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ASSOCIATION OFCYTOMEGALO VIRUS WITH TYPE I DIABETES MELLITUS AMONG CHILDREN IN MINIA GOVERNORATE

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ABSTRACT

Background: Type I diabetes (T1D) is the most common form of diabetes in most parts of the world.Aim: The association between cytomegalovirus (CMV)and T1D mellitus was studied, with comparison to healthy subjects and to correlate its level with different clinical and laboratory parameters.

Materials & Methods: This study included 68 children and adolescents who were classified into two groups. GroupI comprised 53 patients diagnosed with T1D and having regular follow up in the pediatric endocrinology out-patient clinic, Minia University children's hospital. Group II comprised 15 apparently healthy subjects, age and sex matched to the diseased group. According to the onset of diabetes, we divided the diabetic group into two sub-groups. Group Ia (newly diagnosed) comprised 20 patients, with ages ranging between 7 and 18 years; 10 were males (50 %), and 10 were females (50 %). Whilst group Ib (duration of disease >1 year) comprised 33 patients, with ages ranging between 6 and 17 years; 17 were males (49%) and 18 were females (51%). The studied groups were subjected to the following: thorough history taking, clinical examination and laboratory investigations (random blood glucose levels) and HbA1c%. DNA was extracted using QIAamp Min elute kit protocol for detection of cytomegalo virus by RT-RCR.

Results:The frequency of cytomegalovirus was significantly higher in T1D children than the control and in group Ia than group Ib.

Conclusion: T1D children had significantly higher serum cytomegalovirus than the control group, as did those newly diagnosed compared to those with longer duration of illness.

Keywords:T1D, Cytomegalo-virus, Haemoglobin A1C, Polymerase chain reaction

Key Messages: Dose of insulin, significant +ve correlations, RT-PCR

ASSOCIATION DU CYTOMEGALOVIRUS AVEC DIABETE SUCRE DE TYPE 1 CHEZ LES ENFANTS DANS LE GOUVERNORAT DE MINIA.

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RESUME

Contexte : Diabète de type 1(DT1) est la forme la plus courante du diabète dans la plupart des régions du monde.

But : L'association entre le cytomégalovirus et DT1 sucre a été étudiée avec une comparaison aux sujets sains et pour corréler son niveau avec les divers paramètres cliniques et laboratoires.

Matériaux et Méthodes : Cette étude a inclus 68 enfants et adolescents qui ont été classés en deux groupes. Le Groupe I a compris 53 patients diagnostiqués avec DT1 et ayant suivi régulier dans la clinique endocrinologie pédiatrique ambulatoire, l'Université hôpital d'enfants de Minia. Le Groupe II a compris 15 sujets apparemment sains, l'age et le sexe adapté au groupe malade. Selon l'attaque du diabète, nous avons divisé le groupe diabétique en deux sous – groupes. Le Groupe Ia (nouvellement diagnostiqué), a compris 20 patients, dont l'âge est compris entre 7 et 18 ans ; 10 étaient males (50%), et 10 étaient femelles (50%). Alors que le Groupe Ib (la durée de malade >1 ans) comprenait 33 patients ; 17 étaient males (49%), et 18 étaient femelles (51%). Les groupes étudiés ont été soumis a la suivante : grâce a la prise de l'histoire, examen clinique et examen de laboratoire (nouveau de glycémie aléatoire) et HbA1c%. L'ADN a été extrait en utilisant QIAamp Min elute protocole du kit pour le dépistage du cytomégalovirus par RT – RCR.

Résultats : La fréquence du cytomégalovirus était considérablement plus élevée dans les enfants DT1 que le contrôlé et dans le Groupe Ia que le Groupe Ib.

Conclusion : Les enfants DT1 ont eu sérum cytomégalovirus plus élevé que le groupe contrôlé, comme ceux nouvellement diagnostiqués par rapport a ceux qui ont une plus longue durée de la malade.

Mots Clés : DT1, Cytomégalovirus, Hémoglobine A1C, la réaction en chaine de la Polymérase.

Messages Clés : Dose d'insuline, Significative +ve corrélations, RT – PCR.

INTRODUCTION

Type I diabetes (T1D) is the most common form of diabetes in most parts of the world; variations exist between the incidence rates of different populations. In type 1 DM, a viral infection in genetically susceptible individuals was hypothesized as triggering the disease. Human cytomegalovirus (HCMV) can infect and alter functions of poly morphonuclear leukocytes, lymphocytes and macrophages.Type 1 diabetes (T1D) is one of the most common chronic diseases in developed countries and represents about 10% of all cases of diabetes. It is caused by a selective destruction of insulin-producing beta cells (β-cells) in the pancreas (1). An increasing incidence of T1D has been observed in the last few decades, especially in young individuals (less than five years old) (2). The cause of T1 Dis still unknown. Several factors interact and lead to the development of the disease. An inflammatory is let infiltrate (insulitis) can be observed at the symptomatic onset of T1D, and reflects the immune response to β-cells (3). An autoimmune destructive process, which plays a central role in the development of T1D, is facilitated by the subject's own genetic susceptibility and by non-genetic factors. Non-genetic factors include viral infections, toxic chemicals, and others (4). Specific viruses can infect humans and may cause diabetes mellitus through different mechanisms, such as pancreatitis or hepatitis and their subsequent complications (5). Cytomegalo-virus (CMV) is an important factor thought to be associated with type I diabetes mellitus, owing to its ability to induce immunological damage to β-cells (6) CMV is an ubiquitous virus in the herpes group, causing chronic life-long infection in affected participants. It is a widely distributed virus, belonging to herpes virinae, subfamily of herpes viridae. Molecular mimicry is one of the principal immunological mechanisms that lead to destruction of pancreatic β-cells. This mimicry could be in volvedincy to megalo virus-induced diabetes by inducing is reactive antibodies. The loss of T-cell tolerance to self (GAD65) may be due to processing and presentation of a molecular mimic of cytomegalo virus protein pUL57 by dendritic cells (7). A Real-time PCR assay was described as an accurate and rapid test for CMV quantization by Leruez-Ville et al (2004) (8) The purpose of this study is to find out the association between cytomegalovirus and type 1 diabetes mellitus with comparison to healthy subjects, and to correlate its level with different clinical and laboratory parameters.

MATERIALS & METHODS

This study included 68 children and adolescents who were classified into two groups. Group I comprised 53 patients who had already been diagnosed as diabetic according to the standard ADA criteria and had regular follow up in the pediatric endocrinology outpatients' clinic, Minia University children's hospital, Egypt. Group II comprised 15 apparently healthy subjects, age and sex matched to the diseased group. Subjects were collected during the period from September 2013 to December 2013. According to the onset of diabetes, we divided the diabetic group into two sub-groups. Group Ia (newly diagnosed; duration of disease < 1 year) comprised 20 patients; 10 were males (50 %), and 10 were females (50 %), with ages ranging from 6-18 years (mean 11.4 ± 4.2). Group Ib(their duration of disease >1 year) comprised 33 patients; 17 were males (49 %), and 18 were females (51%), with ages ranging from 6 to 17 years (mean 12.3± 5.1). Informed consent was obtained from every subject enrolled in this study. The studied groups were subjected to the following: history taking, clinical examination and laboratory investigations. Random blood glucose levels (Colorimetric, Human, Germany) were assayed using a fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland) (9). HbA1c % was determined, as a parameter for glycemic control, by using resin column chromatography. Kit contents were supplied by TECO DIAGNOSTICS, California; USA (10). Five mL of blood samples from the diabetic children were collected and centrifuged at 5000 rpm. Serum samples were collected in sterile tubes; then DNA was extracted using the QIAamp Min elute kit protocol.

Principle Real time PCR

Procedure: CMV Quantitative Real Time PCR was used according to literature (OD-0002-02) from Life River. 17.5 µL of Reaction Mix CMV was used. Reaction was mixed with 0.2 µL of PCR enzyme mix and 0.5 µL of internal control then 6.8 µL of extracted DNA for a total volume of 25 µL. A positive control was defined as 10^7 copies/mL. To generate a standard

curve, a four-dilution standard was used. The real-time PCR instrument was operated according the thermal profile in the manual Ref (OD-0002-02). Each sample was spun down briefly in order to collect the Master Mix in the bottom of the reaction tubes, then the following protocol was performed in the instrument: 37°C for 2 min 1 cycle, 94 °C for 2 min 1 cycle at 94°C for 15 sec and at 60 °C for 1 min 40 cycles

Statistical Methods: The data were coded and verified prior to data entry. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS/Windows Version 19.0.0, SPSS Inc., an IBM Company). Microsoft excel 2013 was used for drawing figures. Continuous variables were presented as mean followed by standard deviation (SD), and categorical variables were presented as frequency and percentage. For qualitative data, chi-square (κ^2) was used: For quantitative data: Independent-Samples T test (for two groups) used in person correlation for correlation between two quantitative variables. Two-tailed partial correlation coefficients (r) adjusted for age, sex and BMI were used to assess the relationships between 25-hydroxy vitamin D and other variables. P-value < 0.001 was considered significant, and P-value > 0.001 was considered in significant. Kendl's test: was used for correlation between quantitative and qualitative variables.

RESULTS

Diabetic children had significantly higher weight and lower height than the control group (P < 0.001 for both). On the contrary, the differences between the diabetic and control groups as regards BMI and waist circumference was insignificant (P =0.4 and 0.3 respectively; Table 1). Table 2 shows that diabetic children, compared to the control group, had significantly higher fasting blood sugar, HbA1c %, and positivity of cytomegalovirus (45% *vs*7%).

***Duration*:** The current study found that newly diagnosed diabetic children, compared to those with longer duration of diabetes, had significantly higher frequency of cytomegalovirus (70% *vs* 30%), lower fasting blood sugar, and higher HbA1c% (P< 0.001, Table 3).

TABLE 1.COMPARISON BETWEEN THE DIABETIC CHILDREN AND THE CONTROLS AS REGARD SOME CLINICAL PARAMETERS

Clinical Parameter	Group I (Diabetic children)(N=53)	Group II (Control)(N=15)	P-Value
Age:(year)			
6-12 years. No (%)	**17 (32 %)**	**5 (33%)**	**0.9**
12-18 years. No (%)	**36 (68 %)**	**10 (67 %)**	
Sex:			
Male No (%)	**27 (51 %)**	**10 (67 %)**	**0.2**
Female No (%)	**26 (49 %)**	**5 (33 %)**	
Wt t(kg) mean±(SD)	**40.4±16.2**	**37.9±12.6**	**<0.001***
Height(cm)Mean±SD)	**142.8±21.1**	**149.3±13.1**	**<0.001***
BMI(kg/m²)(Mean±SD)	**18.9±4.3**	**16.8±2.7**	**0.4**
Waist circumference (cm)(Mean±SD)	**65±10.9**	**68.1±8.4**	**0.3**

***Significant**

TABLE 2.COMPARISON BETWEEN THE DIABETIC CHILDREN AND THE CONTROLS AS REGARD SOME LABORATORY PARAMETERS

Laboratory parameter	Group I, No=53(Mean±SD)	Group II, No=15 (Mean±SD)	P Value
Cytomegalovirus positivity No (%)	24(45 %)	1(7 %)	0.003*
Fasting blood sugar(mg/dl)	229.5±92.6	92.8±7.6	<0.001*
HbA1c (%)	8.7±1.8	5.7±0.6	<0.001*

- *Significant*

TABLE 3.COMPARISON BETWEEN NEWLY DIAGNOSED AND THOSE WITH DURATION > 1 YEAR AS REGARD SOME CLINICAL AND LABORATORY PARAMETERS

Parameter	Group Ia (duration < 1 year) No=20/cases	Group Ib (duration > 1 year) No = 33 (+ve CMV)	P value
Age (year)			
6-12. No (%)	4(20%), 3 (75%)	10 (33%), 4 (40%)	0.41
12-18 No (%)	16(80%), 11(68.7)	23 (67%), 6 (26%)	
Gender			
Male No (%)	10 (50%), 3 (30%)	16 (48%), 3 (18%)	0.915
Female No (%)	10 (50%), 10 (100%)	17 (52%), 7 (43%)	
Dose of insulin:(IU/kg/day) (Mean±SD)	0.9±0.14	0.9±0.12	0.8
Family history of DM:			
Positive No (%)	4(20%)	6(22%)	0.9
Negative No (%)	16 (80%)	27(78%)	
Cytomegalovirus positivity No (%)	14(70%)	10(30%)	<0.002
HbA1c% Mean±SD)	10.7±1.3	7.7±0.4	<0.001*
Fasting blood sugar(mg/dl) (Mean±SD)	121.2±7.9	259.6±75.5	<0.001*

- *Significant*

On the contrary, there were insignificant differences between these groups as regards age, dose of insulin, and family history of DM. Table 4 shows that there were significant positive correlations of cytomegalovirus positivity with fasting blood sugar and HbA1c%, and significant negative correlations with duration of disease and dose of insulin.

TABLE 4. CORRELATIONS BETWEEN POSITIVITY OF CYTOMEGALOVIRUS AND SOME CLINICAL AND LABORATORY PARAMETERS AMONG THE DIABETIC CHILDREN

Parameter	positivity of cytomegalovirus	
	R	Pvalue
Duration of diabetes (months)	-0.24	0.02*
Fasting blood sugar (mg/dl)	0.76	<0.001*
HbA1c (%)	0.86	<0.001*
Insulin dose (IU/kg/day)	-0.38	0.02*

DISCUSSION

T1D is an autoimmune disease, which implies a role of immune response effectors in the pathogenic processes and a failure of tolerance towards β-cell antigens. There is interplay between immune response, genetic and environmental factors. Several teams paid attention to the relationship between viruses and type 1 diabetes, and their role in the pathogenesis of the disease.[11]In the current study, the diabetic children (group I)were found to have higher weight and shorter height than the control group (group II; Table 1). This result was in agreement withPaulinoMFVM et al (2006)[12]who found that diabetic children were higher in weight and shorter in height in comparison to healthy children. Concerning BMI and waist circumference, there were insignificant difference between group I and group II (P> 0.05). This result was in agreement with that reported in lit.[13]Regarding laboratory parameters. Table 2 showed that diabetic children (group I) had significant higher fasting blood sugar and HbA1c % than the control group (group II). This was in agreement with CiechanowskiPC (2002)[14]Cytomegalovirus tested positive in 45 % of the diabetic patients versus (7%) of the control group, a statistically significant difference (P = 0.003). This finding was in agreement withAhmad-AbakurEH(2014), (15) who found that the positive rate of IgG against cytomegalo virus in the study group (diabetic patients) was 37% while it was 14.8% among a control group, indicating statistically significant association between IgG antibodies of cytomegalovirus and diabetes mellitus type I (*P* value 0.025). The study reveals significant relation (*P* value 0.003) of cytomegalovirus IgG antibodies with type I diabetes mellitusin age group (5-9 years) in Sudanese children. (15)

Regarding the duration of diabetes, we found that newly diagnosed diabetic children had significantly higher frequency of cytomegalovirus, compared to those with longer duration of diabetes, by 70% over 30%. This can be explained in that Human CMV (HCMV) plays an important role in diabetogenesis, where it was postulated that there is T-cell cross-reactivity between Human CMV (HCMV) and GAD 65 in pancreatic islet β-cells. HCMV-derived epitope could be naturally processed by dendritic cells and recognized by GAD65 reactive T-cells. Thus, HCMV may be involved in the loss of T-cell tolerance to autoantigen GAD65 by a mechanism of molecular mimicry leading to autoimmunity.[7]In 2008, Aarnisaloet al (16) analyzedspecific anti-CMV IgG antibodies in 169 serum samples from children who had developed the first T1D-associated autoantibody by the age of 2 years, and, in parallel, in 791 serum controls from healthy children. Serological, immunological, histological signs of autoimmunity and allograft rejection appeared concomitantly with early CMV infections in type 1 diabetic patients receiving pancreas allograft. This observation suggests that persistent CMV infections might be relevant to the pathogenesis of type 1 diabetes. Chen et al, (2012) (16) indicated that cells are damaged directly by viral infection, as the pancreas is a target organ of viral infection through induction of pro-inflammatory cytokines, or the cytotoxic activated effects or lymphocytes.

Regarding the duration of diabetes, the current study found that newly diagnosed diabetic children had significantly higher levels of cytomegalovirus than those with duration >1 year (P=002). This could be explained by the finding by Hjelmesaeth et al (2004) (17) who foundthat asymptomatic cytomegalovirus infection is associated with increased risk of new-onset type I diabetes and impaired insulin release after renal transplantation. As regards other parameters, the newly diagnosed had significantly lower fasting blood sugar and higher HbA1c% than those with long duration of diabetes (P < 0.001, Table 3). On the contrary, there were insignificant differences between these groups as regards age, dose of insulin, and family history of DM. Table 4 shows that there were significant positive correlations between cytomegalovirus levels with fasting blood sugar and HbA1c% and significant negative correlations with the duration of disease and insulin dose. This could be explained in that CMV infection apparently did not cause direct destruction of β-cells but was leading to changing in production of insulin.

