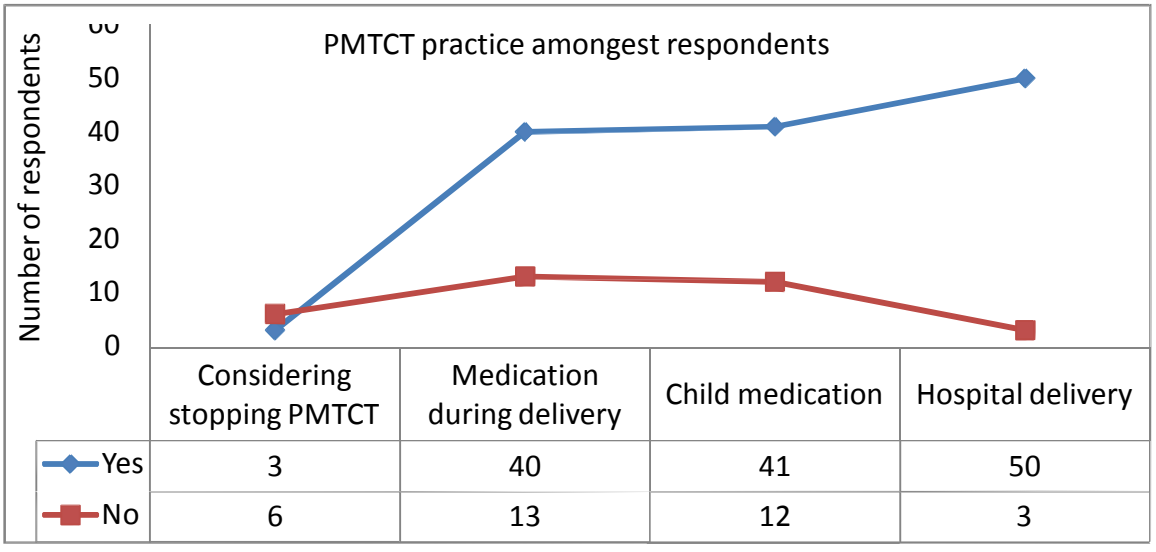
KNOWLEDGE

The majority of the respondents (79.3%) had knowledge about PMTCT, most of which (52%) first had of it from hospital, 34% from radio, 9% from community health workers and 5% from other sources. The majority of the respondents (77.9%) had attended ANC before and this contributed to the largest proportion of respondents (52%) who first heard of PMTCT from hospital. Despite the low level of education among most of the respondents, our study shows a relative increase in the knowledge of PMTCT among women. The increase in knowledge is attributed to the increased number of ANC attendance where health education is always provided to the mothers and their husbands on each visit.

Of the respondents who had knowledge about PMTCT, 30% new about Exclusive breast feeding, 30% knew about ARVs, 27% about replacement feeding and 13% knew about Elective caesarean section as methods of PMTCT. Most of the respondents (98.6%) new the importance of breast feeding to the child just as most of the respondents (90.9%) knew that breast feeding was a means of mother to child transmission of HIV.

Among the respondents there were perceived advantages of PMTCT which included prevention of transmission of HIV from mother to the baby, helps support children who get infected from their mothers, provides good health to the child, you get to know the HIV status of the baby early, it generally reduces chances of transmission of HIV, it is easy to raise a child free from HIV, saves the child born to an HIV positive mother from early death, eases doctors' work and it prevents HIV in children.

Fig.6: Shows the willingness of the respondents who are HIV positive to practice the various PMTCT methods in Laroo division Gulu municipality.



However a small proportion of the respondents were not sure and others did not know of any advantages of PMTCT. On the other hand there were perceived disadvantages among the respondents which include: The available methods are expensive for example replacement feeding, the child misses value in breast milk ,the child can still get HIV, it can lead to divorce, Children can lose the love from their mothers, it can lead to death of the child, Mother can forget to swallow the drugs or give it to the child, it is not widely known to most mothers, it can lead to mental retardation of the child, it can lead to delivery of a sickly child, it leads to child with very low immunity, people talk about you if they discover that you are practicing PMTCT (stigma), Child does not grow healthy, in case of a bad operation, child can get infected and exclusive breast feeding can lead to infection of the child through breast milk. However the majority of the respondents (52%) thought there was no disadvantage of PMTCT and 19.2% of the respondents were not sure of any disadvantage.

ATTITUDES

Among the respondents that heard of PMTCT, the majority (96.3%) thought that PMTCT was beneficial. This to these respondents was because of the same advantages of PMTCT listed above. Some of the respondents(16.7%) who had knowledge about PMTCT thought PMTCT causes abnormalities in children, 74.7% did not think PMTCT could cause abnormalities in children and 8.6% were not sure of any.

However, PMTCT was thought to cause infertility by 10.5% of the respondents who had knowledge about PMTCT. Among the respondents who thought that PMTCT methods cause infertility, Exclusive breast feeding was sited to be the leading cause of infertility followed by Elective caesarean section, ARVs and abnormalities caused by PMTCT. Among the respondents who thought that PMTCT causes abnormalities in children, ARVs were the leading cause followed by replacement feeding.

Despite the low level of education among the majority of the respondents, the attitude towards PMTCT was generally good. This can be attributed to the increased number of ANC attendance among respondents with most of them having heard of PMTCT for the first time in hospital and radio stations where correct PMTCT is disseminated through health education and radio health talks respectively.

PRACTICE:

Of the 208 respondents interviewed, the majority of the respondents (81.7%) had tested for HIV of which 65.3% had tested more than once and 34.5% had tested only once. This may be attributed to the increased ANC attendance where routine counseling and testing services are offered.

Among the respondents who had tested for HIV before, 31.2% had a positive result from their last HIV test and 68.8% tested negative in their last HIV test. From our study, we can attribute this to the low level of education and early marriages among the respondents.

Among the respondents who tested positive in their last HIV test, the majority (64.2%) had ever used contraceptives methods to prevent pregnancy and 35.8% had never used any contraceptive method to prevent pregnancy while they are HIV positive. However, among the respondents who tested positive in their last HIV test, the majority (83%) had had pregnancies after testing positive and 17% had never gotten pregnant after testing positive (Fig. 5).

From the study, we realized that there was increased number of pregnancies among the respondents who were HIV positive despite increased use of contraceptive methods. This may be attributed to irregular use of contraceptives, improper use of contraceptive methods and Cessation of use of contraceptives after initiation.

Among the respondents who got pregnant after testing HIV positive, the majority(68.2%) practiced PMTCT in all the pregnancies, 25% practiced in some and 6.8% did not practice PMTCT (table 1). This may be attributed to the thought among some respondents that it causes infertility and abnormalities in children, and also the disadvantages amongst the respondents as discussed above.

Among HIV positive respondents who got pregnant after testing positive, the majority (84.9%) had all their children free from HIV and 15.1% had some children who got infected with HIV. This may be attributed to the 6.8% of the respondents who did not practice PMTCT.

Of the respondents who practiced PMTCT, 22% experienced problems with PMTCT of which 55.6% attributed to ARVs, and 44.4% to replacement feeding. These problems were attributed to the side effects and the expenses respectively. However, only 33.3% of those who experienced problems with PMTCT were considering stopping irrespective of its advantages (figure 6) Among the respondents who got pregnant after testing HIV positive, 92.7% had their deliveries in hospital and 7.3% had their deliveries at home. (figure 6).

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TRANSFUSION TRANSMISSIBLE VIRAL INFECTIONS AMONG POTENTIAL BLOOD DONORS IN IBADAN, NIGERIA.

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ABSTRACT
It is evident that proper screening procedures prior blood transfusion is a cost-effective approach for prevention and control of transfusion-transmissible infections (TTIs). Also, it has been documented that sub-standard test kits are mostly used in resource limited settings for transfusion related diagnosis. However, the role of such practice in epidemiology of transfusion transmissible viral infections in a tertiary health care facility would give an insight to the rates of blood transfusion associated viral transmission in the community at large. Therefore, the study was designed to determine the prevalence of Human Immunodeficiency Virus (HIV), Hepatitis B and Hepatitis C viruses among blood donors in a tertiary hospital where quality diagnostic procedures are considered prior recruitment of donors. Post ethical approval, counselled and consenting 507(M= 426; F=81) aged 19 to 68 years (Median age:39) potential blood donors were recruited and tested for HIV, HBsAg and anti-HCV using commercial ELISA testkit in strict compliance with the manufacturer'sprocedures. Overall results show rates of 2.0%, 5.9% and 1.4% for HIV, HBsAg and HCV respectively. Also, highest prevalence rates were recorded among age group 26 to 35 years as 2.6%, 7.2% and 2.1% for HIV, HBV and HCV respectively. Furthermore, higher prevalences rates were noted among unmarried individuals as 2.6%, 6.8% and 2.1% for HIV, HBV and HCV respectively.

Key words: Transfusion Transmissible Infections, HIV, Hepatitis B, Hepatitis C, Blood Donors, University College Hospital (UCH), ELISA.

INTRODUCTION

The most common diseases transmitted in blood transfusions are viral infections. Transfusion-transmissible infectious agents such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are among the greatest threats to blood safety for the recipient. The discovery of transfusion-transmissible infections (TTIs) has heralded a new era in blood transfusion practice worldwide with emphasis on two fundamental objectives, safety and protection of human life [1]. Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are of great concern as transfusion-transmissible infections because of their prolonged viraemia and carrier or latent state. They also cause fatal, acute, chronic and life-threatening disorders. Blood transfusion accounts for 5-10% of HIV infections in sub-Saharan Africa [2]. Similarly, 12.5% of patients who received blood transfusion are at risk of post-transfusion hepatitis [3]. Therefore, this study was carried out to determine the seroprevalence, among selected group and trends of HIV, HBV and HCV among donors at University College Teaching Hospital in Ibadan Nigeria.

MATERIALS AND METHODS

Study Centre

The Study centre was located in Ibadan, Oyo State, situated in the tropical belt of Southern Western part of Nigeria. The study is at the Blood Bank of University College Hospital, Ibadan, Nigeria.

Study Population

The study participants were potential blood donors presenting at the blood bank of the University College Hospital, Ibadan between February and July 2010. Total of Five hundred and seven samples was collected from the prospective donors.

Sample Collection (Blood)

Samples were collected in a tube without anticoagulant. A tourniquet was firmly tied to the upper arm of the donor while sitting and skin sterilizer with 70% alcohol. The sterile needle was inserted into conspicuous antecubital vein and the plunger of the sterile syringe was withdrawn and pressure applied to the puncture site with a cotton wool to stop bleeding. Blood sample was spun on a bench centrifuge at 3,000rpm for 10 minutes to obtain serum. Serum or plasma was separated immediately.

Data Collection and Laboratory Methods

A survey of the blood sample of the prospective donors at the Blood Bank, University College Hospital (UCH) Ibadan was conducted between February – July 2010 using structured questionnaire. Descriptive statistics and correlation analysis was performed to check the relationship between the data and laboratory test results observed and inference was drawn using an inferential statistics (SPSS version 15).

A cross-sectional study was conducted from February to July, 2010 at blood bank of the University College Hospital, (UCH) Ibadan. The total samples included all the donors, presented and consented to participate during the study period. Prior to blood collection, the blood donors were required to answer questionnaire. After consent was obtained and pre-test counselling was done, and the questionnaire was completed. 5mls of blood sample was collected in sterile tubes without anticoagulant from each study participant using a vacutainer needle. Serological examination was conducted on each sample.

Serum samples were tested as follows: for HIV 1 and HIV 2 using BIO-RAD GenscreenELISA made in France; for HBsAg using BIO- RAD *monolisa ultra*made in France; for HCV-AbDIA.PRO ELISA made in Italy was used.

RESULTS

Table 1 shows prevalence of HbsAg, HIV and HCV antibody in the study population. Out of 507 participants whose sera were tested using ELISA, 30 (5.9%) were positive for HbsAg, 7(1.4%) for HCV and 10 (2.0%) for HIV.The highest prevalence of HBsAg (7.2%) was found in persons whose ages were between 26-35 years as shown in Table 4. The prevalence of HbsAg in theother age groups was as follows; 16-25 years (6.6%), 36-45 years (4.2%), 46-55 years (4.7%).

The prevalence of HbsAg in age group 26-35 years was significantly higher than the other age groups. The difference observed in the prevalence of HbsAg among different age group, was not statistically significant $P > 0.05$.

TABLE 1: PREVALENCE OF HEPATITIS B SURFACE ANTIGEN (HBSAG), HEPATITIS C (HCV) AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) AMONG THE STUDY PARTICIPANTS.