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EPIDEMIOLOGY OF HEPATITIS B AND HEPATITIS C VIRUS INFECTIONS AMONG HIV COUNSELING AND TESTING CLIENTS IN JOS, NORTH CENTRAL NIGERIA

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ABSTRACT

Hepatitis B and hepatitis C virus infection are common in Nigeria; where they are a major cause of both acute and chronic liver disease, as well as hepatocellular cancer. Persons at risk of acquisition of Human Immunodeficiency Virus (HIV) infection are also at risk of acquisition of infection with Hepatitis B virus (HBV) and Hepatitis C virus (HCV). We set out to determine the epidemiology of HBV and HCV infection among HIV Counseling and Testing (HCT) clients at the Jos University Teaching Hospital (JUTH), Nigeria.

This was a cross-sectional study conducted at the HCT unit of the AIDS Prevention Initiative in Nigeria (APIN) Jos University Teaching Hospital (JUTH), Jos, Nigeria between November, 2012 and April 2013.

Subjects were recruited consecutively at the HCT unit of APIN JUTH. Included were subjects 18 years of age and above, antiretroviral (ARV) drug naive, who accepted and signed the consent form.Clients who declined to sign the consent form were excluded. The study involved collecting demographic data, exposure to risk factors and laboratory determination of HBV and HCV sero-prevalence in the subjects using Enzyme Linked Immunoassay (ELISA) and Polymerase chain reaction (PCR) assay methods.

Chi-squared test was used to determine significance of association between categorical variables.

One hundred and thirty two (56.9%) were females, 100 (43.1%) were males. Thirty six (15.5%) tested positive for HBsAg by ELISA, 31 (13.4%) were confirmed positive by DNA PCR. Nine (3.9%) tested positive by ELISA to HCV antibody, 7 (3.0%) were confirmed positive by RNA PCR. Co-infection rate of HIV / HBV was 5.2%. Infection was more common among those younger than 36 years in the case of HBV and those older than 36 years in the case of HCV.We concluded the prevalence of HBV infection was high. Study was limited by the cross sectional design.

L'EPIDEMIOLOGIE DES INFECTIONS PAR LE VIRUS DE L'HEPATITE B ET HEPATITE C PARMI LES CLIENTS DE VIH EN CONSULATATION ET AU DEPISTAGE A JOS, AU NORD – CENTRAL DU NIGERIA.

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RESUME

Les infections de virus de l'hépatite B et C sont communs au Nigeria ; où ils sont une cause majeure de la maladie de foie aiguë et chronique ainsi que le cancer hépatocellulaire. Les personnes à risque d'acquisition d'infection de virus d'immunodéficience humaine (VIH) sont aussi à risque d'acquisition de l'infection par le virus de l'hépatite B(VHB) et hépatite C(VHC). Nous avons cherché a déterminer l'épidémiologie de l'infection par VHB et VHC parmi les clients de VIH en consultation et au dépistage (HCT) a l'université hôpital d'enseignement de Jos(JUTH), Nigeria.

C'était une étude transversale menée a l'unité de HCT de l'Initiative de la prévention du SIDA au Nigeria (APIN) a l''Universite hôpital d'enseignement de Jos (JUTH) , Jos, Nigeria entre novembre 2012 et avril 2013.

Les sujets ont été recrutés consécutivement a l'unité de HCT de APIN JUTH. Inclus étaient des sujets de dix – huit ans au – dessus, antirétroviral naïve (ARV), qui ont acceptée et signé le formulaire de consentement. Les clients qui ont refusé a signer le formulaire de consentement ont été exclus. L'étude a impliqué recueillir de données démographiques, l'exposition aux facteurs de risques et la détermination en laboratoire du VHB et VHC en utilisant dosage immuno – enzymatique lie (ELISA) et les méthodes d'essai de réaction en chaine de la Polymérase (PCR). Chi – squared test a été utilisé pour déterminer l'importance de l'association ente les variables catégoriques. Cent trente – deux (56,9%) étaient femelles, 100(43,1%), étaient males. Trente – six(15,5%) ont été testés positifs pour HBsAg par ELISA, 31 (13,4%) ont été confirmés positifs par DNA PCR. Neuf (3,9%) ont été testés positifs par ELISA pour l'anticorps du VHC, 7 (3,0%) ont été confirmés positifs par RNA PCR. Le taux de co – infection du VIH/VHB a été 5,2%. L'infection aa été plus commun parmi les moins de 36 dans le cas de VHB et ceux âgés de plus de 36 dans le cas de VHC. Nous avons conclu que la prévalence de l'infection de VHB était élevée. L'étude était limitée par la conception transversale.

INTRODUCTION

Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immune Deficiency Virus (HIV) are among the top 10 leading causes of infectious disease deaths worldwide (1,2,3). Hepatitis B and C virus infections are frequent causes of chronic hepatitis worldwide and they create a significant burden to healthcare systems due to the high morbidity and mortality, and costs of treatment (4,5,6,7).

Hepatitis B and C virus infections can cause chronic hepatitis, liver cirrhosis and hepatocellular carcinoma - all of which are of serious public health concern (4,6). Infection by HBV and HCV cause serious mortality, morbidity and place financial burden on patients and governments and are thus a major global health problem (8). Nigeria is a hyperendemic area of infection with both HBV and HCV which are major causes of both acute and chronic liver disease associated with the development of hepatocellular cancer.[9]These viruses share common routes of transmission, but they differ in efficiency by which certain types of exposures transmit them and in their prevalence by geographic region (10,11).

The overall prevalence rate using the spot (rapid) test in a rural Ghana for HBV was highest in 2006 (13.8%), but decreased in 2008 to 6.9%.[12] However, the overall prevalence of HCV was highest in 2007 (11.1%) but decreased to 7.0% in 2008 in the same rural community (12).
Determining and monitoring the prevalence of viral hepatitis and its distribution in any community is useful for policy makers in order to formulate effective healthcare policies for patients with HBV, HCV and HIV infections(13).
Based on anecdotal evidence there are few reports on the prevalence or co-infections of HBV and HCV in Jos metropolis. This study therefore is aimed at documenting the prevalence of these infections in subjects accessing HIV counseling and testing (HCT) at JUTH.

The aim and specific objectives of this study was to determine the sero-prevalence of HBV and HCV infection among HCT subjects at JUTH using the ELISA method of assay. Also to determine the serum viral load of HBV, HCV and confirm identified seropositive samples using the PCR method.

MATERIALS

The study subjects were recruited from the HCT clients coming to JUTH, Jos for services. The JUTH HCT clinic attends to clients who voluntarily come for HIV counseling and testing on a daily basis.
Subjects 18 years and above were recruited consecutively at the HCT unit of APIN JUTH. The HCT unit offers a continuing enrolment for volunteer clients who are 18 years or older into the ARV treatment programme. The HIV status of each subject was obtained from the clinic register.
The total number of subjects enrolled was obtained by using the standard statistical formula for calculating sample size.[14,15] Blood samples obtained from 232 HCT subjects were used for this study.

All subjects starting from 18 years of age and above were included, these subjects were Antiretroviral drug (ARV) naive subjects. Only subjects who satisfied these criteria and accepted to join by signing the study consent form were included.

METHODS

About 5mls of blood was collected from each study subject into sterile plastic vacutainer with EDTA(16). Plasma was obtained from the whole blood by centrifugation at 1,500 RPM for 10 minutes. This was separated into 2 vials and kept in a -20˚C refrigerator until use. The laboratory evaluation on blood sample obtained from each of the 232 subjects as well as kit reagent positive and negative controls supplied by the kit manufacturer was performed.The Hepatitis B Surface Antigen (HBsAg),antibody to HCV (antiHCV) as well as Viral load (PCR)resultswas recorded on each individual's report form.

The Epi Info 3.5.1 (CDC Atlanta, USA) statistical software was used for data analysis. Continuous variables with normal distribution were expressed as means with standard deviations. For continuous and skewed variables, median value with ranges was stated. The chi-squared test was used to determine significance of association between categorical variables. Where the cell frequency is <5, Fisher's exact test was applied. Where more than two groups are being compared, ANOVA was used. P values of <0.05 was considered statistically significant.

Ethical clearance for this study was obtained from the Jos University Teaching Hospital Ethics Committee.
A consent form was signed by each subject who accepted to participate in the study. It was made clear to each subject that he/she was free to opt out from the study at any time without any prejudice to their care.
All subjects benefited from this study by having their results disclosed to them in confidence. Those found to be infected with HBV or HCV were referred to a Consultant Physician for specialist management.

RESULTS

Of the 36 (15.5%) who tested positive for HBsAg by ELISA, 31 (86.1%) samples were confirmed positive by DNA PCR giving a concordance rate of 86.1%.Among the 9 (3.9%) who tested HCV antibody positive by ELISA, 7 (77.8%) were

confirmed positive by RNA PCR giving a concordance rate of 77.8%).(Figure 1)

The median HBV viral load count was 282 with a range of 0 to 413x10^6 counts per millilitre. The median HCV viral load count was 12.68x10^4 with a range of 0 to 1142x10^4(Table 1).

A total of 36 (15.5%) subjects were found to HBV positive, 13 (5.6%) were male and 23 (9.9%) were females. A total of 9 (3.9%) were found to be HCV positive, 4 (1.7%) were males and 5 (2.2%) were female. Among the female subjects 23 (17.4%) and among the male subjects 13 (13.0%) tested positive for HBV.

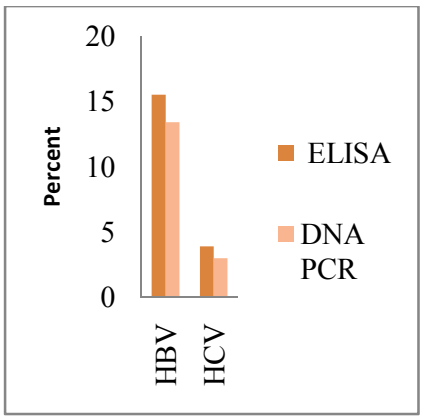

FIGURE 1: SERO-PREVALENCE OF HBV AND HCV BY BOTH ELISA AND DNA PCR AMONG ALL THE SUBJECTS STUDIED.

TABLE 1: HCV (ELISA AND RNA PCR) RESULTS FOR SUBJECTS STUDIED.

Serial Number	ELISA result	IU/ml	log /ml
1	Positive*	Not Detected*	-
2	Positive	49140.9	5.5
3	Positive	1962199.3	7.1
4	Positive	21786.9	5.1
5	Positive	11821.3	4.8
6	Positive	3333.3	4.3
7	Positive	324054.9	6.3
8	Positive	250171.8	6.2
9	Positive*	Not Detected*	-

Positive ELISA, Positive HCV RNA = Acute or Chronic HCV depending on clinical context. *Positive ELISA, Negative HCV RNA = Resolution of HCV; Acute HCV during period of low viraemia.

DISCUSSION

The prevalence of HBV infection among the HCT subjects studied was 15.5% by ELISA and 13.4% by DNA PCR. This difference of 2.1% in favour of virologic result, seem to indicate the virologic end point of HBV infection (i.e., a log10 reduction in the HBV DNA level or suppression of HBV DNA to an undetectable level [<10 to 100 IU per milliliter] (17).

From our study, HBV prevalence from this high risk group for HBV and HCV infections is much higher than the findings in Ogbomosho(18) among pre degree science students of a higher institution and in Nassarawa state (19) in two rural communities in 2010. The result of the present study is similar to the findings in 2007,[8] among male seminary student subjects of Jos and in 2011,[20] among blood donors in Jos. This study involved both sexes as subjects, but gave a similar HBsAg prevalence rate as other studies from Jos that were male dominated (20,21).

The diagnosis of chronic HCV infection generally requires testing of serum for both antibody to HCV (anti-HCV) and for HCV RNA. (22). These two markers of HCV infection may be present in varying permutations in patients, requiring careful analysis for interpretation (22). In this study, the prevalence of HCV is 3.0% by PCR RNA, our concordance rate (77.8%), compares well with the concordance of studies from other parts of the world (23,24) and is consistent with the WHO estimated HCV prevalence of 2.2% - 3.0%.[26,27] This HCV prevalence however, is low compared with previous findings reported from Jos by Egesie et al[28] in 2011 and Egah et al (29) in 2004 both studies were done among blood donors. HCV prevalence findings from other parts of the country (30,31) including report on blood donors from three hospitals in Kaduna state (32) in 2012; and report on blood donors from Lagos state (33) in 2006 were all higher than our findings.

Conclusion

The prevalence of HBV and HCV infection by ELISA and by DNA PCR among HCT clients in JUTH was determined to be high. The infection was substantial among the study population especially in those younger than 37 years in the case of HBV and those older than 36 years in the case of HCV.

Recommendations

Hospital authorities should invest more in the training of Physicians in the use of molecular diagnostic methods fora more effective monitoring of infectious diseases. It is only the use of PCR in screening that would identify infected subjects in their window period; this has implications for blood transfusion and tissue transplant services.Viral load should be used to monitor

patients in the management of HBV, HCV and HIV infected patients. Promotion of HBV screening and of the expanded programme on immunization should be encouraged.

This study was limited by the cross sectional design - a direct observation of the sero-prevalence of vaccination in the general population and continuation of HBV vaccination of children as part HBsAg and HCV only; the implication of the effect of one virus over the other was not deduced.

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BURDEN OF INTESTINAL PARASITES AMONGST HIV/AIDS PATIENTS ATTENDING BAMENDA REGIONAL HOSPITAL IN CAMEROON

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ABSTRACT
Background: Intestinal parasitic infections cause severe diarrhea especially in debilitated subjects with clinical complications of dehydration, malabsorption and severe weight loss, complicating treatment schemes.
Materials and Methods: This was a cross-sectional, hospital based study during which data were collected by the use of questionnaires and laboratory tests of stool and blood samples respectively.
Results: A total of 200 volunteer patients participated in this study of which 132 (66.0%) were females and 68 (34.0%) males. Eight different intestinal parasites were identified in 69 (34.5%) participants. The most prevalent parasite was *Entamoeba histolytica* with 8.0% of infected cases. Opportunistic parasites were identified in 15.5% of the study population. Seven percent of patients were infected with *Cryptosporidium parvum*, 6.5% with *Isospora belli*, and 2% with *Microsporidium* species. Diarrhea was found in 38.5% of the study participants 62.3% of whom had at least a single intestinal parasite. Opportunistic parasites were significantly more prevalent in patients with diarrhea and participants with CD4+ T cell counts less than 200 cells/µl (P < 0.05). Diarrhea was significantly more prevalent in participants who were on antiretroviral drugs than in those who were not (66.5% vs. 33.5%, P<0.05).
Conclusion: Though opportunistic parasites were found in the majority of HIV/AIDS patients attending the Bamenda Regional Hospital, *Entamoeba histolytica* and other intestinal parasites represented a common burden. It was therefore recommended appropriate diagnosis before initiating the routine treatment which is usually practiced in our health settings.
Key words: Intestinal parasites, HIV/AIDS patients, Bamenda Regional Hospital

FARDEAU DES PARASITOSES INTESTINALES CHEZ LES PATIENTS INFECTES PAR LE VIH/SIDA ET CONSULTANT L'HOPITAL REGIONAL DE BAMENDA AU CAMEROUN

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RÉSUMÉ
Contexte: Les infections parasitaires intestinales provoque des diarrhées sévères particulièrement dans des sujets débilités avec les complications cliniques de déshydratation, malabsorption et la perte de poids sévère compliquant parfois le traitement de la maladie
Matériels et méthodes: Il s'agissait d'une étude transversale conduite en milieu hospitalier pendant laquelle les données étaient rassemblées par l'utilisation de questionnaires et les tests de laboratoire effectués sur des prélèvements de selles et de sang respectivement.
Résultats: Un total de 200 patients volontaires ont participé à cette étude parmi lesquels 132 (66.0 %) était de sexe feminin et 68 de sexe masculin (de 34.0 %). Huit parasites intestinaux différents ont été identifiés chez 69 participants (34.5 %). Le parasite le plus répandu était *Entamoeba histolytica* avec 8.0 % de personnes infectées. Les parasites opportunistes ont été identifiés dans 15.5 % de la population d'étude. Sept pour cent de patients étainet infectés par *Cryptosporidium parvum*, 6.5 % par *Isospora belli* et 2 % par des espèces de *Microsporidium*. La diarrhée a été présente chez 38.5 % des participants, 62.3 % de ceux ci ayant au moins un parasite intestinal. Ces parasites opportunistes étaient significativement plus répandus chez les patients souffrant de diarrhée et chez les participants ayant un taux de CD4 inférieur à 200 cellules / µ L (P< 0.05). La diarrhée était significativement plus répandue chez les sujets sous traitement par les antirétroviraux que chez ceux qui ne l'aient pas (66.5% vs. 33.5%, P<0.05)
Conclusion: Quoique des parasites opportunistes aient été trouvés dans la majorité de patients de VIH/SIDA consultant à l'hôpital Régional de Bamenda, *Entamoeba histolytica* et d'autres parasites intestinaux représentaient un fardeau commun. Il a été recommandé

le diagnostic approprié avant l'introduction du traitement ordinaire qui est d'habitude pratiqué dans nos institutions sanitaires
Mots-clés: Parasitoses intestinales, Patients infectés par le VIH/SIDA, Hôpital Régional de Bamenda

INTRODUCTION

Enteroparasitic infections constitute a serious public health problem in developing countries with inadequate sanitary conditions. Many different types of intestinal helminthes and protozoa infect man, provoking a wide range of symptoms that are generally associated with gastrointestinal tract disorders and are dependent on demographic, socio-economic, physiological and immunological factors [1]. Patients with some types of immunocompromised conditions and those subjected to immunosuppressive therapy have an increased probability of presenting with parasitic infections, generally manifesting with a high degree of severity [2]. The immune response of an immunocompetent host against parasites is a complex system in which both cellular and humoral defense mechanisms intervene [3].