Virus	Total NoTested	Seropositive %	No
HbsAg	507	5.9	30
HCV	507	1.4	7
HIV	507	2.0	10

TABLE 2:*PREVALENCE OF HEPATITIS B SURFACE ANTIGEN (HBSAG), HEPATITIS C (HCV) AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODIES AMONG DIFFERENT AGE GROUPOF STUDY PARTICIPANT*

	AGE IN YEARS				
	16-25 No (%)	26-35 No (%)	36-45 No (%)	46-55 No (%)	TOTAL No (%)
HBsAg	9(6.6)	14(7.2)	5(4.2)	2(4.7)	30(6.0)
HCV Positive	2(1.5)	4(2.1)	1(0.8)	0(.0)	7(1.4)
HIV Positive	2(1.5)	5(2.6)	2(1.7)	1(2.3)	10(2.0)

TABLE 3:*PREVALENCE OF HEPATITIS B SURFACE ANTIGEN (HBSAG), HEPATITIS C (HCV) AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODIES AMONG THE MARRIED AND UNMARRIED PARTICIPANTS*

Marital Status	No Tested	HbsAg No (%) Positive	HCV No (%) Positive	HIV No (%) Positive
Married	297	17(5.7)	3(1.0)	4(1.3)
Unmarried	192	13(6.8)	4(2.1)	5(2.6)
Total	489	30(6.1)	7(1.4)	9(1.8)

The results of tests for HCV antibody in the study population is shown in Table 1. Out of the 507 sera tested for antibody to HCV 7(1.4%) were positive. The age distribution of HCV antibody test result in the study is shown in Table 4. The highest prevalence of HCV antibody (2.1%) was found in 26-35 years age group. Prevalence of HCV antibody in the other age groups are as follows; 16-25 years (1.5%) and 36-45 years (0.8%).

Out of 507 study participants (individual blood donors) whose sera were tested for HIV antibody 10 (2%) were positive. The age distribution of HIV antibody in this study is shown in Table 2. The highest prevalence of HIV antibody (2.6%) was found in 26-35 years age group. Prevalence of HIV antibodies in the other age groups was as follows; 16-25 years (1.5%), 36-45 (1.7%) and 46-55 years (2.3%). There was no significant difference between prevalence of HIV antibody among the different age groups, $P > 0.05$.

A total of 489 study participants with different marital status were tested for HbsAg and antibodies to HCV and HIV. The prevalence of HbsAg, HCV and HIV antibody in married and unmarried participants is shown in Table 3. Out of 297 married study participants 17(5.7%) had HbsAg in their

sera, 3(1.0%) and 4(1.3%) were positive of HCV and HIV antibodies respectively .Out of 192 unmarried, 13(6.8%) were positive for HbsAg, 4(2.1%) and 5(2.6%) were positive for HCV and HIV antibodies respectively. The prevalence of HbsAg, and HCV antibody was higher among the unmarried participants than in married individuals, the difference is statistically significant $P > 0.05$. Prevalence of HIV antibody among the unmarried was higher than married. There was a statistical significance ($P < 0.05$) difference between HIV prevalence among the unmarried and the married participants.

DISCUSSION

Blood transfusion is the process of transferring blood or blood-based products from one person into circulatory system of another, to save lives in some medical conditions. Although blood transfusion saves million of lives worldwide each year, recipients of the blood or blood product transfusions stand the risk of becoming infected with blood-borne diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), through transfusion of infected blood and blood products [4].The discovery that HIV, HBV and HCV could be transmitted by blood transfusion has provoked a greatly heightened emphasis on two fundamental objectives, safety and protection of human life [5]. Transfusion transmissible infections (TTIs) are a very serious complication of blood transfusion [6]. These infections continue to pose a great challenge to transfusion medicine, most especially in Africa, due to a high transfusion demand [7]. Hepatitis B and C viruses and HIV are distinct, but share a similar mode of transmission, primarily; through unscreened and contaminated blood and blood products by contact or transfusion. Other routes include sexual intercourse and vertical transmission from mother to foetus in the immediate pre-natal period [8,9].

Prevention of transfusion transmitted infections (TTIs) in developed countries has been achieved by reducing unnecessary blood transfusionand exclusion of donors with specific risk of viral infections.By contrast, in many developing countries, none of these interventions is applied uniformly and the risk of (TTIs) remains high. The high incidence and prevalence of (TTIs) in the blood donors in developing countries means that, some agents escape laboratory detection,[10].

In this study 507 donors were recruited into the study with ages ranging from age 16 years to 68 years. In general blood donors must fulfil certain requirements, one of which is the age requirement of between 18 – 60 years. This explains why no study subject was below age 19 and only a few were above 60 years old. There was a predominance of males among the study participants because females are not encouraged to donate blood in Nigeria society. Also, most of the replacement donors are men. Prevalence of HbsAg (6.8%) and antibody to HCV (2.1%) in this study was significantly higher among the unmarried participants than in married individuals. The prevalence of HIV antibody in unmarried was higher than the married. Higher prevalence values for HbsAg, HCV and HIV antibody in unmarried may be due to the fact that the unmarried individuals are more likely to engage in behaviours that put them at risk of all these infections than the married participants.

The findings of this study showed that hepatitis B and C viruses and HIV are circulating in Ibadan and infecting the residents of the city, including the blood donors. Hepatitis B surface antigen, HCV and HIV antibodies found were 5.9%, 1.4%, and 2.0%, respectively. The result of this study on the prevalence of HbsAg in blood donors in UCH, Ibadan isdifferent from that ofOlaleye, *et al.,*[11], which showed a very high prevalence (30.8%) of HbsAg in Ibadan. The prevalence HbsAg in blood donors in Ibadan is also lower than that found by Okerengwo and Mudasiru[12], when they tested sera of blood donors for HbsAg in the same location, this may be due to the awareness and effect of HIV control programmes in the society, for the fact that they share the same route of transmission.The seroprevalence of HCV antibody found among blood donors in this study is lower than that reported by previous workers. Fashola, *et al.,*[13] showed the prevalence of HCV antibody among blood donors in UCH, Ibadan was 4.96%. Studies carried out in other location in Nigeria also showed a higher prevalence of HCV antibody in blood donors than that found in the present study. Isa, *et al.,*[14] found HCV antibody prevalence of 1.8% among blood donors in Kaduna, Kaduna state, Nigeria. Studies carried out by Egah, *et al.,* [15] in Jos, Plateau state, Nigeria showed that the prevalence of HCV antibody among blood donors was 6%. Chukwurah, *et al.,*[16] and Ayolabi, *et al.,*[17] also found higher prevalence of HCV antibody among blood donors. A study carried out in South-Eastern state of Nigeria, Chukwurah, *et al.,* [16] found that 7.6% of blood donors had HCV antibody in their sera. In similar study carried out in Lagos, Nigeria Ayolabi, *et al.,*[17] reported that 8.4% of blood donors were positive for HCV antibody.

Marked variation in the prevalence of HIV antibody of HIV antibody has been reported in different part of Nigeria. Some workers have reported prevalence of HIV to be lower than the 2% found in this study, while others reported higher prevalence of HIV antibody from some locations [18, 19,13]. Ejele, *et al.,* [18] found that 4% of blood donors in Port Harcourt, South-Southern Nigeria had HIV antibody.

Overall, the prevalence of HbsAg, HCV and HIV antibodies found in this study is one of the lowest

ever reported from Nigeria. This observation may probably be due to the positive impact of HIV education and public enlightenment campaign which have been in place in Ibadan and everywhere for many years and which helped in reducing the spread of HIV in Nigeria. Since HIV, HCV and HBV have similar modes of transmission, the reduction in prevalence of HIV infection in the community will also lead to a reduction in HBV and HCV transmission. Another factor that may account for the low prevalence of HbsAg, HCV and HIV antibody is donor recruitment. Infections by these three viruses are more common in commercial and replacement donors than voluntary donors. It is possible that most of the donors whose sera were tested in this study were voluntary donors.

Age distribution of HbsAg, HCV and HIV revealed that there was no significant difference in the prevalences of HbsAg, HCV and HIV antibody among the different age groups of blood donors. Youths aged 21-30 constituted the highest number of HIV and hepatitis B infection, HBV had 21, {1.4%} seropsitivity; HIV had15 {1.0%}seropisitivity and age 31-40 had 1 (0.1%) for HCV.

Results of the study which showed that the prevalence of HbsAg, HCV and HIV antibody is 5.9%, 1.4% and 2.0% suggest that these three major transfusion transmissible viral infections are still causing considerable morbidity in the communities and continue to pose a major challenge for blood safety. It seems that the prevalence of these viral infections is lower than that was reported by previous workers. This suggests that the disease control measures of the Federal government of Nigeria and Non-governmental organizations (NGO) are making a positive impact in the spread of these viral infections.

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ANTIMALARIAL USE AND THE ASSOCIATED FACTORS IN RURAL NIGERIA FOLLOWING IMPLEMENTATION OF AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM) PRICE SUBSIDY

RUNNING TITLE: ANTIMALARIAL USAGE FOLLOWING AMFM SUBSIDY

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ABSTRACT

Purpose

This study was set out to find out the pattern of antimalarial drug use in a Nigerian rural community following the aggressive price subsidy of Artemisinin Combination Therapy(ACT) recently embarked upon by Roll Back Malaria partners through Affordable Medicines Facility-malaria (AMFm).

Methods

Questioners were administered to 310 adult members of the community with the most recent malaria episodes so as to find out about the drugs used and some of the factors associated with the choice of the drug.

Result

Although the overall use of ACT (13.55%) in this community was about 4 times higher than what it used to be, Chloroquine 123(39.62%) and sulphadozine/pyrimathamine 120(38.71%) were the mostly used antimalarial agents. Choice of drug used was significantly associated with perception of efficacy and price among other factors. Respondents liked the price of ACT (33.3%) most, CQ was the drug most liked in terms of efficacy (44.2%) while SP was the drug most liked in terms of lack of side effect (38.9%), taste (61.6%) and convenience (35.7%).(P= 0.001)

Conclusion

In addition to sustaining the current price control, there is a need to continuously monitor and effectively regulate the quality of the ACTs in circulation so as to gain the confidence of both the prescribers and the end users regarding efficacy and adherence to ACTs. This will help to safeguard the huge investment in ACT subsidy by the Roll Back Malaria partners.

Key words: ACT, Subsidy, Affordable Medicines Facility-malaria

INTRODUCTION

The 2008 World Malaria Report showed that only 3% of children with suspected malaria were treated with Artemisinin Combination Therapy (ACT), suggesting that many children were still receiving chloroquine and sulphadoxine/pyrimethamine for malaria treatment despite the recommended change in treatment guidelines [1]. A published study that evaluated 21 African countries for their antimalarial use in 2006-2007 showed that only three countries reported high use of the regimen that was in agreement with the national treatment guideline [2]. The study further revealed that chloroquine was the most common anti-malarial reported in 14 of 21 countries , and reported use was particularly high in West Africa.

A descriptive study that evaluated the anti-malarial drug prescribing practice in private and public health facilities in South-East Nigeria also showed that only 3.0% of patients were treated with artemisinin combination therapy. Most of the patientswere treated with chloroquine (30.2%), sulphadoxine/ pyrimethamine (22.7%) orartemisininmonotherapy (15.8%) despite the change in the national malaria treatmentpolicy [3].

Treatment of malaria in pregnancy was not exempted from the protocolviolation. A recent survey of the patterns of anti-malarial drug treatment among pregnantwomen in Uganda showed that only 5.6% of the pregnant women in their first semesterwere treated according to the national guideline while 70% were treated withcontraindicated anti-malarial drugs. Recommended antimalarial were used according tothe guidelines in only 30.1% of all second and third trimester episodes [4].Some of the factors that that

have been found to positively influence the use of clinical
guidelines include clarity of guidelines, strong evidence, adequate funding of guidelines and support by opinion leaders especially professional bodies [5].The reluctance to abandon chloroquine is based, in part, on its low cost, wide availability, and acceptance. Chloroquine results in rapid initial improvement of clinical symptoms, which has contributed to its widespread acceptance and the perception that it remains effective, leading to an unwillingness of both health workers and patients to discontinue using chloroquine despite its reduced efficacy [6].In 2004, Nigeria changed from the use of chloroquine and sulphadoxine/pyrimethamine as first line anti-malarial drugs in line with the recommendation of the World Health Organization as a result of unacceptably high level of resistance to the formal drugs [7].Adherence of patients as well as health care providers to the new guidelines remain poor in many parts of Nigeria [8-10].