HIV infections result in severe destruction of $CD4^+$ T cells as the virus undertakes lytic replication cycles in the infected $CD4^+$ T cells. The cellular arm of the immune system thus becomes weakened. It is therefore thought that in advanced stages of HIV infection (AIDS), the $CD4^+$ T cells are drastically diminished and as such, parasites which produce self limiting diseases in immunocompetent hosts tend to result in severe outcomes in immunocompromised subjects [4]. The main clinical manifestation of diseases resulting from intestinal tract parasitic infections is diarrhea [5]; this is an intestinal bowel movements accompanied by loose watery stools. Diarrhea has several etiologic agents, ranging from parasites, through viral, bacterial, to conditions that may inflame the intestinal mucosa such as HIV enteropathy. Diarrhea in immunocompromised patients is usually profuse, generally accompanied by weight loss, anorexia, malabsorption syndrome and in some cases fever and abdominal pain. In such patients, parasites such as *Cryptosporidium parvum, Enterocytozoon bieneusi*, *Encephalytozoon intestinalis* and *Strongyloides stercoralis* may disseminate to other organs such as the bronchia, bile and liver ducts, producing symptomatology specific to the organ affected [6].

Cyclospora cayetanensis, Cryptosporidium parvum, microsporidium species *such as Enterocytozoon bieneusi* and *Isospora belli* have been incriminated as causes of prolonged diarrhea, especially in AIDS patients, although they are thought to cause self limiting diarrhea in immunocompetent individuals. These are referred to as opportunistic parasites [6]. Different species of protozoa have been associated with acute and chronic diarrhea in HIV infection and AIDS. They include *Cryptosporidium parvum, Isospora belli, Microsporidium* species… and account for a significant number of cases of diarrhea [7,8]. Infective causes of chronic diarrhea may satisfactorily be managed because with the exception of cryptosporidiosis and HIV-related enteropathy, good response to treatment can be expected [9], but all etiologic agents are not easily diagnosed in Africa on routine basis because of limited diagnostic facilities and trained personnel [10]. It is known that the pathogens responsible for diarrhea are different according to geographical location; therefore laboratory diagnostic evaluations are required to determine prevalence in each population so that it can provide guidelines for therapy for treatable etiologic agents and necessary data for planning and evaluation of HIV-positive/AIDS patients' care. The objective of this study was therefore to determine the prevalence of these intestinal parasites burden among HIV/AIDS patients who came for medical attention at the Bamenda Regional Hospital.

MATERIALS AND METHODS

Study area and sampling

This study was carried out at the Bamenda Regional Hospital HIV treatment Centre. Bamenda is the North West Regional capital of Cameroon. Hygiene and sanitation within the municipality has been severely compromised by the rapid migration of students since the creation of the University of Bamenda four years ago. They usually reside in numerous clutters around the university, generating large quantities of domestic waste. The sudden population increase has also led to permanent water scarcity that is generating many public health problems. Study participants who were patients already known as HIV/AIDS carrier and seeking medical attention at the Bamenda Regional Hospital were briefed on the modalities of the study with the aid of an informed consent form. After receiving information on what participation entailed, the advantages and inconveniences involved as well as possibility for compensation for damages, those who freely accepted to donate blood and stool samples for the study were the effective study participants.

Study design and ethical considerations The study was a cross-sectional, hospital based study in which HIV/AIDS patients visiting the Bamenda Regional Hospital were provided information on the purpose/objectives of the study, the possible benefits and discomforts that were to go with their participation in the study. The samples collected from participants were identified and processed using codes. Names were not used through out the study. This ensured individual confidentiality. Participants in this study were not billed for the tests and the physicians were provided with the findings for necessary action. The samples collected were solely used for the defined purpose. The ethical clearance was approved by the Ethical Committee of the Hospital prior to sample collection. The study obtained ethical clearance and administrative authorization from the University Ethical Review Board and the Regional Delegation of Public Health for the North West respectively prior to sample collection. The minimum acceptable sample size was derived using the

formula for sample size determination as described by Eng [11]

Sample collection and processing

Stool samples were collected into clean wide mouth specimen containers from male and female patients. A single freshly voided stool sample was collected from participants. A portion of the stool was preserved in 10% formalin. Five mL of venous blood was collected into coded EDTA tubes for $CD4^+$ T cell counts. Stool specimens were processed with saline wet mount and examined microscopically using X10 and X40 objectives to detect motile trophozoites. Formol-ether concentration technique for stool parasites was used and the sediment examined as iodine wet mounts to detect ova, larvae and cysts of intestinal tract parasites. Smears were prepared from the sediments, air dried and stained by a modified acid-fast stain to detect *Crytosporidium* and *Isospora* species as earlier previously described elsewhere [12]. Blood samples c from the same participants who provided stool samples were analyzed for CD4+ T lymphocyte cell estimation using flow cytometry. On the basis of the CD4 + T cell counts, the participants were categorized by their immune status according to the 1993 revised classification system for the HIV infection by CD4 T-cell categories [13].

Laboratory Procedure

Preparing wet mounts of stool for direct microscopy

One gram of freshly voided stool sample, (accepted only when duration between collection and examination was less than or equal to three hours) was emulsified on 50ul of physiologic saline (0.85% NaC1 solution) on a glass slide. The preparation was covered with a coverslip and examined using the X10 and the X40 objectives. To facilitate identification of parasite ova, cysts or trophozoites, lugols iodine was glided under the coverslip to stain and differentiate parasite cytoplasm [14]. Attempt was made to go through all the fields of the preparation before samples were reported negative of parasites.

Formol ether concentration technique

One gram of faeces was emulsified in 3mls of 10% formol water. A further 4mls of formol water was added to the preparation and mixed. The emulsified preparation was sieved and filtrate collected in a beaker and transferred to a centrifuge tube.4mls of ethyl acetate was added to the preparation. The tube was stoppered and its content mixed for a minute by gently inverting the stoppered tube and returning it to its upright position. The stopper was gently removed and the preparation centrifuged at 300rpm for a minute. Using a stick, the layer of faecal debris from the side of the tube was gently loosened and the tube was rapidly inverted to discard the supernatant, consisting of fecal debris, fomol and ether. The sediments remaining consisted of cysts and ova of faecal parasites. The tube was then reverted to its upright position and allowed for the sediments to return to the bottom. To re-suspend the ova, the tube was gently taped and its content transferred to two slides, one was covered with a coverslip and observed using the X10 an X40 objectives. A drop of iodine was run under the slide to increase visibility of parasite ova [12]. The second slide was air dried and stained for opportunistic parasites by the modified Ziehl Neelsen staining technique.

Modified Ziehl Neelsen staining technique

A smear of stool sample sediment from formol ether concentration was made on a clean glass slide and allowed to air dry. The preparation was fixed with absolute methanol. The slide was then stained with carbol fuchsine for 10 minutes, followed by rising of stained slides with water. The preparation was decolorized with acid alcohol (99 mL of 96% alcohol and 1ml hydrochloric acid), followed by rinsing in tap water and counterstaining in methylene blue for one minute. The slide was then rinsed, dried and observed using the oil immersion objective [15].

Data analysis and management

Data management prior to analysis involved the use of workbooks for direct raw data entries prior to keying into Microsoft excel for separation and arrangement of raw data generated. Analysis was done using the SPSS Version 11.0, SPSS Inc statistical software package. Data was summarized using tables and and the strength of association measured by using the chi-square and its associated P value. Associations were considered to be statistically significant when $P< 0.05$.

RESULTS

A total of 200 volunteer patients participated in this study of which 132 (66.0%) were females and 68 (34.0%) males. Table 1 shows the distribution of study participants according to age and sex. The highest number of males (30.7%), and females (69.3%) were between 41 and 60 years of age. The difference between age groups and sex was not significant. By distributing study participants according to occupation, house wives constituted 11 (5.5%) of the study participants, business persons 54 (27.0%), employed persons 16 (8.0%), farmers 62 (31.0%), students 6 (3.0%), unemployed 26 (13.0%) and unskilled labourers 25 (12.5%) (Table 2).

TABLE 1: DISTRIBUTION OF STUDY PARTICIPANTS ACCORDING TO AGE AND SEX.

Age group, (years)	Number (%)* of participants		Total n (%)
	Males	Females	
20 – 40	30 (40.0)	45 (60.0)	75 (37.5)
41 -60	35 (30.7)	79 (69.3)	114 (57.0)
> 60	3 (27.3)	8 (72,7)	11 (5.5)
Total	68 (34.0)	132 (66)	200 (100.0)

*Percentages based on number of participants within age group

TABLE 2. DISTRIBUTION OF STUDY PARTICIPANTS ACCORDING TO OCCUPATION

Occupation	Number (%)* of participants
House wife	11 (5.5)
Business	54 (27.0)
Employed	16 (8.0)
Farming	62 (31)
Student	6 (3.0)
Unemployed	26 (13.0)
Unskilled labour	25 (12.5)
Total	200 (100.0)

* Percentages based on the total number of participants

Table 3 shows the Distribution of study participants with respect to residence**.** Most of the study participants 90 (45.0%) were from semi urban settings, while 63 (31.5%) came from rural communities and 47 (23.5%) from urban areas.

TABLE 3. DISTRIBUTION OF STUDY PARTICIPANTS WITH RESPECT TO RESIDENCE SETTING

Residence	Number (%)* of participants
Urban	47 (23.5)
Semi – urban	90 (45.0)
Rural	63 (31.5)
Total	200 (100)

* Percentages based on the total number of participants.

TABLE 4. PREVALENCE OF INTESTINAL TRACT PARASITES IN STUDY PARTICIPANTS

Parasites	Number (%)* of patients with parasites
E. histolytica	14 (7.0)
E. histolytica and *C. Parvum*	2 (1.0)
A. lumbricoides	14 (7.0)
C. parvum	12 (6.0)
I. belli	13 (6.5)
G. lamblia	7 (3.5)
Microsporidium spps	4 (2.0)
T. trichiura	2 (1.0)
D. fragilis	1 (0.5)
Total	69 (34.5)

* Percentages based on total study population.

Eight (8) different parasites were identified in 69 (34.5%) participants. The most prevalent parasite was *E. histolytica*, 16, (8.0%); [14 (7.0%) solely and 2 (1.0%) in co-infection with *C. parvum*]. In decreasing prevalence, the parasite trend was, 14 (7.0%) *A. lumbricoides,* 14 (7.0%), *C. parvum;* [2 (6.0%) solely and 2 (1.0%) in co-infection with *E. histolytica*], 13 (6.5%) *I. belli,* 7 (3.5%) *G. lamblia,* 2 (1.0%) *T. trichiura and* 1 (0.5%) *D. fragilis* (Table 4).

There were a total of 69 parasite positive stool samples. Diarrhea was diagnosed in 77 study participants. 21 (10.5%) 0f the study participants had parasitic infections without diarrhea, 48 (24.0%) had both intestinal parasites and diarrhea whereas 29 (14.5%) had diarrhea without intestinal parasites. Table 5 shows the association between parasites identified and diarrhea in study participants. *Entamoeba. histolytica* had a significantly higher prevalence in diarrheic participants, 16 (100.0%) compared to their non diarrheic counterparts, 0 (0.0%), ($P < 0.05$). *Ascaris lumbricoides* was significantly less prevalent in diarrheic, 2 (14.3%) than in non diarrheic patients 12 (85.7%) ($P = 0.05$). The prevalence of *C. parvum* was significantly higher in diarrheic, 13 (92.9%) than in non diarrheic participants 1 (7.1%) ($P < 0.05$). Eight, 8 (61.5%) of the participants infected with *I. belli* had diarrhea against 5 (38.5%) without diarrhea. The association between *I. belli* and diarrhea was not statistically significant ($P > 0.05$). *G. lamblia* and *Microsporidium* were significantly associated with diarrhea, ($P < 0.05$) as opposed to *T. trichiura and D. fragilis* ($P > 0.05$).

TABLE 5: ASSOCIATION BETWEEN PARASITES IDENTIFIED AND DIARRHEA IN STUDY PARTICIPANTS.

Parasite	Number of infected patients	Number (%)with diarrhea
+*E. Histolytica**	16	16 (100.0)
A. lumbricoides	14	2 (14.3)
+*C. parvum**	14	13 (92.9)
I. belli	13	8 (61.5)
*G. lamblia**	7	6 (85.7)
*Microsporidium sp**	4	4 (100.0)
T. trichiura	2	1 (50.0)
gilis		(0.0)
	1	50 (70.4)

+ Represents co-infections. *P<0.05

Table 6 shows the association between parasites solated, and level of immunesupression. Patients with $CD4^+$ T cell counts less than 200cells/ul had the highest parasite prevalence, 33 (46.5%), compared to 27 (38.0%), and 11(15.5%) for Participants with $CD4^+$ T cell counts between 200 and 499 cells/ul and greater than or equal to 500cells/ul respectively. *C. parvum* and *Microsporidium spps* were significantly more prevalent in severely immunosupressed subjects with $CD4^+$ T cell counts less than 200cells/ul ($P < 0.05$).

TABLE 6: ASSOCIATION BETWEEN PARASITES ISOLATED, AND LEVEL OF IMMUNOSUPPRESSION.

Parasite	$CD4^+$ T cell counts (cells/ul)			Total
	< 200 n (%)	200- 499 n (%)	≥ 500 n (%)	
$^+$*E. Histolytica*	8 (50.0)	5 (31.3)	3 (18.7)	16
A. lumbricoides	2 (14.3)	8 (57.1)	4 (28.6)	14
$^+$*C. Parvum**	11(78.6)	3 (21.4)	0 (0.0)	14
I. belli	4 (30.8)	6 (46.2)	3 (23.0)	13
G. lamblia	3 (42.9)	3 (42.9)	1 (14.2)	7
*Microsporidium sp**	4 (100.0)	0 (0.0)	0 (0.0)	4
T. trichiura	1 (50.0)	1 (50.0)	0 (0.0)	2
D. fragilis	0 (0,0)	1 (100.0)	0 (0.0)	1
Total	33 (46.5)	27 (38.0)	11 (15.5)	71

$^+$ Represents co- infections.
***P<0.05**

Table 7 shows the $CD4^+$ T cell counts and diarrhea profiles in study participants. The prevalence of diarrhea with respect to $CD4^+$ T cell counts was 44 (64.7%) for patients with $CD4^+$Tcell counts less than 200, 23 (26.7%) with $CD4^+$ T cell counts between 200 and 499cells/ul and 10 (21.7%) for patients with $CD4^+$ T cell counts greater than, or equal to 500 cells/ul ($P < 0.05$).

TABLE 7: $CD4^+$ T CELL COUNTS AND DIARRHEA PROFILES IN STUDY PARTICIPANTS.

Diarrhea status	number (%)* with $CD4^+$ T cell count			Total
	< 200	200-499	≥ 500	
Diarrheic	44 (64.7)	23 (26.7)	10 (21.7)	77 (38.5)
Non Diarrheic	24 (35.3)	63 (73.3)	36 (78.3)	123 (61.5)
Total	68	86	46	200 (100)

*** Percentages based on patients within same CD4 counts**

The occupations with the highest proportion of parasitic infections were business persons 21 (30.4%) and farmers, 17 (24.6%). The proportions of parasitic infections were least in employed persons 7 (3.5%), and students, 2 (1%), but the difference in parasite prevalence was not statistically significant between the different occupations ($P > 0.05$).

Intestinal parasites were significantly more prevalent in patients not yet on antiretrovirals than in those who were (59.1% Vs 14.5%, $P < 0.05$) and diarrhea was significantly more prevalent in patients on antiretrovirals, 51 (66.2%) than in those who were not, 26 (33.8%) ($P < 0.05$)

DISCUSSION AND CONCLUSION

In this cross-sectional study the overall prevalence of intestinal parasites from stool samples was 34.5% This finding show some degree of similarity with those earlier obtained in Chennai and Yaounde [16-17] respectively. It is however far below the 62.5% prevalence reported some years back by Zalem et al [18]. The relatively lower parasite prevalence in this study could be explained by the fact that the study was conducted in semi urban area where the levels of hygiene are far higher as compared to the rural setting in which the previous studies were carried out.

The parasite most associated with diarrhea was *Entamoeba histolytica, Microsporidium* species, *Cryptosporidium parvum* and *Isospora. beli.* This trend was similar to that reported by Haileeyesus and Beyene [19] in Ethiopia. These protozoa usually seek protection from human digestive enzymes in the intestinal lumen by causing the host's intestinal lining to form folds around them, leaving the impression that they are intracellular. Due to the fact that intracellular pathogens are destroyed by the cellular arm of the immune system they profit from the weakened

cellular immune system to cause diarrhea. These folds also reduce the intestinal surface area available for absorption, resulting in malabsorption. Opportunistic parasites were identified in 15.5% of the study participants. This finding was higher than the 9.7% reported in a similar study by Sarfati et al [17]. Diarrhea profiles followed the opportunistic parasite profiles, and were significantly associated with $CD4^+$ T cell counts, below 200cells/ul as earlier reported by Haileeyesus and Beyene [19] in Ethiopia. In our study, business persons and farmers constituted the highest proportions of parasite infected participants. The higher prevalence of infection in business persons could be explain by their regular feeding in with food provide by street food vendors, irrespective of sanitary conditions [20]. Farmers were equally on top of the parasite infection chart, probably due to their contact with the soil, which predisposes them to geohelminthes.