Research data from Kenya provided strong evidence that continued use of chloroquine in areas with resistance was contributing to excess *Plasmodium falciparum* related deaths [6].Apart from vector and parasite biology, pharmacokinetics, and economics reasons;human behavior is another factor that has been associated with antimalarial drugresistance [11].In addition to low usage of ACTs imposed by its high cost, quality of ACTs in circulationis another major challenge. Recent survey by the World Health Organization (WHO) inconjunction with Nigerian drug regulation agency showed that Nigeria has the highestrate of sub-standard antimalarials (63.9%) in Sub-Sahara Africa [1]. The survey furtherrevealed that a common problem for ACTs was that of lower content of active pharmaceutical ingredients (APIs). This was further supported by the findings of the Global Funds which showed that availability of quality-assured ACTs in Nigeria is only 28% [1].

The first attempt to improve the use of ACT in Nigeria was in 2008 when the government took a step forward in subsidizing child doses of Amodiaquine-artesunate combination (ASAQ) via the private sector in 18 of the 37 states. Retailers in these 18 states could purchase subsidized ACTs for 5 naira ($0.03 USD) per treatment with an approved retail price set at 30 naira ($0.20 USD). This was followed by widespread stockouts of ACTs across Nigeria as a result of inadequate supply. This resulted in reduced availability of ACTs in the public sector and increased prices in the private sector. End users were eventually forced to revert back to the cheaper monotherapy [13].

Efforts to remove the obstacle imposed by high cost and stockouts of ACTs received another boost when Nigeria initiated Affordable Medicines Facility-malaria (AMFm) activities on October 5, 2010. AMFm was set by the Roll Back Malaria Partnership (RBM) in 2007 and managed by the Global Fund to Fight AIDS, Tuberculosis and Malaria (the Global Fund).

The AMFm aims to increase use of ACTs by subsidizing prices of ACTs at the "factory gate". The subsidy will in turn be passed along the supply chain to the consumer, lowering ACT prices so that they are comparable to chloroquine, sulfadoxine-pyrimethamine or artemisininmonotherapy. Reduced prices should, in theory, "crowd out" sales of these other drugs and thus increase ACT use [13].
The aim of this study is to find out the current malaria treatment practices following the introduction of AMFm and some of the factors that are associated with such practices in a rural community in South-West Nigeria.

METHODS
This study was carried out between March and April 2012 at Oke-iho, a rural community in Oyo State, South-West Nigeria with a population of about 12,964 inhabitants. It is situated at 8.03° North latitude, 3.35° East longitude and 314 meters elevation above the sea level, the climate is tropical rainforest with malaria transmission occurring most of the year [13-14]. Information about the number of streets and houses in this community was obtained from the local government authority. Eleven houses were randomly selected from each street by simple random sampling method, and then an adult with the most recent episode of malaria was chosen as participant per house after informed consent was obtained. Pretested questionaires were administered by trained research assistants to participants after translation of the contents to their local dialects.

Statistical analysis
Data was analyzed with the free GNU PSPP Statistical Analysis Software version 0.7.9-gd4ae90. Using the descriptive statistics in the analysis command, frequency tables were generated for the general characteristics of the participants and expressed in percentages. Association between the drug used to treat the last malaria episode and other variables was assessed using Chi-Square, and $p < 0.05$ was taken to be significant.

RESULTS
Majority (58.71%) were females while 201 (64.84%) were married (table 1).Malaria was the 4th common health problem 49 (15.16%) experienced by participants and it was experienced once to twice a

year in most cases (41.61%) presenting as fever 126 (40.65%) and headache 86 (27.74%)(Table 2).

TABLE 1: SOCIODEMOGRAPIC PARAMETER OF THE RESPONDENTS

Character	frequency	%
Age		
18-20	50	(16.13)
21-30	114	(36.77)
31-40	87	(28.06)
41-50	31	(10.02)
>50	28	(9.03)
Sex		
Male	128	(41.24)
Female	182	(58.71)
Marital status		
Married	201	(64.84)
Single	09	(35.16)
Separated	0	(0)
Level of education		
No formal education (Nil)	27	(8.71)
Primary school certificate	40	(12.90)
Secondary school certificate	163	(52.58)
Diploma	69	(22.26)
Degree	11	(3.55)
Occupation		
Unemployed	0	(3.23)
Students	68	(21.94)
Trading	81	(26.13)
Farming	20	(6.45)
Artisan	61	(19.68)
Teaching	37	(11.94)
Apprentice	7	(2.26)
Civil servants	10	(3.23)
Others	16	(5.16)
Tribe		
Yoruba	284	(91.61)
Ibo	7	(2.26)
Hausa	14	(4.52)
Fulani	4	(1.27)
Ghanaian	1	(0.32)

Sulphadozine/pyrimathamine 92(29.68%) was mostly regarded as the currently recommended drug for treating malaria followed by chloroquine 85(27.42%). The drug used to treat the last episode of malaria by respondents included chloroquine 123(39.62%), sulphadozine/pyrimathamine 120(38.71%), ACT 82(13.55%) and Artesunatemonotherapy 8 (2.58%) (Table 3).CQ was mostly used by males (42.2%) while SP was mostly used by females (39.0%).

Use of ACT observed among females (15.9%) was about 1.6 times higher than in the males (10.2%), p= 0.56. While the use of SP has no definite pattern with the level of education, ACT use increased with the level of education, with the highest among those with university degree (27.2%) while the use of CQ was highest among those with no formal education 14 (15.8%), P= 0.33, (Table 4).

TABLE 2:. MALARIA FEATURES AMONG PARTICIPANTS

Features	Frequency	%
What is your commonest health problem?		
Headache	95	(30.65)
Back pain	50	(16.13)
General body aches	94	(30.32)
Malaria	49	(15.16)
Menstrual pain	3	(0.79)
Abdominal pain	2	(0.65)
Others	19	(6.13)
How often do you experience malaria attack?		
< once per year	39	(12.58)
1-2 times per year	29	(41.61)
3-4 times per year	91	(29.35)
4-6 times per year	49	(15.81)
>6 times per year	2	(0.65)
What major sign suggest that you have malaria?		
Fever	126	(40.65)
Headache	86	(27.74)
Diarrhea	11	(3.55)
Loss of appetite	50	(16.13)
Body weakness	36	(11.61)
Others	1	(0.32)

Friends 7(50.0%) and Chemists 15 (33.3%) were more likely to prescribe CQ than any other drug. Doctors 26 (19.3%) were the most likely to prescribe ACT followed by nurses (15.4%). P= 0.001. ACT was mostly liked for its good price (33.3%), CQ was the drug mostly liked in terms of efficacy (38.1%) while SP was the drug mostly liked in terms of lack of side effect (38.9%), taste (61.6%) and convenience (35.7%). P= 0.001.

The use of CQ 76(89.4%) and SP 70(76.1%) were highest among those who believed that they were the recommended drugs for malaria while ACT 10(55.5%) use was also highest among those who were of the opinion that it was the current antimalarial of choice. P= 0.001. (Table 5).

DISCUSSION

The overall use of ACT(13.55%) in this community was about 4 times higher than recorded in 2008 [1]. This may be a reflection of user's satisfaction with the

price and probably improved availability following implementation of AMFm .

The higher rate of ACT use and the lower rate of CQ use among females compared to males in this study may be due to the fact that females are more likely to receive health education regarding malaria control as a result of antenatal care visit [15-16].

TABLE 3: KNOWLEDGE AND ATTITUDE TOWARD MALARIA TREATMENT.

Knowledge/Attitude	frequency	%
What is the currently recommended drugin malaria treatment policy?		
Chloroquine	**85**	**(27.42)**
S/P-Fansidar, Amalare.t.c	**92**	**(29.68)**
ACT e.gCoartem	**18**	**(5.81)**
Artesunate alone	**18**	**(5.81)**
Ampicilin	**32**	**(10.32)**
Cotrimoxazole	**28**	**(9.03)**
Others	**37**	**(11.94)**
Drug used during the last episode of malaria?		
Chloroquine	**123**	**(39.62)**
S/P-Fansidar, Amalare.t.c	**120**	**(38.71)**
ACT e.gCoartem	**42**	**(13.55)**
Artesunate alone	**8**	**(2.58)**
Ampicilin	**7**	**(2.26)**
Cotrimoxazole	**5**	**(1.61)**
Others	**5**	**(1.61)**
Who prescribe the drug to you?		
Doctor	**133**	**(42.90)**
Nurse	**39**	**(12.62)**
Chemist	**73**	**(23.62)**
Self	**48**	**(15.53)**
Friend	**16**	**(5.16)**
What did you like most about the used drug?		
Good price	**15**	**(4.84)**
Efficacy	**231**	**(74.52)**
No side effect	**18**	**(5.81)**
Taste	**18**	**(5.81)**
Convenient.	**28**	**(9.02)**

S/P= Sulphadoxin-Pyrimethamine, ACT=ArtemisininCombination Therapy.

Though closely followed by SP 4(26.7%) and CQ 3(20.0%), the fact that the price of ACT 5(33.3%) was what users liked most about these drugs suggested that effect of the recent aggressive subsidy mechanism by AMFm to lower the price ACT is being felt in this part of the country [13].
Surprisingly, participants rated the efficacy of CQ (44.2%) and SP (38.1%) above that of ACT (12.6%) contrary to available evidence from clinical trials [17-18]. This may explain why these older drugs were still largely used in this community despite the perceived satisfaction with the price of ACT.

This contradiction is probably because therapeutic efficacy trials evaluate clinical and laboratory evidence in children below the age of 5 years while this study sought testimonies of adult antimalarial users. It may also be due to the fact that ACTs used in therapeutic efficacy trials are usually of certified quality [19] while drugs used by patients come from the open market. The latter possibility is supported by the recent findings of widespread substandard and fake ACTs in Nigerian drug market [12-13]. Testimonies of the end users may also explain the observed high rate of CQ and SP prescription by doctors and nurses in this study.

Conclusion

This study showed that though chloroquine and sulphadoxine/pyrimethamine were still the major anti-malarial drugs currently used in this part of Nigeria, there was an improvement in ACT use when compared to years before AMFm implementation. Antimalarial use practices are significantly associated with who prescribe the drugs,
knowledge of the current treatment guidelines as well of perception of efficacy by the users. Users liked the price of ACT but still believed in the efficacy of the older drugs. In addition to sustaining the current price control, there is a need to continuously monitor and effectively regulate the quality of the ACTs in circulation so as to gain the confidence of both the prescribers and the end users regarding efficacy and adherence to ACTs. This will help to safeguard the huge investment in ACT subsidy by the Roll Back Malaria partners.

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Conflict of Interest

We declare that there are no conflicts of interest associated with this study

Contribution of Authors

We declare that this work was done by all the 6 authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Authors AME and AF conceived the study. Authors EAM, FA, KB andMOB designed the study. The data presented in this study was collected and analyzed by Authors; EAM, OOO, MOB andOEI. The write-up was done by EAM and KB.

TABLE 4-: RELATIONSHIP BETWEEN SOCIODEMOGRAPHIC PARAMETERS OF THE PARTICIPANTS AND THE DRUG USED TO TREAT THE LAST MALARIA EPISODE.

(Drug used during the last episode of malaria) CQ	S/P	ACT	AM	Amp	Cotrim	Others	Total (%) Frequency (%)
Age							
18-20	22(44.0)	19(38.0)	1(2.0)	2(4.0)	2(4.0)	2(4.0)	2(4.0) 50(100)
21-30	42(36.8)	47(41.2)	15(13.2)	5(4.4)	4(3.5)	0(0.0)	1(0.9) 114(100)
31-40	29(33.3)	34(39.1)	19(21.8)	1(1.1)	1(1.1)	2(2.3)	1(1.1) 87(100)
41-50	14(45.2)	12(38.7)	5(16.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0) 31(100)
>50	16(57.1)	8(28.6)	2(7.1)	0(0.0)	0(0.0)	1(3.6)	1(3.6) 28(100)
Pearson Chi Square = 33.65, *df* = 36, *P* = 0.58							
Sex							
Male	54(42.2)	49(38.1)	13(10.2)	5(3.9)	2(1.6)	3(2.3)	2(1.6) 128(100)
Female	69(37.9)	71(39.0)	29(15.9)	3(1.6)	5(2.7)	2(1.1)	3(1.6) 182(100)
Pearson Chi Square = 4.89, *df* = 6, *P* = 0.56							
Level of education							
Nil	14(51.8)	10(37.0)	1(3.7)	0(0)	0(0)	1(3.7)	1(3.7) 27(100)
Primary	21(52.5)	12(30.0)	3(7.5)	2(5.0)	1(2.5)	1(2.5)	0(0) 40 (100)
Secondary	63(38.7)	68(41.7)	19(11.7)	3(1.8)	6(3.7)	1(0.6)	3(1.8) 163 (100)
Diploma	21(30.4)	26(37.7)	16(23.2)	3(4.3)	0(0)	2(2.9)	1(1.4) 69 (100)
Degree	4(36.4)	4(36.4)	3(27.2)	0(0)	0(0)	0(0)	0(0) 11(100)
Pearson Chi Square = 26.54, *df* = 24, *P* = 0.33							

CQ-choroquine,S/P –sulphadoxin/pyrimethamine, ACT –Artemisinin combination therapy, AM- Artesunatemonotherapy, Amp - Amoxicillin , Cotrim-Cotrimoxazole.