Intestinal tract parasites were more prevalent in patients with $CD4^+$ T cell counts less than 200cells/ul compared to patients with $CD4^+$ T cell counts between 200 and 499 and greater than or equal to 500 cells/ul respectively. This trend is similar to previous reports [19,21] in which increased parasite prevalence was found in patients with $CD4^+$ T cell counts less than 200cells/ul and could be explained by the fact that at lower $CD4^+$ T cell counts, the immune system is weakened; as such the host becomes unable to eliminate pathogens which thrive and cause diseases. Intestinal parasites were significantly more prevalent in patients who were not on antiretrovirals, compared to those who were on treatmentThis is in accordance with previous report from Haileeyesus and Beyene [19]. Antiretrovirals is known to improve on $CD4^+$ T cell counts, thus decreasing the incidence of opportunistic parasites. Diarrhea was significantly more prevalent in patients on antiretroviral drugs than in patients who were not on treatment. The trend was similar to that reported by Call et al.[21] while investigating diarrhea post highly active antiretroviral therapy (HAART) in HIV/AIDS patients in the United states. This trend could be explained by the fact that antiretrovirals such as protease inhibitors are thought to increase intestinal tract electrolyte Though opportunistic parasites were found in the majority of HIV/AIDS patients attending the Bamenda Regional Hospital, intestinal parasites represented a common burden**.** It was therefore recommended appropriate diagnosis before initiating the routine treatment which is usually practiced in our health settings.

concentration, thus increasing diarrhea prevalence. This perception is however not shared by Poles et al.[23] according to who the prevalence of in diarrhea is decrease with HAART.

The prevalence of intestinal parasites was highest in illiterates and persons who had only attained primary education followed by those who had attained secondary education. The least parasite prevalence was recorded in those who had attained university or other higher education level of studies. This was in line with previous other findings [19, 24] that related the prevalence of parasitic infections to the knowledge of the transmission pattern of the causative organism and the level of education of the respondents.

The present study has shown a prevalence of intestinal parasites among HIV/AIDS patients attending the Bamenda Regional Hospital at 34.5% portraying diarrhea as a common clinical manifestation of parasitic infections in these patients. The spectrum of enteric parasites causing diarrhea included opportunistic parasites such as *Cryptosporidium parvum*, *Isospora belli* and *Microsporidia* and conventional pathogens such as *Entamoeba histolytica*, *Giardia lamblia, Trichiuris trichiura* and *Ascaris lunbricoides*. Opportunistic intestinal parasites were more prevalent *in patients with* $CD4^+$ T cell counts less than 200cells/ul and they play a major role in causing chronic diarrhea. The prevalence of infection with a particular enteric parasite in HIV/AIDS patients was largely influenced by occupation, general hygiene practices and levels of education.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

Bissong M. E. A. conceived and designed the study, conducted the literature search, drafted the manuscript and substantially revised it. Nguemain, N. F and Ng'awono, T. Epse Nkoa contributed to the design and substantially revised the manuscript. Kamga, F. H. L substantially revised the manuscript and prepared it for publication. All authors read and approved the manuscript.

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RENAL AND HEPATIC PROFILES IN NIGERIAN MULTIDRUG RESISTANT TUBERCULOSIS PATIENTS WITH OR WITHOUT HIV CO-INFECTION

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ABSTRACT

Tuberculosis (TB) is primarily a lung disease (pulmonary tuberculosis, PTB) but the bacilli can also develop in other places in the body, such as the bones, liver and kidney as extra pulmonary tuberculosis. Hepatic and renal involvements in PTB patients are mostly secondary to TB chemotherapy that is potentially hepato- and nephro- toxic. In this study, the biochemical parameters that indicate renal and hepatic involvements were analyzed in the sera of MDR-TB patients with and without HIV co-infection prior to commencement of chemotherapy. Out of 115 MDR-TB patients (76 males and 39 females) recruited for the study, 22 patients (11 males and 11 females) were co-infected with HIV. Serum levels of sodium (Na^+), potassium (K^+), chloride (Cl^-) were analyzed using Easylite (ISE technology), bicarbonate (HCO_3^-) was analysed using back titration method, urea and creatinine were determined spectrophotometrically using Diacetlymonoxime (DAM) method and Jaffe's alkaline picrate method respectively. Total and direct bilirubin, serum glutamate oxaloacetate transferase (SGOT), serum glutamate pyruvate transferase (SGPT), alkaine phosphatase(ALP), total protein (TP) and albumin (Alb) were determined using Hitachi 912 autoanalyzer. There were no statistical significant differences in the renal and hepatic parameters of TB patients with HIV compared with TB patients without HIV. However, significantly higher proportions (89%) of MDR-TB patients had their SGOT within reference range. The mean values indicate that HIV infection did not significantly alter renal and hepatic profiles in MDR-TB patients prior to treatment.

Key words: Kidney, Liver, Electrolytes, Tuberculosis, Human Immunodeficiency Virus, Co-infection.

LES PROFILS RENALS ET HEPATIQUES DANS MULTIRESISTANTE PATIENTS TUBERCULEUX NIGERIANS AVEC OU SANS CO – INFECTION PAR LE VIH.

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RESUME :

La tuberculose est principalement une maladie du poumon (Tuberculeuse pulmonaire TBP) mais les bacilles peuvent être également se développer dans d'autres endroits dans le corps, tels que les os, le foie et les reins comme la tuberculose extra pulmonaire. L'atteinte hépatique et rénale chez les patients atteints de TBP est surtout secondaire a la chimiothérapie de la tuberculose(TB) qui est potentiellement hépato – et néphro – toxique. Dans cette étude, les paramètres biochimiques qui indiquent les implications rénales et hépatiques ont été analyses dans les sérums des patients atteints de MDR – TB avec ou sans Co – infection par le VIH avant le commencement de la chimiothérapie. Sur les 115 patients atteints de MDR – TB (76 males et femelles), recrutés pour l'étude, 22 patients (11 males et femelles) ont été co – infectes par le VIH. Le niveau sérique de sodium (Na^+), de potassium (K^+), le chlorure($C1^-$) ont été analyses en utilisant Easylite(technologie ISE), le bicarbonate ($HC0_3^-$) a été analyse en utilisant la méthode de titrage de retour, l'urée et de la créatinine ont été déterminés spectrophotométrie rn utilisant la méthode Diacétyle monoxime(DAM) et la méthode de picrate alcaline de Jaffer respectivement. Bilirubine totale et directe, glutamate oxaloacetate transférase sérique(SGOT), transférase sérique de glutamate pyruvate(SGPT), phosphatase alcaline(ALP), protéines totale(TP) et albumine(Alb) ont été déterminés en utilisant Hitachi 912 autoanalyseur. Il n' ya pas de différence statistiquement significative dans les paramètres rénales et hépatiques des patients tuberculeux vivant avec ke VIH par rapport aux patients atteints de tuberculose sans VIH. Néanmoins, les proportions significativement élevées (89%) des patients atteints de MDR – TB ont eu leurs SGOT a porté de référence. Les valeurs moyennes indiquent que l'infection par le VIH n'a pas modifié significativement les profils rénaux et hépatiques chez les patients de TB – MRD.

Mots – clés : Les reins, le foie, l'électrolyte, la tuberculose, Virus de l'immunodéficience humaine, Co – infection.

INTRODUCTION

TB is an air-borne infectious disease caused by bacteria *Mycobacterium tuberculosis*, which primarily affects the lungs. The World Health Organization (WHO) declared Tuberculosis (TB) a global emergency in 1993 and it remains one of the world's major causes of illness and death. One third of the world's population carries the TB bacteria and more than nine million of these become sick each year with active TB (1). TB disproportionately affects people in resource-poor settings, particularly in Africa and Asia during their most productive years (2). Coupled with the pandemic of HIV, TB has become an important cause of morbidity and mortality world-wide (3). TB is a major public health problem in Nigeria, with the country ranking 5th among the 22 high TB burden countries which collectively bear 80% of the global burden of TB. The TB burden in Nigeria is further compounded by HIV/AIDS epidemic and the emergence of multi-drug resistant tuberculosis (MDR-TB) (2).

It was reported that systemic effects especially on vital organs such as the kidneys and liver are later events that characterize tuberculosis (TB) and Human Immunodeficiency (HIV) infections. There can be direct renal and hepatic involvement in TB patients but this rarely causes marked impaired renal and hepatic functions. Occasionally, local signs and symptoms may be prominent in renal and hepatic tuberculosis, and may constitute the initial or sole presenting feature of the disease (4). Anti- tubercular drugs was reported to cause renal and hepatic injuries. Rifampicin, pyrazinamide, isoniazid and ethambutol were shown to benephro- and hepato-toxic drugs (5). A study showed that patients with chronic kidney and liver disease can also develop tuberculosis (6), thus posing special management problems for the TB. Since many of the potent anti-tubercular drugs are nephrotoxic and hepatotoxic, they may aggravate the underlying disease processes. Thus, anti-TB drug regimens need to be monitored to prevent further renal and hepatic insult in the patients.

Moreover, literatures have shown that a single anti-TB drug has side effects on liver and kidney (5, 6), thus it is likely that combination of drugs in MDR-TB patients or co-existence of MDR-TB with HIV may have severe effects on kidneys and liver of these patients. This therefore demands for the need to evaluate the status ofliver and kidney functions in MDR-TB patients before commencement of multi-drug treatment.

MATERIALS AND METHODS

After obtaining informed consent, a total of 115 TB patients consisting of 76 males (37±11 years) and 39 females (30±8 years) admitted into MDR-TB Treatment Center, University College Hospital, Ibadan, Nigeria, were recruited into the study. Twenty-two(22) patients (33±9 years) made up of 11 males ((32±9 years) and 11 females(26±8years)were co-infected with HIV. Five (5) ml of blood samples were collected to plain bottles. Serum was obtained following centrifugation. Serum bilirubin was estimated by photometric method based on the diazo reaction. This was based on the principle that bilirubin reacts with diazotized sulphanilic acid to form the red colour azobilirubin (7).Serum protein was analyzed using Biuret method in which divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-coloured Biuret complex. The colour intensity is directly proportional to the protein concentration which was determined photometrically (8). Albumin was determined by dye binding method whereat a pH value of 4.1 albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG), an anionic dyestuff to form a blue –green complex (9). Urea was estimated using Diacetyl monoxime method where heating urea with substances such as diacetyl formed coloured compound measurable spectrophotometrically at 520nm (10). Creatinine was determined using Jaffe's alkaline picrate method where creatinine reacts with picric acid in an alkaline solution toproduce a deep yellow complex directly proportional to the level of creatinine present in the sample (10). ALP activity was assayed using kinetic methodby measuring the concentration of p-nitrophenol released from the cleavage of p-nitrophenyl phosphate into phosphate and p-nitrophenol(11). ASTand ALT activitywere determined following the principle described by Reitman and Frankel(1953). AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine while ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine (12). GGTactivity was assayed by monitoringthe concentration of 5-amino-2-nitrobenzoate released (13). Serum levels of sodium (Na^+), potassium (K^+), chloride (Cl^-) were analyzed using Easylite (ISE technology), bicarbonate ($HC0_3^-$) was determined using back titration method (14). The data were presented in mean ± S.D. The differences between the means were compared using Mann-whitney U test.

RESULTS

Table 1 shows demographic characteristics of MDR-TB patients with and without HIV infection and their gender distribution. Age, height, weight and BMI were similar in MDR-TB patients compared with patients having MDR-TB/HIV co-infection.

Table 2 shows that there were no significant differences in renal and hepatic profiles of the MDR-TB patients compared with patients having MDR-TB/HIV co-infection.

Table 3 shows percentage of parameters below, within and above reference range (RR). SGOT was significantly above normal reference range in most(74.2%) of MDR-TB patients.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF MDR-TB PATIENTS AND MDR-TB/HIV PATIENTS

	MDR-TB (n= 93)	MDR-TB/HIV (n= 22)	p-values
Age (yrs)	**35.44±11.07**	**33.18±9.18**	**0.377**
BMI (kg/m²)	**16.95±4.91**	**18.13±2.58**	**0.276**
Height (m)	**1.63±0.26**	**1.66±0.11**	**0.617**
Weight (kg)	**48.40±13.26**	**50.27±8.75**	**0.530**
Gender (males)	**65**	**11**	
Gender (females)	**28**	**11**	

TABLE 2: RENAL AND HEPATIC PROFILES IN MDR-TB PATIENTS AND MDR-TB/HIV PATIENTS

	MDR-TB (n= 93)	MDR-TB/HIV (n= 22)	p-values
Urea (mg/dl)	**31.83±9.66**	**29.45±8.62**	**0.293**
Creatinine (mg/dl)	**0.80±0.24**	**0.81±0.21**	**0.804**
Sodium (mmol/l)	**129.77±5.81**	**130.91±2.62**	**0.374**
Potassium (mmol/l)	**3.62±0.51**	**3.55±0.54**	**0.591**
Chloride (mmol/l)	**97.49±2.48**	**97.00±1.20**	**0.366**
Bicarbonate (mmol/l)	**22.88±1.92**	**22.95±1.56**	**0.869**
Total Bilirubin(mg/dl)	**0.62±0.26**	**0.56±0.28**	**0.362**
Direct Bilirubin(mg/dl)	**0.29±0.18**	**0.33±0.22**	**0.351**
SGOT (IU/l	**51.45±23.45**	**55.77±22.62**	**0.436**
SGPT (IU/l)	**38.18±16.08**	**39.91±18.81**	**0.662**
ALP (IU/l)	**88.94±34.99**	**86.23±23.63**	**0.731**
Total protein (g/dl)	**6.55±0.85**	**6.36±0.56**	**0.204**
Albumin (g/dl)	**4.73±0.26**	**4.75±0.14**	**0.699**

TABLE 3: RENAL AND HEPATIC PARAMETERS IN MDR-TB AND MDR-TB/HIV PATIENTS WITHIN AND OUTSIDE REFERENCE RANGES (RR)

	MDR-TB	MDR-TB/HIV	N	p-values
Urea:				
Within RR	**89(80.2%)**	**22(19.8%)**	**111**	**1.000**
Above RR	**4 (100%)**	**0 (0.0%)**	**4**	
Creatinine:				
Within RR	**81(81.8%)**	**18(18.2%)**	**99**	**0.504**
Above RR	**12(75.0%)**	**4 (25.0%)**	**16**	
Potassium:				
Below RR	**32(76.2%)**	**10(23.8%)**	**42**	**0.333**
Within RR	**61(83.6%)**	**12(16.4%)**	**73**	
Chloride:				
Below RR	**3(100.0%)**	**0(0.0%)**	**3**	**1.000**
Within RR	**90(80.4%)**	**22(19.6%)**	**112**	
Bicarbonate:				
Within RR	**93(80.9%)**	**22(19.1%)**	**115**	
Total Bilirubin:				
Within RR	**88(81.5%)**	**20(18.5%)**	**108**	**0.617**
Above RR	**5(71.4%)**	**2(28.6%)**	**7**	
Direct Bilirubin:				
Within RR				
Above RR	**87(82.1%)**	**19(17.9%)**	**106**	**0.370**
SGOT:	**6(66.7%)**	**3(33.3%)**	**9**	
Within RR				
Above RR	**44(89.8%)**	**5(10.2%)**	**49**	**0.036***
SGPT:	**49(74.2%)**	**17(25.8%)**	**66**	
Within RR				
Above RR	**34(77.3%)**	**10(22.7%)**	**44**	**0.440**
ALP:	**59 (83.1%)**	**12(16.9%)**	**71**	
Within RR				
Above RR	**70(79.5%)**	**18(20.5%)**	**88**	**0.515**
Total protein:	**23(85.2%)**	**4(14.8%)**	**27**	
Below RR				
Within RR	**47(77.0%)**	**14(23.0%)**	**61**	**0.268**
Albumin:	**46(85.2%)**	**8(14.8%)**	**54**	
Below RR				
Within RR	**1(100.0%)**	**0(0.0%)**	**1**	**1.000**
	92(80.7%)	**22(19.3%)**	**114**	

Renal and hepatic profiles of male MDR-TB compared with female MDR-TB patients were shown in Table 4. Serum creatinine was significantly raised in male MDR-TB patients compared with female MDR-TB patients. Tables 5 and 6 respectively show hepatic and renal profiles in male or female MDR-TB patients compared with MDR-TB/HIV co-infection. There were no statistical significant differences between the parameters in male patients compared with female patients.