TABLE 5: RELATIONSHIP BETWEEN THE DRUGS USED TO TREAT THE LAST MALARIA EPISODE AND THE DRUG PRESCRIBER, WHAT THE USER LIKED MOST ABOUT THE DRUG AND THE USER'S KNOWLEDGE OF CURRENTLYRECOMMENDED ANTI-MALARIA DRUGS.

(Drug used during the last episode of malaria) CQ S/P ACT AM Amp Cotrim Others Frequency (%)							
Who prescribed theused drug							
Friend	7(5.7)	3(2.5)	1(2.4)	1(12.5)	**2(28.6)**	1(20.0)	1(20)
Doctor	52(42.3)	56(49.1)	24(57.1)	1(12.5)	**0(0)** 0(0)	0(0) Nurse	
16(13.0)	16(13.4)	6(14.3)	1(12.5)	0(0)	0(0)	0(0) Chemist	
32(26.0)	29(24.4)	4(9.7)	4(50.0)	1(14.3)	3(60.0)	0(0) Self	
16(13.0)	15(12.6)	7(16.7)	1(12.5)	4(57.1)	1(20.0)	4(80) Total	
123 (100)	120(100)	42(100)	8(100)	7(100)	5(100)	5(100) Pearson Chi Square	
52.46 , *df* = 24, *P* = 0.001							
What do you like mostabout the drug used?							
Cheap price	3(2.4)	4(3.3)	5(11.9)	1(12.5)	0(0)	2(40.0)	0(0)
Efficacy	102(82.9)	88(73.3)	29(69.0)	3(37.5)	3 (42.9)	2(40.0)	4(80.0)
No Side effect	6(4.9)	7(5.8)	4(9.5)	0(0)	0(0)	1(20.0)	0(0)
Taste	3(2.4)	11(9.2)	0(0)	2(25.0)	2(28.6)	0(0)	0(0)
Convenience	9(7.3)	10(8.3)	4(9.5)	2(25.0)	2(28.6)	0(0)	1(20.0)
Total	123 (100)	120(100)	42(100)	8(100)	7(100)	5(100)	5(100)
Pearson Chi Square	52.24,	*df* = 24, *P* = 0.001					
Currently recommendeddrug in malarial treatmentpolicy,							
Chloroquine	76(61.8)	6(5.0)	3(7.1)	0(0)	0(0)	0(0)	0(0)
S/P	7(5.7)	70(53.8)	10(23.8)	3(37.5)	0(0)	2(40.0)	0(0)
ACT	3(2.4)	4(3.3)	10(23.8)	0(0)	1(14.3)	0(0)	0(0)
AM	2(1.6)	3(2.5)	11(26.2)	0(0)	1(14.3)	0(0)	1(20.0)
Ampicilin	11(8.9)	10(8.3)	2(4.8)	4(50.0)	2(28.6)	1(20.0)	2(40.0)
Cotrimoxazole	7(5.7)	17(14.2)	0(0)	1(12.5)	2(28.6)	0(0)	1(20.0)
Others	17(13.8)	10(8.2)	6(14.3)	0(0)	1(14.3)	2(40.0)	1(20.0)
Total	123 (100)	120(100)	42(100)	8(100)	7(100)	5(100)	5(100)
Pearson Chi Square	258.52,	*df* = 36, *P* = 0.001					

CQ-choroquine, S/P –sulphadoxin/pyrimethamine, ACT –Artemisinincombination therapy,

AM- Artesunatemonotherapy, Amp - Amoxicillin , Cotrim-Cotrimoxazole

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NEONATAL INFECTIONS CAUSED BY *ESCHERICHIA COLI* AT THE NATIONAL HOSPITAL, ABUJA: A THREE-YEAR RETROSPECTIVE STUDY.

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ABSTRACT

Background: *Escherichia coli* (*E.coli*) has been implicated as a common cause of both early and late onset neonatal infections. The emergence of different strains of E.coli that are multiply resistant to commonly used antibiotics has made continuous antibiotics surveillance relevant. Knowledge about common infections caused by E.coli as well as its antibiotics susceptibility pattern will guide paediatricians in choosing appropriate antibiotics for empirical treatment of neonatal infections.

Methods: A retrospective study of *E.coli* neonatal infections in NHA was conduct for the period 1st January 2010 to 31st December 2012. The records of all specimens submitted to the Medical Microbiology laboratory within the neonatal period (first 28 days of life) were examined and data about *E.coli* isolates and their antibiotics susceptibility pattern were retrieved and evaluated.

Results: 251(33.2%) bacteria were isolated out of a total of 757 specimen submitted for analysis within the period under review. 17(6.8%) were E.coli; 16 were from soft tissue specimen and one from blood. There was no isolate of *E.coli* from CSF. Most of the isolates were resistant to commonly used antibiotics for treatment of neonatal infections. Three isolates were resistance to amoxicillin-clavulanate and ceftriaxone. One isolate was resistance to amoxicillin-clavulanate, ceftriaxone and imipenem. 100% and 80% of the strains tested were susceptible to amikacin and imipenem respectively.

Conclusion: *E.coli* is third among the gram negative bacteria isolated within the period under review. Most of them were resistant to commonly used antibiotics for treating neonatal infections but, susceptible to amikacin and imipenem. There is need for regular antibiotics resistance surveillance and stewardship.

Keywords: Neonates, *E.coli* Infections, Antibiotics Resistance, Abuja.

INTRODUCTION

Infections have been identified as the most common cause of death in the neonatal period in both developed and developing countries (1,2). These infections include neonatal sepsis, meningitis, pneumonia, and soft tissue infections. *Escherichia coli* (E.coli) has been implicated as one of the most common isolates recovered from specimen submitted for processing due to infection in this age group(1).It has been implicated as a common cause of neonatal sepsis(1,3), neonatal meningitis(3), neonatal bacteremia (4), and neonatal soft tissue infection(4). *E.coli* is able to cause these infections because they are among the first set of bacteria that colonize the newborn. It exists as a normal flora of the gastrointestinal tract of human(5) and can easily spread from the rectum to the female genital tract through the perineum(1,5). Vaginal *E.coli* colonization is detected in 7-13% of pregnant women and 21% in the blood of fetuses that have died during the third trimester of pregnancy (6,7). Neonates acquire *E.coli* intrapatum and during labour (5,6,7).

The immune system of neonate is not fully developed at birth and till date, no preventive measures have been advocated for *E.coli* colonization of the birth canal (1). *E.coli* and group B β-haemolytic streptococcus (GBS) are the commonest bacteria mentioned as a cause of early onset neonatal sepsis (EONS) in developed countries(8). However, following the introduction of late antenatal screening and intrapatum antibiotics prophylaxis in 1996 which led to a reduction in GBS, the gram negative bacteria such as *E.coli* have become prevalent as a cause of EONS(8). Previous studies in our center implicated *E.coli*, *K.pneumoniae, P.aeruginosa* and *S.aureus* as common causes of infections in the neonatal period similar to what has been reported from other centres in Nigeria(9,10).

The occurrence of antibiotics resistance in E.coli continue to increase nationwide both for single and to multi-class resistance to several antibiotics (multi-drug resistance [MDR]) (11,28). The recent trend in antibiotic resistance has seen the emergence of different strains of *E.coli* that elaborate resistance enzymes such as Extended-Spectrum Beta Lactamases (ESBLs) producer, AmpC β-lactamases and carbapenemases that confer multiple resistance to commonly used antibiotics for treatment of infections(11,12,27,28) . These have made continuous antimicrobial surveillance a priority in clinical microbiology laboratories.

The aim of this study was to evaluate the prevalence of *E.coli* as a cause of neonatal infections as well as their antibiotics susceptibility pattern in the last three years in NHA. The outcome of this study will guide the paediatrician in the management of suspected cases of E.coli infections, and direct further research.

METHODS

This is a retrospective study carried out at the National Hospital Abuja, a 200 bedded tertiary healthcare institution, located in the Federal Capital Territory (FCT), Nigeria. It has a well equipped Special Care Baby Unit (SCBU) and Neonatal Intensive Care Unit (NICU). The hospital serves as a tertiary healthcare and referral centre for the inhabitants of Abuja and neighboring states.

The records of the Department of Medical Microbiology and Parasitology laboratory of the hospital from Jan. 1st 2010 to Dec. 31st 2012 were reviewed. All isolates from blood culture, Cerebrospinal fluid (CSF) and soft tissue (wound swab, eye swab, ear swab, umbilical cord, pus) specimens from neonates that were conclusively identified as pathogens were included in the study, while contaminants were excluded. E.coli isolates were specifically reviewed with emphasis on the age, sex, type of specimen submitted to the laboratory for analysis as well as their antibiotics susceptibility pattern.

In our facility during the study period, blood cultures were usually performed with Oxoid signal system (Oxoid, Basingstoke, UK) or BACTEC 9050(Becton-Dickson, New Jersey, USA) continuous-monitoring blood culture systems. CSF analysis, soft tissue culture and bacterial identification were performed using standard microbiology techniques (13). The Modified Kirby-Bauer disc diffusion method was used for antibiotics susceptibility testing and interpreted based on the Clinical and Laboratory Standard Institute (CLSI) recommendations (14).

RESULTS

A total of 757 specimen comprising blood(245), CSF(127) and soft tissue(385) were submitted for processing during the period under review. 251(33.2%) pathogens were isolated, out of which 247 (98.4%) were bacteria isolates while 4(1.6%) were Candida species (Table 1). 17 (6.8%) were identified as *E.coli*; one from blood culture, non from CSF, 16 from soft tissue specimens (Table 2).

S.aureus was the predominant bacteria isolated, accounting for 143 (56.5%) of the 251 isolates and the most frequently isolated pathogen across all the specimens types submitted for processing (Table 2).

TABLE 1: YIELD OF ISOLATES BY SPECIMENS.

Specimen types	Total No of specimen	Isolates		Total
		Bacteria	Candida spp	
Blood culture	**245**	**94**	**2**	**96**
CSF	**127**	**7**	**0**	**7**
Soft tissue	**385**	**146**	**2**	**148**
Total	**757**	**247(98.4%)**	**4(1.6%)**	**251(100%)**

spp: Species., CSF: Cerebrospinal Fluid.

TABLE 2: PATHOGENS ISOLATED FROM DIFFERENT SAMPLES.

Isolates	Blood culture	CSF	Soft tissue	Total	Percentage
E.coli	1	0	16	17	6.8%
S.pyogenes	0	0	8	8	3.2%
S.aureus	62	4	77	143	57.0%
Enterococcus spp	6	1	7	14	5.6%
L.monocytogenes	1	0	0	1	0.4%
Klebsiellae spp	8	1	12	21	8.4%
P.aeruginosa	5	0	14	19	7.6%
CoNS	4	0	0	4	1.6%
Proteus spp	1	0	4	5	2.0%
Serratia marcescens	2	0	1	3	1.2%
Citrobacter freundii	1	0	3	4	1.6%
M. morgagnii	1	0	2	3	1.2%
Alcaligenes spp	2	0	1	3	1.2%
Neisseria spp	0	1	0	1	0.4%
Acinectobacter spp	0	0	1	1	0.4%
Candida spp	2	0	2	4	1.6%
Total	96	7	148	251	100%

CSF: CerebroSpinal Fluid. CoNS: Coagulase Negative Staphylococci, SPP: Specie

All the 17 isolate of E.coli were from neonates older than 72hours; 8 from females and 9 from males. Wound and umbilical swabs specimen were the predominant specimen received (Table 3). 80%, 81.8%, 83.3%, and 62.5% of the tested *E.coli* strains were resistant to ampicillin, amoxicillin-clavulanate, gentamicin and cefuroxime respectively, while 40%, 33.3%, 66.7% and 20% were resistant to cefotaxime, ceftriaxone, ceftazidime and imipenem respectively. All tested *E.coli* strains were sensitive to amikacin (Table 4).

TABLE 3: DISTRIBUTION OF *E.COLI* ISOLATES

Specimen type	No. of Isolates	Percentage
Blood Culture	1	5.9%
Wound swab	5	29.4%
Umbilical swab	5	29.4%
Eye swab	3	17.6%
skin swab	2	11.8%
Scalp swab	1	5.9%
Total	17	100%

TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *E.COLI* ISOLATED

Antibiotics	No tested	Susceptible(%)	Resistant(%)
Ciprofloxacin	7	5(71.4)	2(28.6)
Ofloxacin	4	2(50)	2(50)
Cefotaxime	10	6(60)	4(40)
Cefuroxime	8	3(37.5)	5(62.5)
Amox-Clav.	11	2(18.2)	9(81.8)
Genticin	6	1(16.7)	5(83.3)
Amoxicillin	5	1(20.0)	4(80.0)
Ceftazidime	6	2(33.3)	4(66.7)
Imipenem	5	4(80)	1(20%)
Amikacin	7	7(100)	0 (0)
Ceftriaxone	6	4(66.7)	2(33.3)

DISCUSSION

It has been revealed in this study that most of the *E.coli* isolated were from soft tissue specimens; it is difficult to ascertain whether they represent true infections or colonization of the newborn. No conclusion can be made concerning the only isolate from blood except to say that it most probably represent a true infection. S.aureus is the most common bacteria cause of infections in this age group. It is the predominant bacterial isolate from blood culture, followed by *K.pneumoniae*. This finding is in agreement with similar studies from Maiduguri and Uganda on neonatal infections which showed *S.aureus* as the predominant pathogen, followed by *Klebsiellae pneumonia* (15,16). It however, contrast with a previous study done in this center between 2002 to 2004 which showed almost equal representation of both gram negative and gram positive bacteria as causes of neonatal sepsis(9) and a prospective study in Port Harcourt which showed *Klebsiella pneumonia* as the predominant isolate (17). S.aureus was the main bacteria isolated from CSF during the study period, and this is in keeping with the result of a previous study in Jos, Nigeria (18). The same study reported the absence of *E.coli* from CSF (18) . *E.coli* is a known cause of neonatal meningitis (3). The reason for this is not clear, and may require further studies. Although, the coagulase negative staphylococci (CoNS) that were isolated were recorded as pathogens they may as well represent contaminants. Difficulties exist in some cases in the differentiation of CoNS infection from culture contamination in clinical specimen because CoNS is a common skin commensal (19). Four of the isolates were candida specie from blood culture and soft tissue which might be responsible for infection in low birth weight neonates as observed in some previous studies(20,21). All the isolates in this study were from the late neonatal period and as such, it is difficult to determine whether *E.coli* is a common cause of infection in the early neonatal period or not. It is possible that delays in decision making by the paediatrician with respect to submission of neonatal specimen for analysis whenever they suspect neonatal infection within 72 hours, may have inadvertently moved early onset infections into late onset period.

The antibiotics resistant pattern in the *E.coli* isolated in this study cannot be interpreted with a high degree of certainty because not all the isolates were equally tested for resistance to the same classes of commonly used antibiotics in our laboratory. However, based on the available data, most of the isolates are resistant to amoxicillin, amoxicillin-clavulanate in agreement with previous studies (9,22,23). Most were also resistance to the third generation cephalosporins with few of the E.coli isolates showing resistant pattern resembling ESBL producers; the prevalence of which has been reported in other studies as11.4% (2009)and 3.8%(2012) by Iroha et al from Enugu (24,25) , while 76.9% was report from Ibadan in 2012(28). The tested isolates were susceptible to imipenem and this agrees with previous studies (9,25,26,27). This may suggest that strains that elaborates carbapenemase enzyme may not be common in this locality. Studies have shown that most *Enterobacteriaceae*, *E.coli* inclusive; even the ESBL-producing strains, are still susceptible to the carbapenems (25,26,27,28).

Seven of the *E.coli* strains tested against amikacin were susceptible. Of these seven isolates, three were tested against imipenem, out of which two were susceptible and one was resistant. Amikacin and

imipenem were the most active antimicrobial agent against isolates of *E.coli* as found in other studies (27,28).

In conclusion, *E.coli* is the fourth most common bacteria isolated in the neonatal period in this study and third among the gram negative bacteria. Most were resistant to commonly used antibiotics for treatment of neonatal infections with a few showing resistant pattern resembling ESBLs producers. Most of the isolates were susceptible to amikacin and the carbapenems. In view of the limitation encountered in this study, a well designed prospective study; as well as continuous surveillance of antimicrobial resistance in *E.coli* isolates are hereby recommended.

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URINE CULTURE CONTAMINATION: A ONE-YEAR RETROSPECTIVE STUDY AT THE NATIONAL HOSPITAL, ABUJA

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ABSTRACT

Background: Urine culture contamination is a significant cause of delay in treatment of patients being investigated for urinary tract infection. Though contamination is not completely avoidable, several measures have been proven to decrease contamination rates. There are few studies detailing urine contamination rates in laboratories in Nigeria.

Aim: To determine the frequency and factors associated with urine culture contamination in samples submitted to the Medical Microbiology Laboratory in National Hospital Abuja (NHA).

Method: Retrospective study of urine culture contamination in which data from Medical Microbiology Laboratory from January 1 to December 31 2012 at National Hospital Abuja were reviewed. Patients' age, gender, location and urine culture result were assessed. Contamination rates for different genders, age groups and departments were assessed and results presented in simple averages and percentages.

Results: Overall contamination rate was 13.1%. Females had a contamination rate of 16.9%, which was significantly higher than the contamination rate of 6.8% in males. The Gynaecology and Antenatal clinics had the highest contamination rates amongst departments with 22.5% and 21.3% respectively. Lowest contamination rates were in Emergency Paediatric Unit (EPU) and intensive Care Unit (ICU) with rates of 5.9%and 9.5% respectively. The female gender was found to be the most significant predictor of higher contamination rate.

Conclusion: Contamination rate of urine cultures in this study is unacceptably high. Appropriate interventions need to be instituted to reduce the current urine culture contamination rate in National Hospital Abuja.

Key Words: Urine, Contamination, National Hospital, Abuja.

INTRODUCTION

Contamination of urine cultures results from poor collection technique and or prolonged time from collection to processing (1). Suprapubic aspiration and straight catheter technique are the best methods to avoid contamination but they are invasive (2). Most urine specimens in adults and children are collected using the clean-catch midstream (CCMS) technique. Proper use of the CCMS technique results in colony counts which correlate with those of specimens collected via suprapubic aspiration (3). Bacterial contamination of urine often has important consequences; overuse of antibiotics, delay in instituting appropriate antibiotics, erroneous diagnosis and added cost of repeat cultures (1,4).

Urine culture contamination has been defined in several ways. The College of American Pathologists (CAP) has defined it as 'any urine specimen that yields $>10^5$cfu/ml of two or more different organisms' (1). Pure culture growth of bacteria in numbers $<10^5$ have been considered as contaminants in other studies (5,6). The rate of urine culture contamination in some studies range from 2- 37% (1, 7, 8, 9).While possibly not being completely avoidable, rates can be reduced by instituting appropriate effective measures. This study was carried out to determine the baseline contamination rate in NHA to guide appropriate intervention measures.

MATERIALS AND METHODS

The study was designed to assess the frequency of bacterial contamination of urine cultures and elucidate factors associated with urine contamination. Laboratory data for urine cultures from January 1 to December 2012 were analyzed using Microsoft Excel. All culture were made on either CLED and blood agar plates or McConkey and blood agar plates and incubated in air for 16-24 hours in air. Variables analyzed were patient age, gender, location and urine culture result. Urine culture contamination as defined by CAP is adopted is our laboratory. Patients with specimens not specifying age, gender or urine culture results in register were excluded

from the study. Factors that could potentially be associated with higher or lower urine contamination rates were identified. Selected variables were examined individually to determine if they were independently associated with urine culture contamination rate.

RESULTS

A total of 4448 specimen were received in the laboratory, out of which 2631 (59.2%) met the inclusion criteria. The sample population was made up of 1593 (60.6%) females and1038 (39.5%) males.

Overall urine culture contamination rate was 13.1% (345/2631). Contamination rate of the female subset was 16.9% while that of the male subset was 6.7% (Table 1).

TABLE 1: URINE CONTAMINATION RATES BYGENDER

	Total No of Specimen	Number of Contaminated	Percent contaminated
Male	**1037**	**70**	**6.6**
Female	**1593**	**269**	**16.9**
Total specimen	**2631**	**345**	**13.1**

P<0.0001

Analysis of the age subset showed children aged less than two years had contamination rate of 10.5% while patients aged 2-60 years had a contamination rate of 12.9%. The contamination rate of patients over 60 years of age was 11.1%. (Table 2)

TABLE 2: URINE CONTAMINATION RATE BY SITE

	Total Specimen	Number Contaminated	Percent contaminated
EPU	**255**	**15**	**5.8**
ICU	**21**	**2**	**9.5**
GYNAE	**178**	**40**	**22.5**
ANTENATAL	**150**	**32**	**21.3**
INPATIENT	**1346**	**179**	**13.3**
OUTPATIENT	**1285**	**161**	**12.5**

The EPU and ICU had contamination rates of 5.5% and 9.5% respectively. Contamination rates of specimen from the Gynaecology and Antenatal subsets were 22.5% and 21.3% respectively. The adult emergency department had a contamination rate of 13.8%. The rate for inpatients was 13.3% while that of outpatients was. 12.5%. (Table 3)

TABLE 3: URINE CONTAMINATION RATE IN DIFFERENT AGE GROUPS

Age	Number of Contaminated Specimen (n)	Total Specimen (N)	Percent contaminated
<2yr	**12**	**114**	**10.5**
2-60yr	**306**	**2374**	**12.9**
>60yr	**12**	**117**	**11.1**

P<0.01

DISCUSSION

This study was designed to elucidate the frequency of urine culture contamination and analyze factors associated with the rates.

Majority (60.0) of the urine specimens analyzed were from females. This increased rate of investigating females is because of their higher risk of having urinary tract infection (1, 10).

Overall urine culture contamination rate in NHA for the period under study was found to be 13.1%. The literature has widely varying estimates of urine contamination (1, 7, 8, 9); this variation may be because of the different characteristics of the populations studied – healthy males, healthy women, prepubescent females, uncircumcised males - and the different criteria used for defining urine culture contamination in the various studies. The largest study done on urine culture contamination rate, the CAP (1) study, used the same definition of urine culture contamination as this study and has the most similar patient characteristics. Median contamination rate in the CAP study was found to be 18.1% (1), with laboratories in the 90th and 10th percentiles of the study having average rates of 5.7% and 36.7% respectively. Thus, relative to that study, the urine culture contamination rate in NHA may appear to be within average. Due to the differing characteristics between this study and the others, no direct comparison can conveniently be made. The finding that females have significantly more urine contamination rate than males is consistent with previous findings (1, 5, 6, 10).

Patients of different ages had slightly different contamination rates; the trend towards higher contamination rates was seen in groups with a higher proportion of females. Although contamination rates differed markedly for different departments, the higher contamination rates were seen in sections with higher female population. This female dominance in urine contamination is likely due to the anatomical features of the external genitalia and its proximity to the perianal region. Patients in the hospital being sent for urine culture are rarely instructed on the collection technique (personal communications); it is therefore, most likely that the contaminations occurred at the time of collection as already established in previous studies (1,11). Studies have shown that patients given instructions on proper collection have lower contamination rates than those who did not receive instructions (6, 7, 8).

Similarly urine specimens were often observed to be delayed at varying points for a total of up to four to eight hours after collection without refrigeration or preservatives before processing. Delayed processing of urine specimen for more than 2 hours post collection results in increased rate of culture contamination unless specimens have been refrigerated or kept in a preservative (1, 11, 12, 13).In EPU, specimens were transported rapidly to the laboratory as against what obtains in other wards and outpatients where samples were kept for hours before being taken to the laboratory. This rapid transport from EPU may be one of the factors responsible for the lower contamination rate observed there, in addition to the very low number of females in this group.

It is concluded that the relatively high contamination rate seen in this study is unacceptable and can be reduced by giving proper instructions to patients and processing specimen within two hours of collection or stored in preservatives or refrigerated. There is need to set a benchmark contamination rate so as to enhance its use as a quality indicator in urine processing.