TABLE 4: RENAL AND HEPATIC PROFILES IN MALE AND FEMALE MDR-TB PATIENTS

	Male (n= 76)	Female (n= 39)	p-values
Urea (mg/dl)	31.84±9.48	30.46±9.53	0.462
Creatinine (mg/dl)	0.84±0.25	0.73±0.17	0.013*
Sodium (mmol/l)	130. 03±5.38	129.92±5.37	0.923
Potassium (mmol/l)	3.63±0.51	3.55±0.52	0.440
Chloride (mmol/l)	97.49±2.18	97.23±2.53	0.574
Bicarbonate (mmol/l)	23.05±1.91	22.59±1.70	0.205
Total Bilirubin (mg/dl)	0.62±0.25	0.58±0.28	0.500
Direct Bilirubin (mg/dl)	0.28±0.18	0.33±0.22	0.259
SGOT (IU/l	52.24±23.09	52.36±23.89	0.979
SGPT (IU/l)	39.16±15.86	37.26±18.00	0.562
ALP (IU/l)	87.11±32.08	90.97±35.14	0.555
Total protein (g/dl)	6.55±0.86	6.44±0.68	0.474
Albumin (g/dl)	4.72±0.27	4.78±0.16	0.200

TABLE 5: HEPATIC AND RENAL PROFILES IN MALE MDR-TB PATIENTS AND MDR-TB/HIV PATIENTS

	MDR-TB (n= 65)	MDR-TB/HIV (n= 11)	p-values
Urea (mg/dl)	31.74±9.66	32.45±8.78	0.819
Creatinine (mg/dl)	0.84±0.25	0.87±0.24	0.646
Sodium (mmol/l)	129.74±5.70	131.73±2.33	0.260
Potassium (mmol/l)	3.64±0.51	3.55±0.56	0.600
Chloride (mmol/l)	97.62±2.29	97.73±1.19	0.214
Bicarbonate (mmol/l)	23.02±1.96	23.27±1.68	0.682
Total Bilirubin(mg/dl)	0.62±0.26	0.57±0.23	0.532
Direct Bilirubin(mg/dl)	0.27±0.17	0.34±0.21	0.279
SGOT (IU/l	51.62±23.19	55.91±23.19	0.572
SGPT (IU/l)	39.32±16.03	38.18±15.50	0.827
ALP (IU/l)	86.77±33.28	89.09±24.98	0.826
Total protein (g/dl)	6.59±0.91	6.32±0.53	0.334
Albumin (g/dl)	4.71±0.29	4.73±0.17	0.789

TABLE 6: HEPATIC AND RENAL PROFILES IN FEMALE MDR-TB PATIENTS AND MDR-TB/HIV PATIENTS

	MDR-TB (n= 28)	MDR-TB/HIV (n= 11)	p-values
Urea (mg/dl)	**32.04±9.83**	**26.45±7.70**	**0.100**
Creatinine (mg/dl)	**0.72±0.18**	**0.75±0.15**	**0.562**
Sodium (mmol/l)	**129.86±6.15**	**130.09±2.74**	**0.905**
Potassium (mmol/l)	**3.55±0.53**	**3.55±0.54**	**0.966**
Chloride (mmol/l)	**97.21±2.91**	**97.27±1.19**	**0.949**
Bicarbonate (mmol/l)	**22.57±1.81**	**22.64±1.43**	**0.916**
Total Bilirubin(mg/dl)	**0.60±0.27**	**0.55±0.32**	**0.619**
Direct Bilirubin(mg/dl)	**0.33±0.21**	**0.33±0.25**	**0.977**
SGOT (IU/l	**51.07±24.46**	**55.64±23.16**	**0.598**
SGPT (IU/l)	**35.54±16.17**	**41.64±22.26**	**0.348**
ALP (IU/l)	**93.96±38.84**	**83.36±23.04**	**0.404**
Total protein (g/dl)	**6.45±0.71**	**6.40±0.61**	**0.828**
Albumin (g/dl)	**4.78±0.18**	**4.77±0.11**	**0.920**

DISCUSSION

Chemotherapy is the basic approach to clinical tuberculosis control. Previous study have reported that antituberculosis therapy (ATT) causes derangement of hepatic and renal functions (15) but influence of HIV co-infection and combination of ATT on renal and hepatic profile was not reported. The primary purpose of this study was to determine the base line serum levels of renal and hepatic parameters in MDR-TB patients without HIV infection compared with MDR-TB/HIV co-infection before commencement of multi-drug therapy for MDR-TB patients. This allows early detection of possible hepatic or renal defect and thus necessitating more appropriate management strategies.

To the best of our knowledge, this is the first study that will monitor renal and kidney functions in patients with co-infection of MDR-TB and HIV at initiation of therapy in this environment. Renal- and hepatic- parameters measured in MDR-TB patients with HIV infection were not statistically different compared with MDR-TB patients without HIV infection. However, higher proportions of MDR-TB patients have their SGOT above normal reference range.Previous authors have reported raised transaminases as early sign of liver involvement in TB/HIV pathology (16). Also, renal- and hepatic-parameters in male or female MDR-TB patients with HIV infection did not show any statistical significance differences when compared with MDR-TB patients without HIV co-infection. However, serum creatinine in male MDR-TB patients was significantly raised compared with female MDR- TB patients. This might be related to higher muscle mass in males compared with females. Although, this has no clinical implication but this study recommends that creatinine should be frequently monitored in these patients since raised creatinine is a sensitive and specific marker of kidney damage. Besides this, regular biochemical investigations should also be performed essentially to predict when the patients are likely to develop hepatic and renal function derangements. According to Ciba Laboratories, kidney and liver function tests should be carried out before initiating ATT (17) as it has been reported that patients with abnormal baseline transaminases levels are at increased risk of developing hepatic injury eventually (16). It was reported that renal and hepatic profiles should be determined twice weekly for the first two weeks followed by weekly monitoring till the end of two months and then monthly investigations till the end of the treatment (18). This is not affordable in patients in resource poor countries, thus a single sensitive parameter like SGOT may be most useful in this situation.

Conclusion

The results indicate that HIV infection did not significantly alter renal and hepatic profiles in MDR-TB patients prior to treatment. However, since the current recommended treatment anti-tuberculosis

drugs are found to be potentially nephrotoxic and hepatotoxic, continuous monitoring of hepatic and renal functions of MDR-TB patients is necessary.

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PREVALENCE OF HIV/SPUTUM AFB POSITIVITY AMONG PATIENTS ATTENDING UNIVERSITY OF BENIN TEACHING HOSPITAL (UBTH), BENIN CITY, NIGERIA.

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ABSTACT

Human Immunodeficiency Virus (HIV) and Tuberculosis(TB) have synergistic interactions that speedily accelerate decline of the host's immune system and accentuate the progression of each other. Eight hundred and ninety five patients referred from different units of University of Benin Teaching Hospital, Nigeria were screened for antibodies to HIV-1 and HIV-2 using ELISA and sputum microscopy for acid-fast bacilli. The result showed that, 92(10.3%) were HIV positive with females 68(14.2%) higher than males 24(5.8%) though not statistically significant (p=0.066), 123(13.7%) patients were AFB positive, with males 75(18.1%) significantly higher than females 48(10%) (p=0.046) and 14(1.6%) patients were co-infected. Although the co-infection rate was higher among females 9(1.9%) than males 5(1.2%), there was no significant difference (p=0.450). The age group 40-49 and 50-59 had the highest TB/HIV co-infection (2.5% each).Regular screening for TB in HIV patients and HIV in TB patients would demonstrate the true burden of TB disease amongst HIV infected patients.

Key words: prevalence, TB, HIV, co-infection, Nigeria.

LA PREVALENCE DE LA POSITIVITE DU VIH/EXPECTORATIONS AFB CHEZ LES PATIENTS QUI FREQUENT L'UNIVERSITE HOPITAL D'ENSEIGNEMENT DE BENIN (UBTH), BENIN CITY, NIGERIA.

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RESUME

Le virus de l'immunodéficience humaine(VIH) et la tuberculose (TB) ont des interactions synergiques qui rapidement accélèrent le déclin du système immunitaire de l'hôte et accentuent la progression de l'un l'autre. Huit cent quatre – vingt quinze patients des différentes unités de l'université hôpital d'enseignement de Benin, Nigeria, ont été criblés pour les anticorps a VIH – 1 et VIH – 2 en utilisant ELISA microscopique des expectorations pour les bacilles acide – Résistant. Le résultat a montrée que 92 (10,3%) étaient séropositifs avec des femelles 68(14,2%) plus élevé que les males 24(5,8%), mais pas statiquement significatif, (p=0,066), 123(13,7%) patients étaient AFB positif, avec les males 75(18,1%) significativement plus élevés que les femelles 48 (10%) (p=0,046) et 14 (1,6%) patients ont été Co – infectés. Bien que le taux de la Co – infection était plus élevé parmi les femelles 9(1,9%) que les males 5 (1,2%), il n y avait pas de différence significative (p=0,450). Le groupe d'âges 40 – 49 et 50 – 59 avait la plus forte Co - infection de TB/HIV (2,5%). Le dépistage régulier de la tuberculose chez les patients VIH et VIH chez les patients tuberculeux démontrerait le véritable fardeau de la maladie de la tuberculose chez les patients infectés par le VIH.

Mots Clés : Prevalence, Tuberculose, VIH, Co – infection, Nigeria.

INTRODUCTION

Data from World Health Organization (WHO) shows that tuberculosis is the most common cause of death from infectious diseases, causing more death than HIV and malaria combined; with Africa harboring about 29% of those infected. Nigeria is one of the countries in sub-Saharan Africa noted to be saddled with a high prevalence of the disease [1]. It is known

to have the highest burden of tuberculosis infection in Africa (311 per 100,000, about 0.31% of the population) [2]. The prevalence of HIV infection in Nigeria is high; approximately 3.6% of the population (about 3 million people) is known to be living with HIV infection [2]. The country has the second largest number of people living with HIV-AIDS and accounts for 10% of the global HIV burden with approximately 215,000 HIV-AIDS related death in 2010 [2]. HIV infection is the single most important factor for the resurgence of TB globally and the major reason for failure to achieve set TB control targets especially in areas with high prevalence [3].

HIV and TB have synergistic interactions that speedily accelerate the decline of the host immune system, accentuating the progression of each other [4]. In the individual patient, HIV infection weakens the immune system and increases the susceptibility to TB. HIV increases the likelihood of reactivation, re-infection and progression of latent TB infection to active disease. It also alters the clinical presentation of TB, complicates the follow up and compromises the response to anti-TB treatment [5].

There were an estimated 1.1 million TB/HIV co-infected patients worldwide in 2011; 79% of these cases were in the African Region [6]. This is due to the high incidence of HIV in this region. HIV and TB are the two leading causes of death and continued to be a serious problem in developing countries [7]. People living with HIV/AIDS (PLWHA) have an exquisite vulnerability to TB and are 30-50 times more likely to progress to active TB, while the likelihood of progressing to full blown AIDS increases by 100 folds in HIV/TB co infected patients [8]. HIV/TB co-infection presently poses serious and major public health challenges especially in the African region, especially Nigeria. With a population of 162 million people, Nigeria is one of five countries with high TB/HIV burdens contributing 60% of the global HIV-associated TB in 2011 [6]. In Nigeria the prevalence of HIV among TB patients increased from 2.2% in 1991 to 19.1% in 2001 and 25% in 2010, indicating that the TB situation in the country is HIV-driven [9]. The need to define optimal timing of antiretroviral therapy during TB treatment and to find better alternatives to current drug regimens has been reasonably answered in Camelia, Sapit trials [10] . To mitigate the dual burden of TB/HIV in populations at risk of or affected by both diseases, the World Health Organization (WHO) published a document on priority research questions in 2010 [11], and an updated policy on collaborative TB/HIV activities in 2012 [6], which emphasize the importance of surveillance of HIV among TB patients and surveillance of active TB patients among people living with HIV in all countries.

In Benin City, Edo state, Nigeria, there is sketchy literature on prevalence of HIV and TB co-infection and associated risk factors. This study was carried out to determine the prevalence of HIV and TB co-infection among patients attending University of Benin Teaching Hospital, Benin City, South-southern Nigeria.

MATERIALS AND METHODS

Study design

This was a prospective cross sectional study carried out between January, 2014 and December, 2014 in which subjects referred from Accident & Emergency unit , Children Emergency unit, Outpatient clinics, Wards and TB centers to Medical Microbiology laboratory for sputum AFB examination and HIV testing were recruited for this study after obtaining inform consent

Study area

The study was conducted at the University of Benin Teaching Hospital, Benin City, Nigeria. The institution is located in the heart of Edo state and serves as a referral centre to other neighbouring health institutions in Western and South-South regions of Nigeria. The facility also serves as a regional center for TB and HIV control programmes in Nigeria.

Collection of samples

Sputum samples were collected based on the conventional method of on the spot day one, early morning and on the spot on day two or 3 (first) early morning specimens obtained after a deep, productive cough on consecutive days using clean transparent wide-mouthed sputum cups. Specimens were brought to the Medical Microbiology laboratory on the day of collection.

Sputum analysis for AFB

The sputum samples were handled in a class I safety cabinet. Sputa accepted were purulent, opaque or greenish in appearance with less than 10 epithelial cells/1 pf on Gram staining. Smears were made on grease free clean frosted slides, air-dried, heat-fixed and stained according to the standard operating procedure for the hot Ziehl Neelsen technique [12]. Smears were examined microscopically for acid fast bacilli (AFB) using oil immersion objective lenses and results were recorded appropriately.

HIV Screening

Blood samples were obtained by vene-puncture and centrifuged, and the sera obtained were screened for

antibodies to HIV-1 and HIV-2 using approved ELISA kits (Alere Determine™ and Double Check Gold™) (Alere industries, Japan), according to the manufacturer's instructions. Samples that tested positive were confirmed by Western blot.

Ethical Approval

Ethical approval was obtained from the Ethics Review Board of UBTH and informed consent was obtained from all study subjects.

Data analysis

The prevalence of HIV, TB and their co- infection was calculated. The data generated was subjected to non-parametric Mann-Whitney statistics and chi-square to determine any significant relationship between infection rate, age and gender. All statistical analyses were carried out using SPSS computer software version 16.0 for Windows. Significant and non-significant difference was determined at $p \leq 0.05$ and $p > 0.05$ respectively.

Inclusion and Exclusion Criteria
Newly diagnosed subjects who gave informed consent and provided sputum and blood for AFB test and HIV screening respectively were included, while those who decline to give consent or were unable to produce sputum were excluded from the study. Also, subjects who had already been diagnosed with TB and HIV and were already on therapy were excluded from the study.

TABLE 1: REFERRAL DISTRIBUTION OF PATIENTS FOR HIV AND AFB TEST FROM DIFFERENT UNITS

S/N	Referred from	Frequency	Percentage
1	A/E	87	9.7
2	CHER	66	7.4
3	CLINIC	289	32.3
4	DOT	285	31.8
5	WARD	168	18.8
	TOTAL	895	100

Key: A/E= accident and emergency, DOT= direct observed therapy, CHER = children emergency

LIMITATIONS OF THE STUDY
The selection of patients from a single center poses a major limitation for the applicability and generalization of the findings. The finding is also limited because participation was voluntary. The analysis was only restricted to smear-positive tuberculosis cases which is not enough to accurately diagnosed Mycobacterium tuberculosis. However, we believe that these not invalidate the study.

RESULTS
Eight hundred and ninety five (895) patients were included in this study. Most of these patients were referred from the outpatient clinic (32.3%) and TB centers (31.8%), while 18.8%, 9.75 and 7.4% were referred from the wards, Accident and emergency and children emergency center respectively (Table 1). The result in Table 2 showed that, of the total populations, 415(46.4%) were males while 480(53.6%) were females, with a male: female ratio of 0.87:1. Ninety-two patients (10.3%) were HIV positive with females 68(14.2%) showing a higher prevalence rate than males 24(5.8%) but with no statistical difference ($p=0.066$). The results further showed that 123(13.7%) patients are AFB positive with males 75(18.1%) showing significantly higher prevalence rate than females 48(10%) ($p=0.046$). The result of the co-infection rate among these populations showed that 14(1.6%) patients were co-infected with both AFB and HIV. Although the co-infection rate was higher in females 9(1.9%), the result however, showed no statistically significant difference ($p=0.450$).

TABLE 2: SEX DISTRIBUTION OF HIV, AFB AND AFB/HIV CO-INFECTION

Sex	No. Tested	HIV+	AFB +	HIV/AFB co-infection
Male	415	24 (5.8)	75 (18.1)	5 (1.2)
Female	480	68 (14.2)	48 (10)	9 (1.9)
Total	895	92 (10.3)	123 (13.7)	14 (1.6)

Age distribution of patients in this study is presented in Table 3. The age group with the highest HIV infection rate was 30-39 years (17.8%), with females (21.2%) having higher prevalence rate than males (13%) (Table 4). Also, TB infection is much more prevalent in the age group 30-39 years (17.8 %), with males (24%) having higher prevalence rate than females (12.4%). The age groups 40-49 years and 50-59 years had the highest TB/HIV co-infection (2.5% each) (Table 4).