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CAUSATIVE AGENTS OF KERATOMYCOSIS IN IBADAN: REVIEW OF LABORATORY REPORTS

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ABSTRACT

Introduction: Fungi are responsible for less than 2.0 % of corneal infection around the globe. Trauma to the cornea is the leading cause of fungal keratitis especially with history of corneal trauma with vegetable or organic matter. Because of the dearth of data on the aetiological agents of Keratomycosis in this hospital, this study was aimed at finding the prevalence and fungal etiological agents responsible for this condition Ibadan, Nigeria. Methods: A retrospective review of the laboratory reports of corneal scrapings of patients that presented with signs and symptoms suggestive of Keratomycosis was carried out. The scrapings were subjected to wet preparation with 10.0 % KOH, Gram staining and Giemsa staining to rule out inclusion bodies. The diagnosis of Keratomycosis was made on the basis of the recognizable and characteristic appearance of fungal hyphae and fruiting bodies under microscopy. The media with no obvious growth after 3-4 weeks of incubation were regarded as negative. Results: A total of 48 specimens from patients with suspected diagnosis of Keratomycosis were included in the analysis. The patients consisted of 42 (87.3%) males and 6 (12.5%) females. The ages at diagnosis ranged from 3 to 73 years with a mean of 36.46 years and a median of 35.5 years. The prevalence of Keratomycosis among this group of patients in this hospital was 8.4 %. *Candida albicans and Fusarium spp* were the fungal isolates in these patients as it occurred in 4.2 % (2/48) of them respectively. Conclusions: Corneal scarring due to trauma or infections is a major cause of monocular blindness, especially in developing countries like Nigeria. Despite the low level of Keratomycosis in this study, high index of suspicion of possible diagnosis should be giving to patients with history of trauma, tissue devitalization with topical steroids and immunocompromised immunity.

Keywords: Keratomycosis, Corneal, *Fusarium and Candida.*

INTRODUCTION

According to the World Health Organization, corneal diseases are a major cause of vision loss and blindness, second only to cataract in overall importance (1). It is estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new cases of corneal blindness annually.[1] Trauma is the most common precipitating factor in most of the cases (2). Trauma leads to destruction of the epithelium and Bowman's membrane, impairing barrier to infection. The underlying stroma becomes, excessively hydrated and possibly altered in such a way to constitute a more favorable site for fungus to grow (2). Keratomycosis caused by filamentous fungi is an occupational hazard of farmers and agricultural workers (3).

Keratomycosis is a major cause of visual disability in developing countries (1, 4). Fungi have replaced bacteria as the predominant cause of infectious keratitis in some developing countries like Nigeria (1). Fungal keratitis is a major blinding eye disease in Asia and other developing countries (2, 5). One report from South India found that 44% of all central corneal ulcers are caused by fungi (2). This high prevalence of fungal pathogens in South India is significantly greater than that found in similar studies in Nepal (17%), Bangladesh (36%), Ghana (37.6%), and south Florida (35%) (6-10).

It has also been postulated that the lower temperature of the cornea relative to the rest of the body may favor the growth of fungi (9). Many ophthalmologists also identified topical steroids as the principal risk factor in enhancing ocular fungal growth (11). Steroid use

as initial therapy was reported in 1 to 30% of patients having microbial keratitis (11).

Keratomycosis can be caused by as many as 60 species of fungi. The predominant etiological agents vary in different geographical areas. However, Aspergillus spp. is the commonest isolate in India (12-13). Most cases of mycotic keratitis are amenable to treatments if effective topical antifungal therapy is started early. The major hurdle to this lies in definitive laboratory diagnosis in clinically suspected case of oculomycosis. False negative diagnostic results may result in delay in institution of anti fungal therapy (2).

Because of the dearth of data on the prevalence of Keratomycosis in this hospital, this study was aimed at finding the prevalence and fungal etiological agents responsible for this condition.

METHODS

This study was carried out in the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria. It was a retrospective review of the laboratory reports of corneal scrapings of patients that presented with signs and symptoms suggestive of Keratomycosis. The materials for culture and microscopic examination were obtained from the lesion.

The scrapings were subjected to wet preparation with 10.0 % KOH, Gram staining and Giemsa staining to rule out inclusion bodies. The diagnosis of Keratomycosis was made on the basis of the recognizable colonial morphology, characteristic appearance of fungal hyphae and fruiting bodies under microscopy.

The corneal scrapings were also cultured on the Sabouraud Dextrose agar at room temperature (27-30^{0}C). Fungal growth occurred within 48 hours to three weeks. Once fungal growth appeared on primary isolation medium, colonies were subcultured promptly to fresh medium for isolation and identification in pure cultures. These fungi were identified with the aid of recognizable colonial morphology, microscopical appearance and biochemical reactions. All fungal cultures plates were kept for at least 3 week after inoculation before being declared negative. The data collected were analyzed with the aid of SPSS version 12.0 computer software**.**

RESULTS

A total of 48 patients with suspected diagnosis of Keratomycosis were included in the analysis. The patients consisted of 42 (87.3%) males and 6 (12.5%) females. The ages at diagnosis ranged from 3 to 73 years (Mean - 36.5 years, median of 35.5 years and SD = 8.6).

The prevalence of positive culture results for Keratomycosis among this group of patients in this hospital was 8.4 %.

Candida albicans and *Fusarium spp* were the fungal isolates in these patients as it occurred in 4.2 % (2/48) of each of them respectively.

There was no statistical significance associated between age of presentation and types of fungi isolated ($P > 0.05$). Though, there was no statistical significance between sex and fungi isolated ($P > 0.05$), it was noted that all the fungi were commonly isolated from male gender in their thirties.

TABLE 1: DISTRIBUTION OF THE FUNGAL PATHOGENS.

Fungal isolates	Frequency (n = 48)	Percentage (%)
Fusarium spp	**2**	**4.2**
Candida albicans	**2**	**4.2**

DISCUSSION AND CONCLUSION

Mycotic keratitis is an infection of the cornea by the fungus that causes ulceration and inflammation, usually following trauma or treatment for a bacterial infection with steroid and antibiotics. Fungal keratitis is responsible for a significant burden of blinding disease in the developing world (14). Despite the known potential for visual impairment and blindness

associated with fungal keratitis, few research studies have evaluated the risk factors and etiological agents in Nigeria. Corneal scarring due to trauma or infections is a major cause of monocular blindness, especially in developing countries like Nigeria.

More than 70 genera of moulds and yeasts have been associated with Keratomycosis (15). Hyaline moulds like *Aspergillus*, *Fusarium*, *Cephalosporium*, *Acremonium* and *Penicillium* are more frequently isolated as causative agents than phaeoids (dematiaceous fungi) moulds like *Alternaria*, *Curvularia*, *Bipolaris* and *Exserohilum* species (16, 17).

In this study, Fusarium and *Candida spp.* are the most common causes of fungal keratitis. This was found to be consistent with other studies in developing countries (10, 18). In India, *Aspergillus* and *Fusarium* are the commonest cause of Mycotic keratitis (16).

Chang *et al. (*19) from Taiwan have reported that *Fusarium* species are common plant pathogens, particularly in corn crops or onion fields. Gopinathan *et al.* (20) from India also reported *Candida* as a rare fungal corneal pathogen (0.7%). In a series of 24 patients also from Wills Eye Hospital, Philadelphia, *Candida* was identified in 45.8% of cases of fungal keratitis; this probably represents the only study reporting *Candida* as the commonest etiologic agent of fungal keratitis (21).

Despite the low level of Keratomycosis in this study, high index of suspicion of possible diagnosis should be given to patients with history of trauma, tissue devitalization with topical steroids and immunocompromised immunity. Corneal scrapings of the patients should be sent to the laboratory as early as possible for prompt diagnosis.

Recommendations

Nigeria is the most populous black African nation .The increased incidence of fungal keratitis warrants further study of the risk factors, antifungal susceptibility testing, and possible pharmacologic combinations to prevent blindness. Research toward rapid diagnosis and specific drug therapy that could minimize the morbidity caused by this preventable disease will be appreciated in University College Hospital, Nigeria.

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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF AMPC AND ESBL PRODUCING CLINICAL ISOLATES AT A TERTIARY HEALTH CARE CENTER IN KANO, NORTH-WEST NIGERIA.

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Abstract

The increase in production of Extended Spectrum Beta Lactamase (ESBL) and Amp C beta lactamase among clinical isolates in our hospitals is of utmost importance. Failure to detect these enzymes in many of our hospitals has greatly led to treatment failure and uncontrolled spread of multi drug resistant pathogens. It was for this purpose that the present study was conducted to determine the prevalence, distribution and susceptibility pattern of Gram negative bacteria producing ESBLs and Amp C beta lactamases in the largest tertiary health care provider in Kano, North-West Nigeria. A total of 75 ESBL and 10 AmpC producing bacteria were involved in the study which were obtained from a study involving 500 Gram negative clinical bacterial isolates from various hospital wards over a period of 9 months from Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. Isolates were screened for ESBLs and AmpC using Double Disc Diffusion Method and Amp C Disc test respectively. All confirmed ESBL and Amp C producing isolates were tested for susceptibility to sixteen (16) different antibiotics by the Disc Diffusion Method (DDM). The prevalence of ESBLs was high in Shigella spp. (1/2 or 50%), followed by Klebsiella pneumoniae (10/50 or /20%), and E. coli (47/247 or 19.3%) while Amp C producers were detected more in Klebsiella pneumoniae (4%) and E. coli (2.8%). Of the specimens screened, distribution varies between ESBL and AmpC producers, but more prevalent in urinary tract pathogens in both. Highest prevalence of ESBLs and AmpC producers was recorded in intensive care units and surgical wards. ESBL and AmpC production in the hospital is not sex dependent statistically, thought higher in males (52 and 60%) than in females (48 and 40%) for ESBL and AmpC respectively. ESBL and AmpC producers were both sensitive to Imipenem, Nitrofurantoin and Levofloxacin and resistance to Amoxycillin, Ceftazidime and Tetracycline. The study indicates the occurrence of ESBL and AmpC producers in our tertiary health provider, widely distributed in various clinical samples, wards and sexes and are multi drug resistant posing serious threat in managing life threatening infections.

Key words: prevalence, distribution, ESBL producers, Amp C producers, antibiotic susceptibility

INTRODUCTION

Beta lactamases are enzymes produced by some bacteria and are responsible for their resistance to ß-lactam antibiotics like penicillins, cephamycin and carbapenem [1]. β-lactamase deactivates the molecular antibacterial properties of β-lactam antibiotics there by breaking and opening the common element in their molecular structure β-lactam. Some of these enzymes include extended spectrum β-lactamase (ESBL), AmpC βL, and carbapenemase. The first β-lactamase was detected during the 1960s [2]. Since then many types of β-lactamases which vary both in their ability to inactivate a given beta lactam drug as well as their susceptibility to inhibitors such as clavulanic acid, sulbactam and tazobactam came into existence.

Extended spectrum beta lactamases (ESBLs) are enzymes that mediate resistance to the third generation cephalosporins (e.g ceftazidime, cefotaxime and ceftriaxone) and monobactams (e.g aztreonem) but do not affect cephamycins. The enzymes are now observed worldwide in all species of Enterobacteriaceae especially *K. pneumoniae* and *Escherichia coli*. Originally, ESBL enzymes were derived from the widespread TEM and SHV β-lactamase family. The first β-lactamase with an extended-spectrum was detected in Germany in 1983

[3] and later in the western parts of Europe, probably because expanded-spectrum β-lactam antibiotics were first used their clinically.

The Class C β-lactamases (AmpC) are an important group of proteins that are broadly distributed and are the second most common β-lactamase group [1]. AmpC β-lactamases, in contrast to ESBLs, hydrolyse broad and extended-spectrum cephalosporins but are resistant to inhibition by β-lactamase inhibitors such as clavulanic acid [4,5,6].

In Nigeria, indiscriminate use of antibiotics, poor hygienic practices in hospitals and lack of monitoring of microbial drug resistance have created suitable conditions for the emergency and uncontrollable spread of the ESBLs and Amp C enzymes [7] and thus make their detection complicated due to the variable affinity of these enzymes for different substrates and inoculum effect.

Resistant to commonly used antibiotics in our hospitals increases at an alarming rate. Awareness of their occurrence and their potential effects in managing infectious diseases is low among the health care providers. Laboratory detections of these enzymes, proper reporting, necessary precautions to avoid their spread is lacking in many of our hospitals. In view of this, the research was conducted to detect the prevalence, distribution and antibiotic susceptibility of ESBL and AmpC producing clinical isolates in a tertiary health care provider which serves as a referral centers for other hospital in the region.

MATERIALS AND METHODS

Collection of specimens

A total of eighty five (85) Gram negative bacterial isolates comprising of 75 ESBL and 10 AmpC producing isolates were involved in the study. The screening was done in a study involving 500 clinical isolates collected from various clinical samples and hospital wards of the Microbiology Department of Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. The isolates were identified according to the procedures described by Cheesbrough [8]. Ethical clearance was granted to conduct the research by the management of the hospital.

Presumptive Test for ESBLs

All the clinical isolates collected were tested for potential ESBL producers using the Cefpodoxime (10µg) and the Ceftriaxone (30µg) antibiotic discs. Results were interpreted based on the CLSI criteria [9].

Confirmatory Test for ESBLs

All isolates suspected of ESBL production in the presumptive test were furthered confirmed using the double discs synergy according to CLSI guidelines [9]. A suspension of the test organism was inoculated on Mueller- Hinton agar. A disc containing 30µg Amoxicillin plus clavulanic acid was placed centrally on the plate. Discs containing cefpodoxime (10µg) and ceftriaxone (30µg) were placed on the agar at a distance of 15mm, centre to centre away from the amoxicillin + clavulanic acid disk. The plates were incubated over night at 35°C. The results were then interpreted as outlined in the CLSI guidelines [9]. A ≥ 5-mm increase in zone diameter for either antimicrobial agent combination compared to its zone when tested alone signified a positive ESBL.

PRESUMPTION TEST FOR Amp C

Sensitivity cefoxitin (30µg) discs were used to test for Amp C production using the disc diffusion method and interpreted as per the CLSI criteria [9]. Isolates that yielded a zone diameter less than 18 mm (screen positive) were further subjected to confirmatory test using the AmpC disc test technique.

AMP C DISK TEST

All confirmed isolates from the presumptive test were then subjected to the Amp C disc test as described by black *et al* [10]. A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Sterile disks (6 mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk. A negative test had an undistorted zone.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotic susceptibility tests were performed on all the confirmed ESBLS and Amp C Beta lactamase positive isolates according to CLSI directives [9]. The results were expressed as susceptible or resistant according to criteria developed by CLSI [9]. Sixteen (16) different antibiotic discs (Oxoid) were used : Gentamicin (10µg), Cotrimoxazole (25µg), Ciprofloxacin (5µg), Cefpodoxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefoxitin (30µg), Imipenem (10µg) Nalidixic Acid (30µg), Amoxycillin (20µg), Ofloxacin (30µg), Levofloxacin (30µg), Nitrofurantoin (300µg), Tetracycline (30µg), Chlorampenicol (30µg) and Augmentin (30µg) (Oxoid). *Escherichia coli* ATCC 25922 strain was used as a control culture.

RESULTS

ESBL Producing Isolates

Out of the 500 isolates screened for ESBL production, 75 were confirmed to produce ESBL giving an overall prevalence of 15.0%. The highest prevalence of ESBLs was found in Shigella *spp* (1/2 or 50%) followed by *Klebsiella pneumoniae* (10/50 or 20.0%), *E. coli* (47/247 or 19.3%), *Salmonella spp* (2/12.5%) and *Proteus mirabilis* (14/11.8%) (Table 1).

TABLE 1: PREVALENCE OF ESBL PRODUCERS AT ATKH, KANO.

Isolates	No. screened	No. of confirmed ESBLs	% confirmed ESBLs
E. coli	247	47	19.3
P. mirabilis	119	14	11.8
K. pneumonia	50	10	20
Ps. Aeruginosa	66	1	1.5
Salmonella spp.	16	2	12.5
Shigella spp.	2	1	50
Total	500	75	15

Plasmid mediated AmpC producing Isolates

The overall prevalence of AmpC β-lactamases was 10%. Highest prevalence was found among *K. pneumoniae* (2/4.0%), followed in that order by *E. coli* (7/2.8%) and *P. mirabilis* (1/0.8%). Amp C Beta lactamases were not detected among *Shigella spp*., *Salmonella spp.* and *Ps. aeruginosa* isolates (Table 2).

TABLE 2: PREVALENCE OF AMP C BETA LACTAMASE PRODUCERS AT AKTH, KANO.

Isolates	No. screened	No. of confirmed Amp C	% confirmed Amp C
E. coli	247	7	2.8
P. mirabilis	119	1	0.8
K. pneumonia	50	2	4.0
Ps. Aeruginosa	66	0	0.0
Salmonella spp.	16	0	0.0
Shigella spp.	2	0	0.0
Total	500	10	2.0

Distributions of β-lactamases

The distribution of ESBL and AmpC producers in clinical samples, clinics/wards and sexes were presented in Table 3, 4 and 5 respectively. ESBL producers were most prevalent in blood (14/22.2%) and urine samples (48/17.6%). This was followed in that order by stool (3/15.8%), urogenital swabs (1/14.3%) and wound swab (5/13.5%) while the least prevalence was observed in ear swab specimens (2/3.2%) (Table 3). However, AmpC was more prevalent in urine and catheter tips.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of ESBL producers to the selected antibiotics showed that both the ESBL and AmpC producing isolates showed appreciable susceptibility to carbapenem (Imipenem) and flouroquinolones (Lev, Cip, Nit and Nal). *E. coli* producing ESBLs are more susceptible to IMI (40/47), followed by LEV (32/47), Nal (30/47), NIT (26/47) and OFL (25/47). Resistance to penicillin and 2nd and 3rd generation cephalosporin were high but total in CAZ, CHL and TET. However, ESBL producing *K. pneumoniae* showed more resistance to more

antibiotics such as AMX, AUG, CTR, CAZ, CHL, and TET when compared with *E. coli* producers (fig 1).

TABLE 3: DISTRIBUTION OF ESBL AND AMPC PRODUCERS AMONG CLINICAL SPECIMENS AT AKTH, KANO

Samples	No. and % of ESBLs	No and % of AmpC
Ear Swab	**2 (3.2)**	**1(10)**
Urine	**48(17.6)**	**5(50.0)**
Catheter Tip	**2(8.7)**	**2 (20.0)**
wound Swab	**5(13.5)**	**0 (0)**
Blood	**14(22.2)**	**1(10)**
Stool	**3(15.8)**	**0 (0)**
Urogenital Swab	**1(14.3)**	**1 (10)**
Total	**75**	**10**

The highest prevalence of ESBLs from the various clinical sections/units at the AKTH, Kano was recorded at the nephrology, dialysis and surgical outpatient units (100%) while Ear, Nose and Throat clinic (5.6%) had the least prevalence (Table 4). AmpC producers also showed similar pattern of ESBL with highest prevalence in ICU, SCBU, MSW and SOPD. The distribution of ESBL and AmpC producers based on gender indicates that males had a higher prevalence rate (37/15.3%) than females (38/14.7%) (Table 5).

Moreover, *Ps. aeruginosa* and *K. pneumoniae* also showed same pattern of susceptibility profile with more susceptibility to carbapenem and flouroquinolones (fig 2 & 3) and total resistance to AMX, AUG, CTR, CAZ, CHL and TET.

TABLE 4: DISTRIBUTION OF ESBL AND AMPC PRODUCERS AMONG CLINICAL SECTIONS/UNITS AT ATKH, KANO.

Clinical wards	No.	No. and % of ESBLs	SOPD	No. and % AmpC	3	3(100)
GOPD	168	22(13.1)	FSSW	1(0.6)	4	1(25.0)
POPD	53	4(7.5)	EPU	0(0.0)	30	3(10.0)
A & E	29	3(10.3)	PMW	0(0.0)	15	6(40.0)
ENT	18	1(5.6)	ANC	0(0.0)	11	3(27.3)
MMW	24	7(29.2)	OPD	0(0.0)	5	1(20.0)
MOPD	8	2(25.0)	PNW	0(0.0)	5	1(20.0)
SCBU	20	6(30.0)	NEPHROLOGY	2(10)	1	1(100.0)
FMW	18	5(27.8)	DIALYSIS	0(0.0)	1	1(100.0)
NEOCLINIC	2	1(50.0)	ICU	0(0.0)	2	1(50.0)
MSW	12	216.7)	Total	3(40.0)	441	75(17.0)
RETAINERSHIP	12	1(8.3)		0(0.0)		

Key : GOPD General Out-Patient Department; POPD Paediatrics Out-Patient Department; A&E Accident and Emergency; ENT Ear ,Nose and Throat Clinic; MMW Male Medical Ward; MOPD Medical Out-Patient Department ; SCBU Special Care Baby Unit; FMW Female Medical Ward; NEOCLINIC Neonatal ward; MSW Male Surgical Ward; RETAINERSHIP Microbiology Laboratory ;SOPD Surgical Out-Patient Department ;FSSW Female Surgical Speciality Ward ; EPU Emergency Paediatrics Unit; PMW Paediatrics Medical Ward; ANC Antenatal Clinic; OPD Out Patient Department ;PNW Post Natal Ward; NEUROLOGY Neurology; DIALYSIS ;ICU Intensive Care Unit.

TABLE 5: GENDER BASED PREVALENCE OF ESBL AND AMPC PRODUCERS AT AKTH, KANO.

Sex	No. & % confirmed ESBLs	No. & % confirmed AmpC
Male	39 (52)	6 (60)
Female	36 (48)	4 (40)

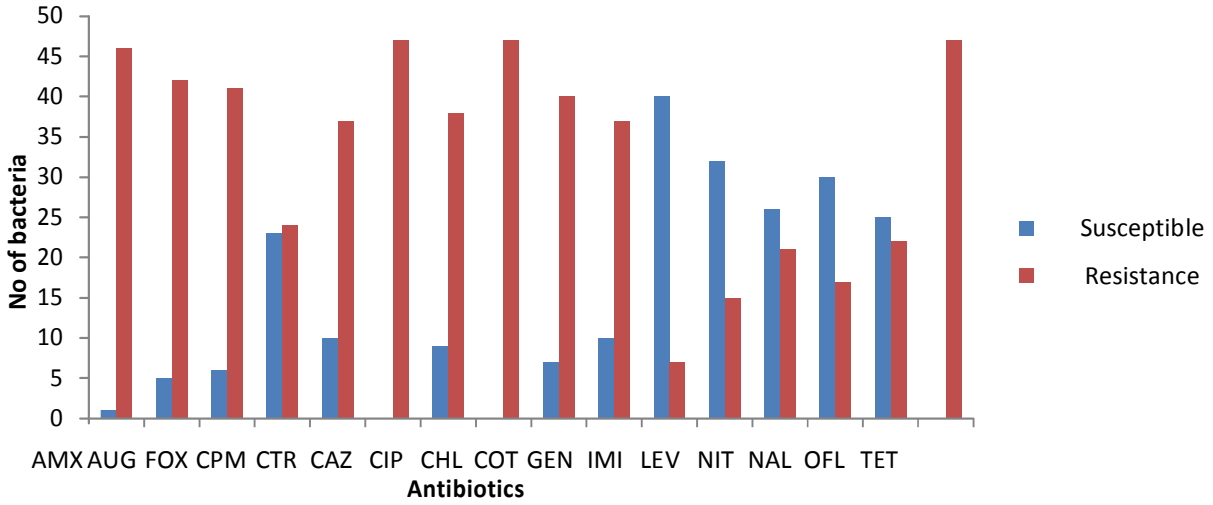

Fig1. Antibiotic susceptibility profile of 47 ESBL producing *E. coli* to commonly used antibiotics in a tertiary health care in Kano, North West Nigeria