TABLE 3: AGE DISTRIBUTION OF HIV, AFB AND AFB/HIV CO-INFECTION

Age distribution	No. Tested	Sex		No. of HIV positive(%)	No. of AFB positive(%)	AFB/HIV Co-infection
		Male	Female			
0-9	18(2.0)	10	8	0 (0)	1(5.6)	0(0)
10-19	63(7.0)	37	26	3(4.8)	8(12.7)	1(1.6)
20-29	167(18.7)	77	90	15(8.9)	25(14.9)	1(0.6)
30-39	213(23.8)	100	113	37(17.4)	38(17.8)	3(1.4)
40-49	118(13.2)	53	65	14(11.9)	13(11.0)	3(2.5)
50-59	120(13.4)	50	70	13(10.8)	16(13.3)	3(2.5)
60-69	118(13.2)	57	61	6(5.1)	15(12.7)	2(1.7)
70 and above	78(8.7)	31	47	4(5.1)	7(8.9)	1(1.3)
Total	895(100)	415(46.4)	480(53.6)	92 (10.3)	123(13.7)	14 (1.6)

TABLE 4: AGE AND SEX DISTRIBUTIONS OF HIV, AFB AND AFB/HIV CO-INFECTION

Age distribution	No. Tested (%)		HIV positive (%)		AFB positive (%)		AFB/HIV Co-infection (%)	
	Male	Female	Male	Female	Male	Female	Male	Female
0-9	10(55.6)	8(44.4)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)
10-19	37(58.7)	26(41.3)	1(2.7)	2(7.7)	4(10.8)	4(15.4)	1(2.7)	0(0)
20-29	77(46.1)	90(53.9)	2(2.6)	13(14.4)	15(19.5)	10(11.1)	0(0)	1(1.1)
30-39	100(46.9)	113(53.1)	13(13)	24(21.2)	24(24)	14(12.4)	2(2)	1(0.9)
40-49	53(44.9)	65(55.1)	2(3.8)	12(18.5)	8(15.1)	5(7.7)	0(0)	3(4.6)
50-59	50(41.7)	70(58.3)	1(2)	12(17.1)	10(20)	6(8.6)	1(2)	2(2.9)
60-69	57(48.3)	61(51.7)	3(5.3)	3(4.9)	9(15.8)	6(9.8)	1(1.8)	1(1.6)
70 and above	31(39.7)	47(60.3)	2(6.5)	2(4.3)	5(16.1)	2(4.3)	0(0)	1(2.1)
Total	415(46.4)	480(53.6)	24(5.8)	68(14.2)	75(18.1)	48(10)	5(1.2)	9(1.9)

DISCUSSIONS

The results of this study showed that more females (53.6%) were screened for both HIV and TB compared with males (46.4%). This is in agreement with a similar study earlier reported from Nnewi, Nigeria [4]. Another similar study in the Niger-Delta region of Nigeria revealed that females (64%) participated more in the study than their male (36%) counterpart [13]. Naturally, females tend to seek medical attention much earlier than males who in keeping with their stronger-sex and bread winner tendencies would not go to hospital until later during the course of their illness[4].

A non-significant rate of HIV infection was found to be higher among females (14.2%) than males (5.8%), a finding contrary to that from Gombe, Nigeria[14] where males had a higher prevalence. This rising trend in female HIV prevalence is not unexpected due to the fact that the penile-vaginal transmission by an infected individual in a single sexual exposure is as low as one in 1000 from woman to man and as high as one in 300 from man to woman [15]. In addition, early exposure to sexual activities, the poor economic status and the pressure on women to provide for their families as well as the lack of ability to negotiate safer sex [16, 17, and 18] might have contributed to the increase in the risk of HIV acquisition among women. A relative reason for the disparity could be the high health-seeking behavior of women. Furthermore, the age group with the highest HIV infection is 30-39 (17.8%). This is consistent with previous report in Nigeria [18 and 19]. Individuals in this age group make up a greater proportion of the workforce in the country thus the impact of HIV/TB co-infection on the economy of the country will certainly become over- whelming if not controlled with appropriate intervention measures such as provision of effective prevention education and early detection and treatment of both infections [18]. However, other report showed that the pattern of HIV infection in the general population is highest among individuals in the age group 20-29 [20]. The result of this study also showed that none of the subjects tested among age group 0-9 is HIV positive. This might be due to the fact that this age group are less vunerable to risk factors for HIV infections . The age group 10-19 (4.8%) has the lowest HIV infection rate among the studied population. This is consistent with earlier report [18] and with previous national data [21] and may indicate less HIV associated acquisition of TB in this age group.

This study showed that TB infection is correlated with gender, with males (18.1%) showing significant higher prevalence rate than females (10%) (p=0.046). Contrary to the findings of this study, Onubogu et al. [22] reported that TB infection rate was statistically higher in females than in males. According to them, the differences in the infection rate in females and males could be as a result of biological factors such as higher susceptibility to infection due to low immunity in women. However, this finding is in agreement with

previous studies [23, 24 and 25] which reported that TB infection was more in males than females. Obiora et al. [26] also reported higher TB infection rates among males in Benin and Irrua, Nigeria. Nnorom et al. [27] reported higher TB infection rates for TB among males in urban and rural communities in Nigeria. The reason for the gender difference in prevalence of TB and HIV is not known from our study. However, it seems probable that a combination of different factors such as biological differences in disease and disease presentation, together with gender related factors like access to health care may play a role. Biological and immunological factors might be contributory as to why evidence which suggest that men may have more infectious TB (smear-positive pulmonary TB) than women [28]. A theory that is often presented as an explanation to the gender differences is that men in general have a wider social network that leads to a greater exposure to the tubercle bacillus. A population study [29] among people with a cough for more than 3 weeks reported that although women did not start seeking health care later than men, they often sought health care from less qualified providers, took more health care actions, and had longer delay to hospital than men. In a study on TB patients by Matsushita et al. [30], in Japan, they observed that the stage and the extent of lung lesions are less advanced among female TB patients. So the prevalence of cough and sputum expectoration among female TB patients was significantly less common than among male patients. In many low-income countries, women often have a lower social position and poorer access to economic resources, education, and information than men. These gender differences influence both health risks among women and care-seeking behaviour [31]. Although our study showed that age group is not a risk factor with regard to TB infection, however, the age group 30-39 (17.8%) are more predisposed to TB infection. This is contrary to previous reports from Nigeria [32] and Turkey [33], but is consistent with a previous study reported from Tanzania [34].

The result of the co-infection rate among these populations showed that 14(1.6%) patients are co-infected with both TB and HIV. The HIV-TB co infection rate of 1.6% obtained in this study could be comparable to 1.42% reported from a tertiary care hospital in Nnewi, Nigeria [4] and 1.23% obtained in a rural tertiary care hospital in Punjab [35]. However, it was quite lower than those obtained from other states of Nigeria where rates ranging from 4.39% to 41.2% have been documented [17, 19, 22, 36-42]. Co-infection rates could vary between study populations and regions, probably due to differences in prominent occupation and other socio-economic factors [43] and differences in study designs [36]. The low prevalence of TB-HIV co-infection as observed in this study may not be unconnected with the increased awareness of HIV infection and increase in the number of free treatment centres, provided by the Government and NGO's in the country. It might also be due to the fact that all the subjects involved in this study are ignorant of their HIV or TB status until confirmed in the laboratory. Although the co-infection rate was higher in females 9(1.9%) than in males 5(1.2%), the result however, showed no statistically significant difference (p=0.450). This observation is in agreement with previous studies that reported 6.85% TB-HIV co-infection in females and 4.95% prevalence rate in males [37]. Contrary to the findings of this study, previous report showed that TB and HIV co-infection rate was also higher among females than males but the difference was statistically significant ([44, 22]. Difference in co-infection rate in males and females could be as a result of early exposure of females to sexual activity due to their economically disadvantaged position, high susceptibility to infection [45], delay in care seeking due to stigma associated with HIV infection [46]; less access to fund for transportation and personal health care [22], high incidence of HIV infection in females which predisposes them to TB as the former is known to activate dormant TB [47]. Other studies contrary to the findings of this study also revealed higher TB/HIV co-infection rates among males than females [48, 49]. This was attributed to the general attitude of males towards indiscriminate sex especially when they travel away from their families and visit sex tourist centres [48].

The rising cases of TB/HIV co-infection no doubt impacts negatively on AIDS and TB control programmes in many ways, this includes increased caseload of active TB attributable to HIV, HIV-related morbidity and mortality in TB patients, higher default rates and low cure rates, high rate of adverse drug reactions, increased risk of TB transmission and delay of access to health services for TB suspects due to the stigma of HIV/AIDS [50].

CONCLUSION

There are genders and age differences in the HIV prevalence and HIV-TB co infection rates among the study subjects. However, the study also demonstrates that gender rather than age group is a risk factor for TB infection with males' preponderance. Therefore it is strongly recommended that Patients with newly diagnosed TB should be tested for HIV infection and patients with newly diagnosed HIV be tested for TB infection.

CONTRIBUTION BY AUTHORS

The study was conceptualized, data was collated and manuscript written by both authors.

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IN VITRO ACTIVITY OF FOSFOMYCIN AGAINST UROPATHOGEN MULTI-DRUG RESISTANT (MDR) *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII*

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ABSTRACT

Urinary tract infections caused by multidrug resistant Gram negative bacilli constitute a major global healthcare problem. Fosfomycin is considered the best treatment option for such infections. Urine samples were collected and cultured in a tertiary care hospital (Urology). Identification of these uropathogens and their antibiotic sensitivity screening were performed according to CLSI guidelines. Urine samples (n=436) were selected in which *Ps. aeruginosa* and *Acinetobacter baumannii* were found to be the significant pathogens and treated-exposed to fosfomycin. Sixty six (15%) were identified as *Acinetobacter baumannii, Ps. aeruginosa* n=370(85%). Forty four percent of all *Ps. aeruginosa* were found to be multidrug resistant while 48.5% of the *Acinetobacter baumannii* strains were found multidrug resistant. Polymyxin B was found to be the most effective drug (100%) against all uropathogens and fosfomycin was found effective against 73% of the multidrug resistant *Acinetobacter baumannii* isolates and 70% of the multidrug resistant *Pseudomonas aeruginosa* strains. It may be concluded that antimicrobial activity (*in vitro*) of fosfomycin, especially against MDR uropathogens, is very effective.

Keywords: Fosfomycin, Multidrug resistant Gram negative bacilli, Urinary tract infections, *Ps. aeruginosa, Acinetobacter baumannii*

L'ACTIVITE IN VITRO DE LA FOSFOMYCINE CONTRE UROPATHOGEN MULTI-DRUG RESISTANT (MDR) *PSEUDOMONAS AERUGINOSA* ET *ACINETOBACTER BAUMANNII.*

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RESUME

Les infections des voies urinaires causées par les bacilles de multi résistants Gram négatifs, constituent un problème majeur de sante mondiale. Fosfomycine est considéré comme la meilleure option de traitement pour telles infections. Les échantillons d'urine ont été recueillis et cultives dans un hôpital de soins tertiaires. Identification de ces uropathogènes et programmation de leur sensibilité aux antibiotiques ont été réalisés selon les directives (CLSI). Les échantillons d'urine (n = 436) ont été choisis dans laquelle *Ps. aeruginosa* et *Acinetobacter baumannii* se sont trouves être l'agent pathogène important et traités – exposés a Fosfomycine. Soixante – six (15%) ont été identifies comme *Acinetobacter baumannii, Ps. aeruginosa* = 370 (85%). Quarante – quatre pourcent de tous les *Ps. aeruginosa* se sont trouvés être multi résistants et 48,5% des souches *Acinetobacter baumannii* se sont trouves multi résistants. Polymyxine B a été trouvé d'être le médicament le plus efficace (100%) contre tous les uropathogènes et Fosfomycine a été trouvé efficace contre 73% des isolats de multi résistants *Acinetobacter baumannii* et 70% des souches de multi résistants *Pseudomonas aeruginosa*. On peut conclure que l'activité antimicrobienne (*in vitro*) de Fosfomycine est très efficace, particulièrement contre les uropathogenes MDR.

Mots – clés : Fosfomycine, les bacilles de Multi résistants Gram négatifs, les infections des voies urinaires, *Ps. aeruginosa*, *Acinetobacter baumannii*.

INTRODUCTION

Urinary infections (UTIs) due to multi-drug resistant Gram-negative bacilli (MDR-GNB) are an increasing clinical problem worldwide (1, 2). The prevalence of multi-drug resistant (MDR) bacterial species of *Pseudomonas aeruginosa and Acinetobacter baumannii* has increased considerably since the introduction followed by arbitrary use of new generation extended

spectrum antibiotics like third and fourth generation cephalosporins, carbapenems, monobactams, broad and extended spectrum penicillins etc (3). During the last few years these organisms are undergoing genetic modifications and result in highly resistant forms that cause untreatable nosocomial infections and healthcare associated complications (4, 5). These bacterial strains create very serious problems for antibiotic treatment especially in critically ill patients admitted in intensive care units. Fosfomycin can be a potentially useful agent for urinary tract sepsis (caused by MDR-GNB), as many such strains remain susceptible to this decades old drug (6, 7). It is for this reason and along with its soft administration that it has been widely recommended and used for the treatment of uncomplicated urinary tract infections (8). It is a well-tolerated drug and has a broad spectrum of activity (9). The objective of this study is to manifest fosfomycin bioactivity against multi drug resistant *Pseudomonas aeruginosa and Acinetobacter baumannii* strains encountered in urinary tract infections.

MATERIALS AND METHODS

Collection sites (anatomical) of the urine samples

Urine samples were collected from patients showing overt symptoms of urinary tract infections (UTIs) in a tertiary care hospital (Urology). A variety of collections were done including Foleys catheter collection, Midstream sample collection, Left and Right Percutaneous nephrostomy (L-PCN and R-PCN) collection and Suprapubic (S/P) collection depending on the patient's condition (10).

Inoculation of urine samples

All urine samples were inoculated on Cystine lactose electrolyte deficient (CLED) agar medium plates by 1µl calibrated loops (Culti loops). Plates were incubated under aerobic conditions at 37^{o}C for 24 hours when colonies were observed for significant count and lactose or non lactose fermentative activity (10).

Identification of uropathogens

Significant counts (100 colonies) were counted on CLED medium plate i.e. equal to 10^5cfu/ml. Gram staining was performed as preliminary step. Pathogens were identified by standard biochemical reactions or by automated profile index (API 20 NE) system (bioMerieux) where needed (10, 11). In this study 436 urine samples (Positive for *Ps. aeruginosa and Acinetobacter baumannii)* were selected for fosfomycin bioactivity.

Antibiotic sensitivity screening and media

Antibiotic sensitivity testing was performed by Kirby-Bauer disc diffusion method on Muller Hinton agar (Oxoid, UK) according to Clinical laboratory standard institute (CLSI) and European committee on antimicrobial susceptibility testing (EUCAST) (12, 13). Amikacin (AK30µg), Ampicillin (AMP10µg), Amoxicillin-clavulanic acid (AMC20/10µg), Aztreonam (ATM30µg), Ceftazidime (CAZ30µg), Cefoperazone-sulbactam (SCF105µg), Cefotaxime (CTX30µg), Ciprofloxacin (CIP5µg), Fosfomycin (FOS300µg), Imipenem (IPM10µg), Nalidixic acid (NA30µg), Nitrofurantoin (F300µg), Pipracillin-tazobactam (TZP100/10µg), Polymyxin B(PB300µg) and Trimethoprim / sulfamethoxazole (SXT1.25/23.75µg) discs were used. All the antibiotic discs were obtained from Oxoid. MacFarland 0.5 suspension of the isolate was made in normal saline that was spread by swab over the Muller Hinton (MH) agar and appropriate discs of the above indicated antibiotics were placed at the 15 mm distance from each other. Quality control strains *E.coli* ATCC25922 *and Pseudomonas aeruginosa* ATCC27853 were used for the standardization of antibiotic sensitivity testing.

RESULTS

In this study 436 urine samples (Positive for *Ps. aeruginosa* and *Acinetobacter baumannii)* were selected for fosfomycin bioactivity. Out of the isolated bacterial strains, a total of n=66(15%) were identified as *Acinetobacter baumannii* and n=370(85%) as *Ps. aeruginosa.* Antibiogram for *Acinetobacter baumannii* is shown in fig-1 and fig-2 depicts the antibiogram for *Ps. aeruginosa.* Data was interpreted in percent by using Microsoft Office Excel 2007.