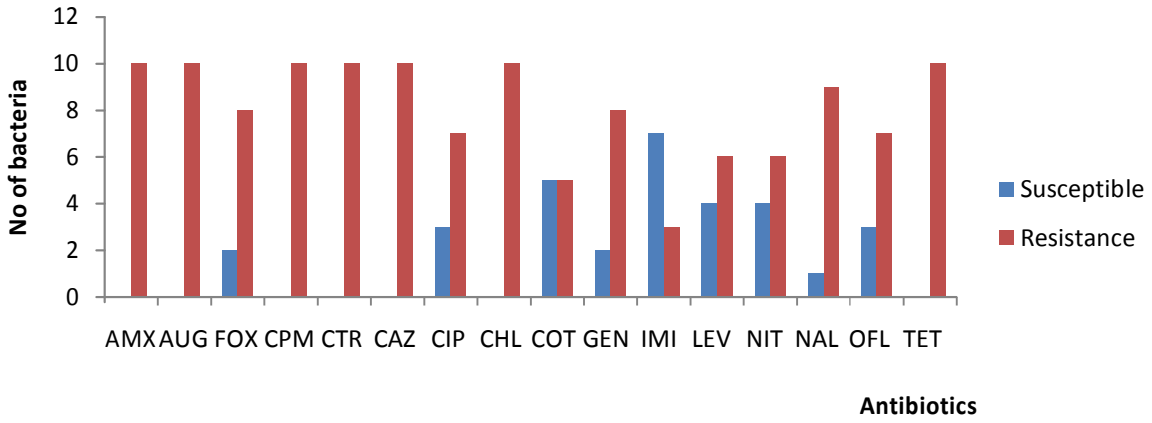

Fig 2. Antibiotic susceptibility profile of ESBL producing *K. pneumoniae* to commonly used antibiotics in a tertiary health care in Kano, North West Nigeria

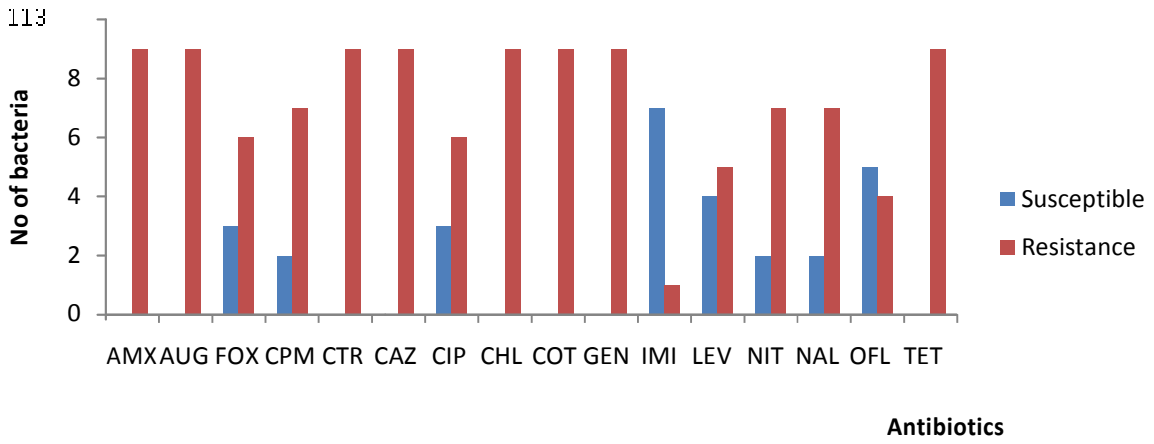

Fig 3: Antibiotic susceptibility profile of ESBL producing *P. aeruginosa* to commonly used antibiotics in a tertiary health care in Kano, North West Nigeria

While AmpC producing *E. coli* are sensitive to IMI, LEV, CIP, NAL, and OFL (fig 4), AmpC producing *K. pneumoniae* are more sensitive to LEV, OFL, GEN and IMI (fig 5). Most of the *K. pneumoniae* are resistant to majority of the tested.

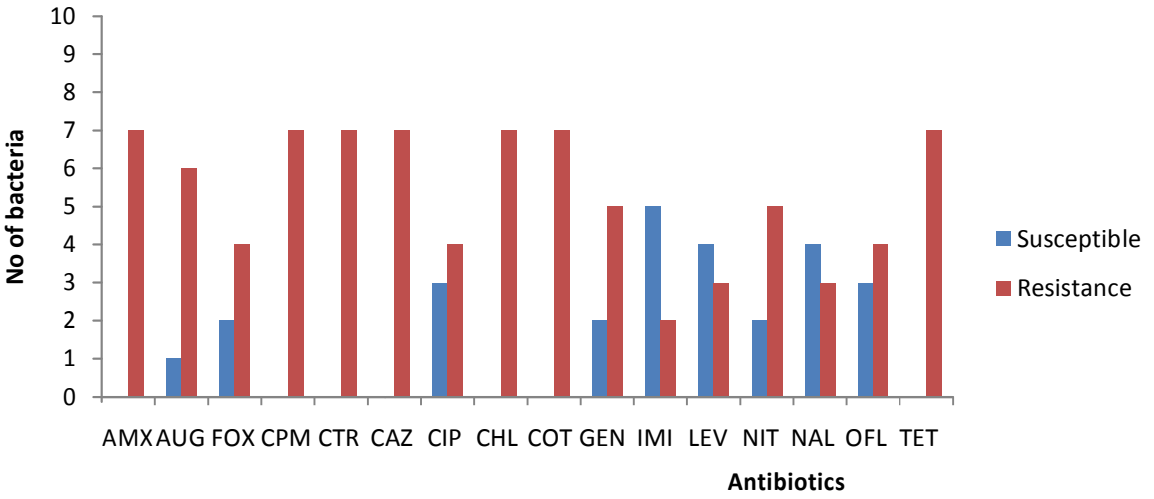

Fig 4: Antibiotic susceptibility profile of AmpC producing *E. coli* to commonly used antibiotics in a tertiary health care in Kano, North West Nigeria

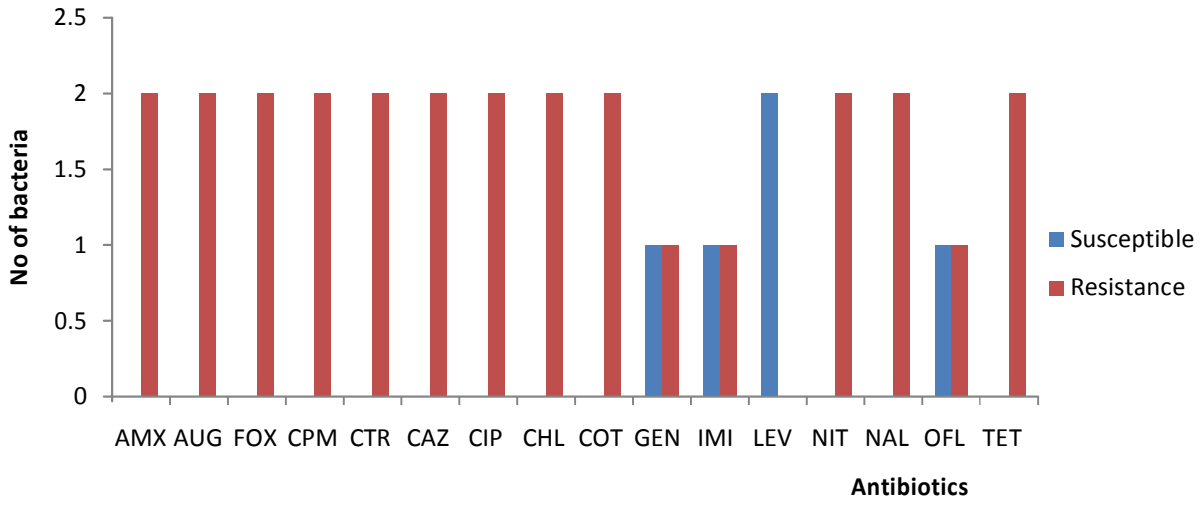

Fig 5. Antibiotic susceptibility profile of AmpC producing *K. pneumoniae* to commonly used antibiotics in a tertiary health care in Kano, North West Nigeria

DISCUSSION

Multi drug-resistant (MDR) Gram negative bacilli induced infections have been reported with an increasing frequency in tertiary health care providers in Nigeria and they have been found to be associated with a significant morbidity and mortality [11]. The numerous β- lactamases such as ESBL and AmpC were encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposones [12]. The overall prevalence of ESBL and AmpC producers in this study was 15% and 10% respectively (table 1,2). Although an increase in ESBL production have been reported in previous studies [13, 14], our results showed comparatively higher overall levels of ESBL- and AmpC producers. Among the Gram negative bacteria screened for ESBL production, Shigella *spp*. had the highest prevalence (50%). This could be attributed to the fact that the bacterium has been progressively acquiring resistance to several antimicrobial agents used for the treatment of infections [15]. Although the number of the isolates recovered during the study was 2, but several reports have indicated an increase in cases of *Shigella* species resistant to beta-lactams, including third-generation cephalosporins [15].

Comparison of the prevalence rate of ESBLs (15%) found in this study with findings of similar studies from same state indicated that the prevalence rate in the present study is higher when compared to previous studies by Yusha'u *et al*. [14, 16] who reported prevalence of 9.25% and 10.31% in 2007 and 2009 respectively, but lower to reports from other parts of the country, such as Lagos, 20% [17], Benin 36.6% and Enugu 44.6% [18]. This variations could be due to the differences in selecting type of antibiotic, antibiotic selection pressure, local antibiotic and prescribing habits, which differ from state to state, country to country and from institution to institution.

Among the clinical specimens, blood (22.2%) had the highest prevalence of ESBL producers followed by urine (17.6%). This observation is in agreement with that of Osazuwa *et al*. [18] who found that ESBL prevalence was highest for blood (61.2%) and urine (16.41%) specimens. This might be attributed to factors like improper use of syringes or needles, inadequate disinfection of skin of prolonged hospital stayed patients during phlebotomy or transfusion, poor hand washing techniques among health care practitioners which are some of the factors that predispose one to blood stream infections.

The distribution of ESBLs and AmpCs in hospital sections/units was highest in the nephrology, dialysis, surgical outpatient wards (100%) followed in that order by the ICU and the SCBU (50%). This indicates that they are more found among the hospitalized patients who may have contact them in

the hospital or from the health care providers. Some of these units mostly deal with seriously ill patients with life threatening infections and those in need of special postoperative techniques and are placed on prolonged antibiotic therapy. High prevalence of ESBL and AmpC in ICUs in the hospital has negative health implications, and could result into prolonged hospital stay, increased cost of treatment and quick spread in the hospital setting. Major risk factors for colonization or infection with ESBL and AmpC producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of third generation cephalosporin use and instrumentation or catheterization [19]. AKTH being a tertiary care that served as referral center for other hospitals within and outside the metropolis, it received many patients from various government owned and private owned hospitals with varying complicated life threatening infections. This has lead to use of higher antibiotics by the clinicians as a common empirical therapy without screening for ESBL and or AmpC. In addition, due to heavy patients load from other hospitals, proper infection control measures cannot be maintained by the paramedical staffs which can lead to transfer of MDR to other hospital. All of these factors lead to the higher rate of ESBL and AmpC β-lactamase producing organisms in our institution.

Based on gender, the distribution of the duo was not statistically significant as prevalence of 52 and 48.7% was recorded for ESBLs while 60% and 40% was recorded for AmpC beta lactamase ($P<0.05$). However, in another study, Erum *et al*. [20] reported increased ESBL production in males as compared to females. It was hypothesized that males in general have more obstructive uropathies leading to frequent urinary catheterization and have more chronic pulmonary and cardiovascular diseases that in turn lead to increased hospitalization and higher prevalence.

The distribution of antibiotic resistance to ß-lactams in this study was very similar to that described by Stéphane *et al*. [21] with nearly all of the isolates being resistant or slightly susceptible to commonly prescribed antibiotics in the hospitals, and many of the isolates showing a decreased susceptibility or resistance to promising antibiotics such as imipenem, levofloxaxin, cefoxitin, cefpodoxime and ceftriaxone (fig 1). The study showed that imipenem and levofloxacin were still the most effective antibiotics against ESBL and Amp C beta lactamase producing bacteria. However, data showed that imipenem usage in the hospital is low when compared with levofloxacin. Thus, these drugs could be used to treat infections caused by bacteria capable of producing these enzymes especially in cases of life-threatening infections such as urinary tract infections and pulmonary pneumonia. However, unless antibiotic therapy is restricted and controlled legally, misuse in the form of self medication may cause the spread of resistance, which will result in the prevalence of resistance against effective antibiotics such as levofloxacin and carbapenems

CONCLUSION

Based on the findings of this study, it could be deduced that**:**

- Prevalence of ESBL and AmpC Betalactamases producing gram negative bacteria is on the increase in a tertiary health care in Kano North West Nigeria.
- Blood and urine samples had a higher prevalence of ESBL producers in Kano
- The resistant pathogens were more found Neurology, dialysis and surgical out patient clinical sections/ units in addition to intensive care units.
- Prevalence of ESBL and AmpC producing bacteria does not differ significantly between the sexes.
- Levofloxacin and Imipenem is choice antibiotic for the treatment of infections caused by ESBL and Amp C producing bacteria in the hospital.

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