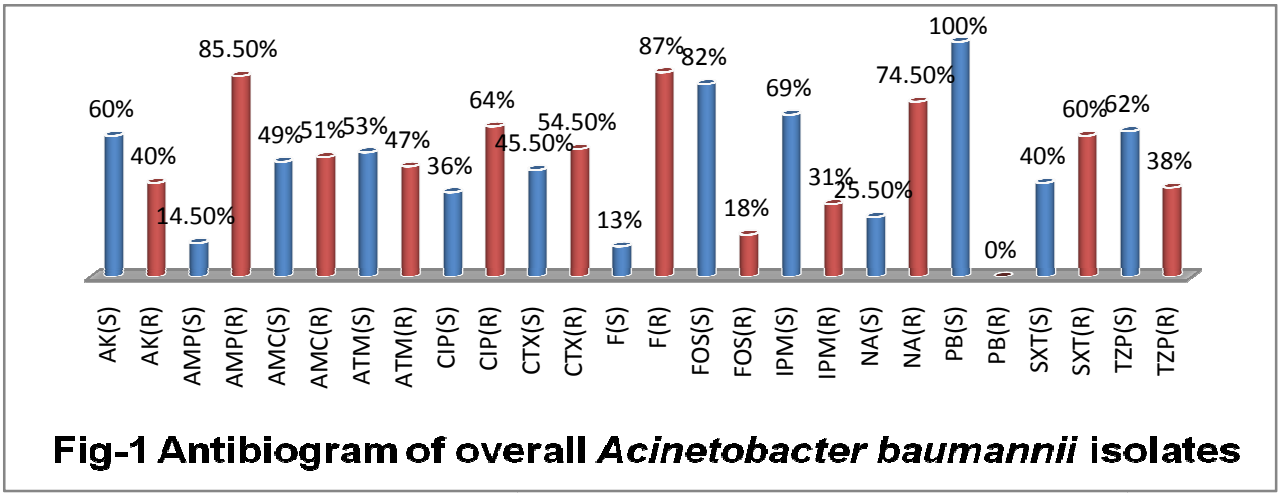

Fig-1 Antibiogram of overall *Acinetobacter baumannii* isolates

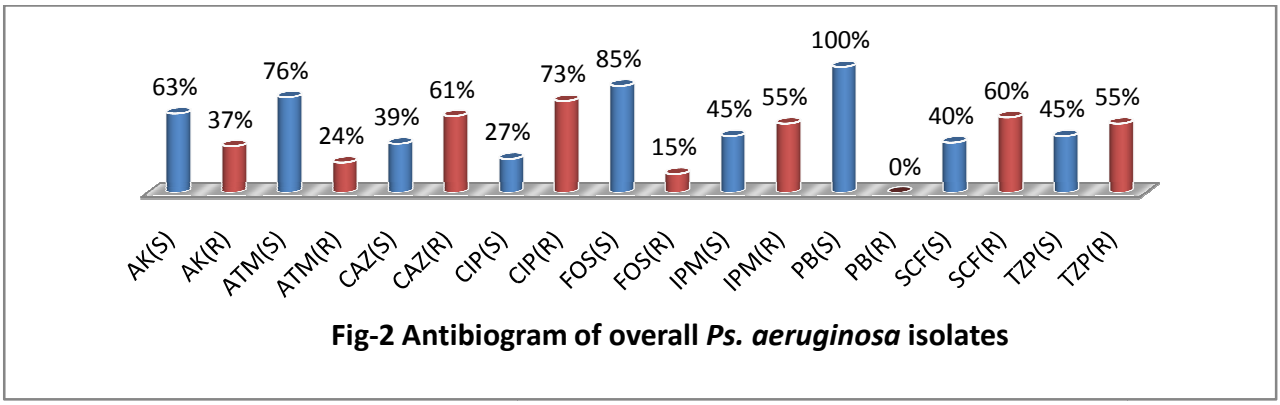

Fig-2 Antibiogram of overall *Ps. aeruginosa* isolates

Key: (S)= Sensitive, (R)= Resistant

The expansions of all the antibiotics abbreviations are given in materials and methods section. A total of 44% of *Ps.* aeruginosa isolates were found to be multidrug resistant while 48.5% of all *Acinetobacter baumannii* isolates were also found multidrug resistant. Results of antibiogram and bioactivity of fosfomycin against the MDR isolates are presented in table.1.

TABLE: 1 ANTIBIOTIC SENSITIVITY PATTERNS OF MULTI-DRUG RESISTANT (MDR) *ACINETOBACTER BAUMANNII* AND *PS. AERUGINOSA*.

Antibiotics	*Acinetobacter baumannii* (MDR) 48.5% Percentage (%) of resistant strains to individual drug	*Ps. aeruginosa* (MDR) 44% Percentage (%) of resistant strains to individual drug
Amikacin	33	38
Ampicillin	0	-
Amoxicillin-clavulanic acid	11	-
Aztreonam	14	49
Cefotaxime	8	-
Ceftazidime	-	2
Ciprofloxacin	11	1
Fosfomycin	73	70
Imipenem	42	6
Nalidixic acid	8	-
Nitrofurantoin	5.5	-
Polymyxin B	100	100
Cefoperazone-sulbactam	-	2
Pipracillin-tazobactam	28	8
Trimethoprim/sulfamethoxazole	18	-

DISCUSSION

The present study was conducted to evaluate the potential of the older antibiotic (fosfomycin) for the treatment of UTIs, especially against MDR-GNB pathogens. Prevalence of MDR *Acinetobacter baumannii (48.5%)* and *Ps. aeruginosa* (44%) in patients of UTI was observed. These findings are on higher side than the previous reports regarding prevalence of MDR-GNB in Karachi (14) which points to an increase in the drug resistance.

The current study demonstrates the resistance of *Acinetobacter baumannii* and *Ps aeruginosa* to therapeutically important antibiotics. Interestingly, higher frequency of resistance was noticed in *Acinetobacter baumannii* as compared to *Ps. aeruginosa.* Compared to other antibiotics, Polymyxin B (100%), Fosfomycin (82%), Imipenem (69%), Pipracillin-tazobactam (62%) and Amikacin (60%) were found to be effective (bioactive) against all the isolates of *Acinetobacter baumannii* (fig.1). For MDR *Acinetobacter baumannii,* many antibiotics showed a decrease in susceptibility (< 50% sensitive) but interestingly, Polymyxin B and Fosfomycin were found bioactive (100%) and (73%) of these isolates, respectively (table 1). For all the isolates of *Ps. aeruginosa,* most effective antibiotics included*:* Polymyxin B (100%), Aztreonam (76%), Amikacin (63%) and Fosfomycin (85%) respectively (fig.2). For MDR *Ps. aeruginosa,* all antibiotics showed decreased bioactivity except Polymyxin B (100%) and Fosfomycin (70%) which showed more bioactivity against these isolates. Very important antibiotics like Amikacin, Amoxicillin-clavulanic acid, Cefotaxime, Ciprofloxacin, Imipenem, Cefoperazone-sulbactam and Pipracillin-tazobactam were found to show decreased bioactivity against both MDR-GNB types (with some variations).

However, Polymyxin B has come out to be the most effective against both MDR-GNB type of the isolated strains but this antibiotic leaves behind many side-effects as well. So, Fosfomycin should be the better choice for MDR-GNB and it has another merit (can be used orally as well as intravenously). In fact, Fosfomycin has emerged as a promising treatment option. It has rare adverse reactions which may develop in 1-8% of all the patients, the most common ones being diarrhea, nausea, vomiting, skin rashes, heartburn, vaginitis, headache, chills and asthenia (15). Fosfomycin has a low molecular weight with a relatively long half‑life post intake (mean half life-SD, 5.7-2.8 h) and therefore, penetrates various tissues with ease, achieving the minimum inhibitory concentrations needed to inhibit the growth of most of the pathogens (16). Resistance emergence rate is low and most frequently acquired by chromosomal mutations that do not spread easily (17).

In previous studies, around 10% of strains of *Ps. aeruginosa* were found resistant to fosfomycin (18). Current studies on *Ps. aeruginosa* isolates demonstrated similar rates of resistance to fosfomycin *in vitro* (19), and this study correlates with these findings. Polymyxin B and colistin also demonstrated good results against *Ps. aeruginosa* and *Acinetobacter baumannii.* Keeping this in view, further trials can be done for combined therapy (Fosfomycin with colistin or Polymyxin B). Further studies to be based on molecular mode of action of fosfomycin are needed. Fosfomycin appears to be picked as an excellent therapeutic choice for the treatment of both MDR-GNB pathogen types.

CONCLUSIONS

Fosfomycin is a bactericidal agent that encounters a low level of resistance as compared to other antibiotics. Antimicrobial activity of fosfomycin, especially against MDR uropathogens, makes it an effective and safe drug for the treatment of UTIs caused by Gram‑negative bacteria, especially against the MDR *Acinetobacter baumannii* and *Ps. aeruginosa* for which previous antibiotics have failed to treat the infections.

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HIGH INCIDENCE OF MULTIDRUG-RESISTANT STRAINS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES IN BENIN-CITY, NIGERIA

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RUNNING TITLE: MRSA SHOWS HIGH RATE OF MULTIDRUG RESISTANCE FROM CLINICAL ISOLATES

ABSTRACT
Infections of methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming an increasingly concerning clinical problem. The aim of this study was to assess the development of multidrug resistant strains of MRSA from clinical samples andpossibilities for reducing resistance. This study included a total of seventy-five (75) isolates comprising fifteen (15) each collected from ear, urine, cervix, blood and wounds. An agar disc diffusion test was used to measure the effects of antimicrobial agents against the bacteria isolates following standardized guidelines. Out of a total of 75 clinical isolates of *S. aureus*collected, 43 (57.3%) were resistant to methicillin with isolates obtained from ear infections showing the highest resistance pattern of 14.7% while the least was from urine sample with incidence of 5.3%. From the 43 isolates that showed resistance to methicillin, 36 (83.7%) were multidrug resistant to various classes of antibiotics tested.MRSA showed an increasing trend of antimicrobial resistance and therefore calls for periodic surveillance of nosocomial infections due to S. aureus and other important bacterial pathogens.
Key Words: methicillin-resistant *Staphylococcusaureus*, MRSA, multidrug resistance, MDR

L'INCIDENCE ELEVEE DE SOUCHES MULTIRESISTANTES DE STAPHYLOCOCCUS AUREUS RESISTANT A LA METHICILLINE ISOLES DES ECHANTILLONS CLINIQUES A BENIN - CITY, NIGERIA.

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TITRE COURANT: MRSA MONTRE UN TAUX ELEVE DE MULTIRESISTANCE DE ISOLATS CLINIQUES.

RESUME
Les infections *Staphylococcus aureus* résistant a la methicilline (MRSA) sont de plus en plus devenir une préoccupation clinique. Le but de cette recherche était d'évaluer le développement des souches multirésistantes de MRSA des échantillons clinique et les possibilités de réduire la résistance. Cette recherche a compris un total de soixante quinze (75) isolats comprenant quinze (15), chaque collecté de l'oreille, de l'urine, du col de l'utérus, du sang et des plaies. Un test de diffusion sur disque d'agar a été employé pour mesurer les effets des agents antimicrobiens contre les isolats bactériens selon les directives normalisées. Sur un total de 75 isolats cliniques de *S. aureus* collectés, 43 (57,3%) étaient résistants a methicilline avec des isolats obtenus des infections de l'oreille montrant le profil de résistance le plus élevé de 14,7% tandis que le moins était de l'échantillon d'urine avec une incidence 5,3%. Des 43 isolats qui ont montré la résistance amethicilline, 36(83,7%) étaient multirésistants aux diverses classes d'antibiotiques testés. MRSA a montré une tendance de plus en plus de la résistance aux antimicrobiens et demande par conséquent la surveillance périodique des infections nosomicales due a*S. aureus* et d'autres agents pathogènes bactériens importants.

Mots - clés: *Staphylococcus aureus* résistant a la methicilline, MRSA, Multrésistance , MDR.

INTRODUCTION

Among all the antibiotic resistance achieved by *Staphylococcus aureus*, two most remarkable ones are methicillin and vancomycin resistance. The methicillin resistance was achieved by interspecies transfer of*mecA* gene from an ancestral *Staphylococcus* species to *S. aureus* mediated by a unique staphylococcal mobile genetic element. Vancomycin resistance was achieved by horizontal transfer of a plasmid-born*vanA*-gene transposon from vancomycin-resistant Enterococcus to *S. aureus* across the genus barrier. Practicallyall *S. aureus* isolates were methicillin susceptible until 1961, when Jevons found three MRSA strains among 5440 clinical *S. aureus* strains in England (1).

MRSA is born when methicillin-susceptible*S. aureus* (MSSA) has acquired the methicillin-resistance gene *mecA* by horizontal gene transfer mediated by a mobile genetic element staphylococcal cassette chromosome (SCC) (2).*S. aureus* colonizes various parts of healthyhumans such as the nares, skin, vagina andgastrointestinal tract (3). Its prevalence have beenseverally reported in healthy populations; 36% and40% were reported in women's urine in two centresin Nigeria, 17.3% in nasal cavity of Turkish children,36% in nares of Japanese adults and 32.4% in nasalcavity of adults in USA (4-7). Colonizing strains mayserve as endogenous reservoirs for overt clinicalinfections or may spread to other patients.

S. aureus have become resistant to variousantimicrobial agents including the commonly usedpenicillin-related antibiotics. Multi-drug resistantstrains of *S. aureus* have been reported withincreasing frequency worldwide. Strains that are resistant to methicillin were found to exhibit varyingresistance to lincosamides, macrolides,aminoglycosides, fluoroquinolones, or combinationsof these antibiotics (8, 9). Vancomycin – a glycopeptidewhich was initially very effective in the treatment ofMethicillin resistant *S. aureus* (MRSA) infections isrecently being witnessed with intermediate resistancefrom MRSA strains (10).
In this study we hypothesize that the constant use of antibioticsin the hospitals could result in high amount of multidrug-resistant strains of MRSA

STUDY AREA

Clinical isolates of *Staphylococcus aureus*from patients' samples were obtained from the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria within a 3-month period from July – September, 2007.

SAMPLE PROCESSING

A total of seventy-five (75) isolates were collected comprising fifteen (15) each from ear, urine, cervix, blood and wounds. Identification and confirmation of isolates were conducted based on growth and fermentation on mannitol salt agar (MSA), colonial morphology, Gram staining and positive results to both catalase and coagulase tests (11).

SUSCEPTIBILITY TEST

The susceptibility of isolates to oxacillin using the E-test strips (AB Biodisk) was carried out bythe disk diffusion method (12).Also tested were commercial antibiotics; amoxicillin 30µg, ampicillin/cloxacillin 30µg, ceftriaxone 25µg, cefuroxime 20µg, ciprofloxacin 10µg, pefloxacin10µg, gentamicin 10µg, streptomycin 30µg, erythromycin 10µg and sulphamethoxazole/trimethoprim 30µg. Methicillin discs 5µg (Oxoid, England) was applied onto the Petri dish alongside with other tested antibiotics. A breakpoint of ≥2 µg was used to define resistance to oxacillin, zone diameter less than 14mm for methicillin while multidrug resistance was defined as strains resistant to three or more drug classes other than beta-lactams.

RESULTS

Out of a total of seventy-five (75) clinical isolates of *S. aureus*collected in this study, 43 representing 57.3% were resistant to methicillin. The distribution according to the site of isolation is shown in **Table 1**. Isolates obtained from ear infections showed the highest resistance pattern of 14.7% while the least was from urine sample with incidence of 5.3%. **Figure 1** shows the resistance pattern of the various isolated to the antibiotic classes with multidrug resistance defined as resistance to three or more classes of antibiotics other than the beta-lactams. From the 43 isolates that showed resistance to methicillin, 36 (83.7%) were multidrug resistant.

TABLE 1: PREVALENCE OF MRSA FROM DIFFERENT CLINICAL SAMPLE

Site	No. of *S. aureus* isolates	No. resistant to methicillin/ oxacillin	Percentage(%)
Ear	15	11	14.7
Cervix	15	10	13.3
Urine	15	4	5.3
Blood	15	10	13.3
Wound	15	8	10.7
Total	75	43	57.3

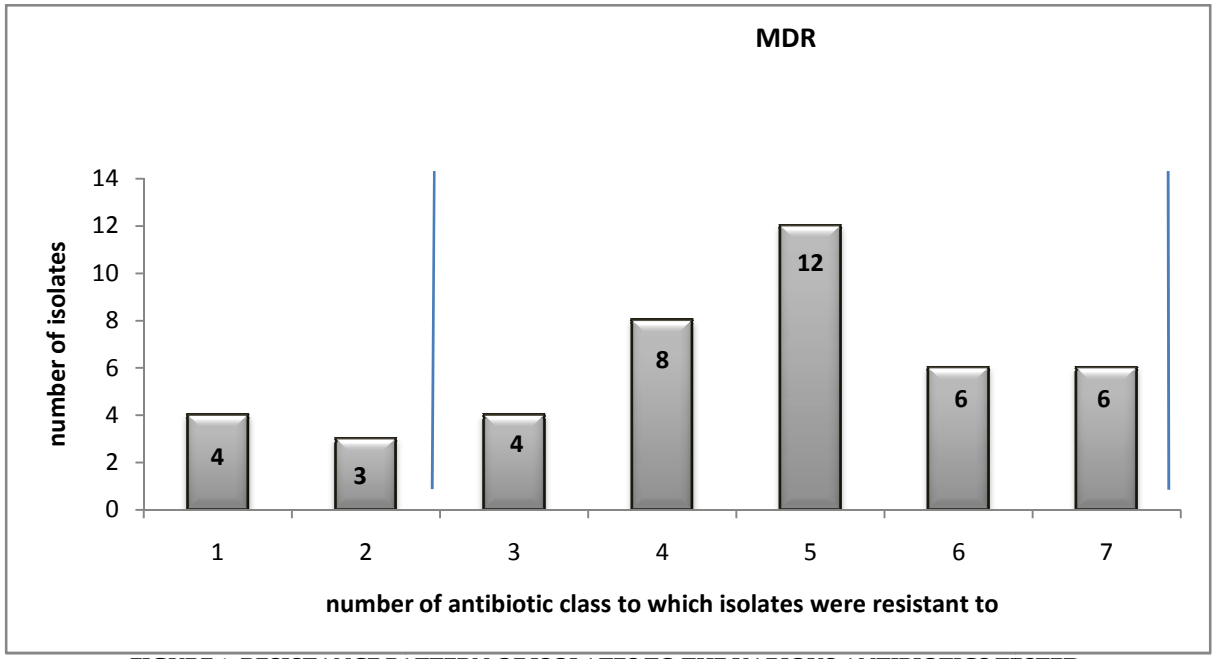

FIGURE 1: RESISTANCE PATTERN OF ISOLATES TO THE VARIOUS ANTIBIOTICS TESTED.
(3-7) – multidrug resistance, N = 36

DISCUSSION

Infections caused by multi-resistant strains of *S. aureus* are identified by their resistance to methicillin. MRSA by definition is any strain of *S. aureus* that has developed resistance to beta-lactam antibiotics which include beta-lactam stable formulations such as methicillin, oxacillin, flucloxacillin, nafcillin and cephalosporin. These MRSA strains are often responsible for several difficult to treat infections in humans (13). Knowledge of epidemiology of bacterial infections is very important for appropriate decision-making in the treatment of infections, such as septicemia, wound infections, and postsurgical infections. Iroha*et al*. (14) investigated cases among neonates in Lagos, Nigeria, in a prospective study. The incidence was 18 per 1,000 live births. *S. aureus* (37.4%) was the predominant etiologic pathogen among the bacteria. Another study investigated the bacteriology of nonsurgical wound infections in Ibadan. *S. aureus* (38%) was the predominant pathogen, followed by gram-negative bacteria (7 to 19% each). High rates of antibiotic resistance were recorded among these isolates (15).

The prevalence of MRSA in our study was higher (57.3%) compared to those in previous studies in South-westernNigeria. However, it should be considered that the presence of the*mecA* gene, which is the "gold standard" for determining methicillin-resistance, was not investigated in some of these studies.

A recent multicentre study in South-western Nigeriaconfirmed resistance to methicillin by the detection of the*mecA* gene by PCR and reported a lower prevalence rate of1.4% (16). Obasuyi also used molecular techniques and reported the prevalence of 11% MRSA from clinical samples with two PFGE types (17).

Despite the high MRSA rate in our study, it is evidentthat multidrug resistant strains occurred frequently in South-western Nigeria. However, the MRSA isolates were predominantly associated with infections (57.3%), since all isolates were from clinicalsamples as also observed in other studies elsewhere (27). Nevertheless, the prevalence of MRSA was higher in this study than that Taiwo*et al*. (18) which showed the rate of 29%.

A major problem in the treatment of *S. aureus*infections is the multidrug resistance pattern of thepathogen to a number of antibiotics. In the last fewyears, understanding of the genetic basis formethicillin resistance has advanced significantly.Multi-resistant MRSA have been reported to be relatively high in African countries including Morocco, Kenya and Cameroun (19). A majority of the MRSA in our study showed multidrug resistance (83.7%). The misuse and misapplication of many antimicrobial agents in many parts of Nigeria may contribute to the high MRSA rate in this community. This poses a significant difficulty in antimicrobial agent choice for patients with this variety of infections which calls for periodic

surveillance of nosocomial infections due to *S. aureus* and other important bacterial pathogen in order to minimize microbial transmission.

CONCLUSIONEffort must therefore be put in place at control measures that should include a renewed awareness, isolation of MRSA infected patients in hospitals and multidrug resistance surveillance and enforcement of empiric use of antimicrobial agents to stem the tide of MRSA

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MICROBIOLOGICAL EVALUATION OF THE POTENCIES OF BRANDS OF FOUR PARENTERAL ANTIBIOTIC PREPARATIONS USED IN THE TREATMENT OF URINARY TRACT INFECTIONS

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RUNNING TITLE: POTENCIES OF PARENTERAL ANTIBIOTIC PREPARATIONS

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ABSTRACT

Urinary tract infection (UTI) is a common disease and sometimes life threatening if not properly treated. In Nigeria, aside adulteration and counterfeiting of antibiotics, potency of antibiotics can also be altered by factors like production errors and storage condition at the Pharmacy stores. This study investigated the potencies of selected brands of four common parenteral antibiotic preparations, in Nigerian drug markets against uropathogens isolated from patients with recurrent UTI.

Ten selected clinical bacterial isolates from patients with recurrent UTI were collected from the Microbiology unit of the University College Hospital, Ibadan and authenticated by standard bacteriological methods. The isolates were subjected to susceptibility test against eight standard antibiotics by disc diffusion method. The selected brands of the four parenteral antibiotic preparations used in this study includes: Ciprofloxacin (Emason® and Uniflox®); Ceftriaxone (Rocephin® and Cefin®); Aminoglycoside (Pe-genta® and Philo-genta®) and Aminopenicillin/inhibitor (Augmentin® and Amoxiclav®). Efficacies of the parenteral antibiotic preparations against the isolates were determined by Minimum Inhibitory Concentrations (MICs) using broth-dilution method.

Antibiotic susceptibility test using standard antibiotic discs showed that all (100%) the bacterial isolates were multidrug resistant (MDR), being resistant to two or more classes of antibiotics. Aside *E. coli* (E1) that was susceptible to the two brands of gentamicin preparations at the Clinical Laboratory Standard Institute (CLSI) susceptibility breakpoint (≤ 4 µg/mL), all the other isolates showed resistance to the four parenteral antibiotic preparations and were only susceptible at higher concentrations (> 2 folds) above the CLSI resistance breakpoints for the different antibiotic preparations. The brands of the parenteral antibiotic preparations used in this study have low potency which varies with different bacterial strains involved.

L'EVALUATION MICROBIOLOGIQUE DES PUISSANCES DES MARQUES DE QUATRES PREPARATIONS ANTIBIOTIQUES PARENTERALES UTILISEES DANS LE TRAITEMENT DE L'INFECTION DES VOIES URINAIRES.

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RESUME

L'infection des voies urinaires (UTI) est une maladie commune et parfois met la vie en danger si pas correctement traitée. Au Nigeria, a part de la falsification et la contrefaçon des antibiotiques, la puissance des antibiotiques peut également être modifiée par des facteurs tels que les erreurs de production et les conditions de stockage dans les magasins de la pharmacie. Cette étude a examiné les puissances des marques sélectionnées de quatre préparations antibiotiques parentérales courantes, aux marchés nigérians de la drogue, contre les uropathogenes isolées des patients avec infection urinaire récidivante.

Dix isolats bactériens sélectionnés des patients avec infection urinaire(UTI) récidivante ont été recueillies de l'unité microbiologie de l'hôpital Universitaire, Ibadan et authentifie par des méthodes bactériologiques classiques. Les isolats ont été soumis à des tests de sensibilité contre huit antibiotiques Standards par la méthode de diffusion sur disques. Les marques sélectionnées de quatre préparations antibiotiques parentérales utilisées dans cette étude comprennent : Ciprofloxacine (Emason® et Uniflox®) ; Ceftriaxone (Rocephine® et Cefin®) ; Aminoside (Pe - genta® et Philo – genta®) ; et Aminopenicilline/inhibiteur (Augmentin® et Amoxiclav®). L'efficace des préparations antibiotiques parentérales contre les isolats ont été déterminés par concentrations minimales inhibitrices(MICs) en utilisant la méthode du bouillon – dilution.

Le test de sensibilité aux antibiotiques en utilisant les disques antibiotiques standard a montré que tous (100%) les isolats bactériens étaient multi résistants (MDR), étant résistant aux deux ou plusieurs classes d'antibiotiques. A part de *E.coli* (E1) qui était sensible aux deux marques de préparations gentamicine a l'Institut de Laboratoire Clinique Standard. Le point d'arrêt de la sensibilité (≤ 4µg/ml) tous les autres isolats ont montré résistance aux quatre préparations antibiotiques parentérales et étaient seulement sensibles a des concentrations plus élevées (> 2 plis) au- dessus des points d'arrêt de résistance CLSI pour les préparations antibiotiques différentes. Les marques des préparations antibiotiques parentérales utilisées dans cette étude ont une faible puissance qui varie avec les souches bactériennes différentes impliquées.

INTRODUCTION

Urinary system is classified into Lower (urethra, bladder) and upper (kidneys, renal pelvis) urinary tract, and the infection of the urinary system is when any one or all parts of the urinary system are infected by microorganisms, mostly bacteria, with significant bacteriuria in the presence of symptoms (1). Urinary tract infection (UTI) is described based on the location of the infection in the urinary system as either lower urinary tract infection (LUTI) or upper urinary tract infection (UUTI). Usually, someone is declared to have urinary tract infection (UTI) only if repeated viable count of the microorganisms in the urine samples of the person concern is $\geq 10^5$ $CFUmL^{-1}$ of the urine (1).

Urinary tract infection caused by bacterial isolates is of global concern and the major setback in its treatment using antibiotic is the emergent of highly resistant bacterial strains (2, 3). Common bacterial isolates usually involved in UTI, either uncomplicated or complicated, are *Escherichia coli*, which is the most common, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Streptococcus saprophyticus*, species of *Enterobacter*, *Serratia*, *Acinetobacter*, and *Pseudomonas aeruginosa* (3, 4, 5, 6). Aside the first line antibiotics such as trimethoprim-sulphamethoxazole, penicillins and nitrofurantoin, which are now obsolete in the treatment of UTI due to high level of resistance (7, 8), three major classes of antibiotics commonly used as second line treatment of UTI are the fluoroquinolones, aminoglycosides and some beta-lactams (9). Resistance to these classes of antibiotics have been reported world-wide including Nigeria by several authors in their laboratory screening of clinical uropathogens and has contributed immensely to the problems encountered by physicians in the treatment of UTI (10, 11). Taken of antibiotics for too short a time, at an inadequate concentration, or for the wrong treatment, all constitute irrational use of antibiotics and thus contribute to the development of resistance among clinical bacterial isolates (2, 12, 13). Another issue of major concern is the use of antibiotics as growth promoters in food-producing animals and poultry flocks (14). Such practices have contributed to the rise in the level of antibiotic resistance and dissemination of resistance traits among microorganisms which in turns can be transmitted from animals to humans (15, 16, 17, 18). This study however, evaluated the potencies of two brands each, of fluoroquinolone (ciprofloxacin), aminoglycoside (gentamicin), cephalosporin (ceftriaxone) and amino-penicillin/inhibitor combination (amoxicillin-clavulanic acid) obtained from local pharmaceutical market in Ibadan against selected multidrug resistant uropathogenic bacterial isolates from patients diagnosed with recurrent UTI.

MATERIALS AND METHODS

COLLECTION OF UROPATHOGENIC MICROORGANISMS

Ten bacteria isolated from ten patients with recurrent urinary tract infection were collected from the Microbiology and Parasitology Department of the University College Hospital (UCH), Ibadan on sterile nutrient agar slants and were authenticated by standard bacteriological techniques. Pure cultures of the authenticated bacterial isolates were sub-cultured on fresh nutrient agar slants and stored in the refrigerator at 4°C. The bacterial isolates include two strains each of *Pseudomonas aeruginosa* (Ps1 & Ps2), *Staphylococcus aureus* (S1 & S2), *Proteus spp* (Pr1 & Pr2), *Klebsiella spp* (K1 & K2), and *Escherichia coli* (E1 & E2).

SUSCEPTIBILITY TEST USING STANDARD ANTIBIOTIC DISCS

Antibiotic sensitivity testing was carried out by the disc diffusion method on Mueller Hinton agar using the following selected standard antibiotic discs: amoxicillin-Clavulanic acid (AMC)- 20/10 µg, cefuroxime (CRX)- 30 µg, cefixime (CXM)- 5 µg, ceftazidime (CAZ)- 30 µg, gentamicin (GEN)- 10 µg, ofloxacin (OFX) - 5 µg, ciprofloxacin (CPR)- 5 µg, nitrofurantoin (NIT)- 300 µg. Pure colonies of each test organism were inoculated into tubes containing 10ml of sterile nutrient broth and incubated at 37°C

for 24hrs. Thereafter, a 10^{-2} dilution of the stock bacterial suspension was made and a sterile cotton swab was used to evenly inoculate the entire dried surface of previously prepared and set Mueller Hinton agar plates. The selected standard antibiotic discs were firmly placed on the set agar plates using sterilized forceps. The agar plates were left for about 30 minutes for effective diffusion of the antibiotics before being incubated at 37°C for 24 hours. The diameters of the zone of growth inhibition were measured to the nearest millimetre and the results interpreted as sensitive or resistant based on the Clinical and Laboratory Standard Institute (CLSI) 2011 guideline (19).

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION OF THE SELECTED PARENTERAL ANTIBIOTIC PREPARATIONS

The parenteral antibiotic preparations used in this study include: Aminoglycoside (gentamicin inj: Philo-genta® and Pe-genta®), Floroquinolone (ciprofloxacin inj: Uniflox® and Emason®), Cephalosporins (ceftriaxone inj: Rocephin® and Cefin®) and Amino-penicillin (amoxicillin-clavulanic acid: Augmentin® and Amoxiclav®). These antibiotics were bought from reputable pharmaceutical stores located within Ibadan.

Stock preparations of the antibiotic under investigation were diluted serially with nutrient broth such that the concentration was halved in each container in a series to give ten concentrations. This was done by adding 5ml of the solution of the test antibiotic aseptically to 5ml of double strength medium and mixed by shaking. With a fresh pipette, 5ml of the mixture was transferred aseptically to the second tube which contains 5ml single strength medium. This was also mixed by shaking and the procedure repeated until the last tube giving the following concentrations: 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5µg/mL. Thereafter, 0.1ml of a 10^{-2} dilution of the overnight broth culture was added to each tube. A tube containing sterile broth only served as a control. The tubes were incubated at 37°C for 24 hours and the minimum inhibitory concentrations (MICs) of the different parenteral antibiotics preparations were determined and result tabulated.

RESULTS

The microbiological characterisation of the isolates confirmed their identities to be: *Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table 1). The results of the antibiotic susceptibility test using standard antibiotic discs shows that 90% of them were resistant to cefixime, cefuroxime, and ceftazidime. Percentage resistance to nitrofuratoin and ofloxacin was 70%, Gentamicin was 60% while for ciprofloxacin it was 20%. Seven (70%) of the isolates showed resistance to three classes of antibiotics while K2 and Pr2 each showed resistance to five and four classes respectively (Table 1). The results of the susceptibility test using different concentrations of the parenteral antibiotics are shown in table 2. All the isolates showed resistance to the four parenteral antibiotic preparations giving MIC values greater than the Clinical Laboratory Standard Institute (CLSI) guideline Resistance breakpoints for each antibiotic except against *E. coli* (E1) that was susceptible to the two brands of gentamicin preparations at 4µg/mL which was within CLSI susceptibility breakpoint of ≤4µg/mL. However, variations in potency exist among some of the different brands of antibiotics used in this study against the clinical isolates. The MIC of Uniflox® brand (4µg/mL) of ciprofloxacin against *Pseudomonas earuginosa* -Ps1 was reduced fourfold compared to that of Emason® brand (16µg/mL) as well as between Pe-gena® brand (64µg/mL) of Gentamicin and Philo-genta® brand (256µg/mL) against Ps1. Also, MIC of Uniflox® brand (64µg/mL) against *Klebsiella pneumoniae* K1 was increased fourfold compared to the Emason® brand (16µg/mL). This also occurred between Augmentin® brand (64µg/mL) of amoxicillin-clavulanic acid and Amoxiclav® brand (256µg/mL) against *Staphylococcus aureus* S1.

TABLE 1: ANTIBIOTIC RESISTANCE PROFILES OF THE UROPATHOGENS TO THE STANDARD ANTIBIOTIC DISCS

Isolate ID	Antibiotic Resistance Profile	No. of Antibiotic classes
E1	AMC, CRX, CXM, CAZ	2
E2	GEN,AMC,CRX, CXM, CAZ	3
S1	OFX, CPR, GEN,AMC	3
S2	AMC, NIT, CRX, CXM, CAZ	3
Ps1	AMC, NIT, CRX, CXM	3
Ps2	AMC, NIT, CRX, CXM,	3
Pr1	AMC, NIT, CRX, CXM,CAZ	3
Pr2	AMC, NIT, GEN, CRX, CXM, CAZ,	4
K1	AMC, GEN, CRX, CXM, CAZ	3
K2	AMC, NIT, GEN, CRX, CXM, CAZ, OFX	5

ID = Identity; E1& E2 = *Escherichia coli*, S1& S2 = *Staphylococcus aureus;* Ps1& Ps2 = *Pseudomonas aeruginosa*; Pr1& Pr2 = *Proteus mirabilis*; K1& K2 = *Klebsiella pneumoniae*, AMC = amoxicillin-Clavulanic acid; CRX = cefuroxime; CXM = cefixime; CAZ = ceftazidime; GEN = gentamicin; OFX = ofloxacin; CPR = ciprofloxacin; NIT = nitrofurantoin.

TABLE 2: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICs) OF PARENTERAL ANTIBIOTIC PREPARATIONS BY BROTH-DILUTION

Isolate ID	Brands of Ciprofloxacin CLSI BP = ≥ 4µg/mL		Brands of Ceftriaxone CLSI BP = ≥ 4µg/mL		Brands of Amoxicillin/clavulanic acid CLSI BP = ≥ 32/16µg/mL		Brands of Gentamicin CLSI BP = ≥ 16µg/mL	
	Emason® Conc. µg/mL	Uniflox® Conc. µg/mL	Rocephin® Conc. µg/mL	Cefin® Conc. µg/mL	Augmentin® Conc. µg/mL	Amoxiclav® Conc. µg/mL	Philo-genta® Conc. µg/mL	Pe-gena® Conc. µg/mL
E1	4	4	> 256	> 256	256	256	4	4
E2	128	128	> 256	> 256	256	256	256	> 256
PS1	16	4	64	64	> 256	> 256	256	64
PS2	16	16	64	64	> 256	> 256	64	64
Pr1	8	8	64	64	128	256	64	64
Pr2	128	128	256	256	256	256	> 256	> 256
K1	16	64	> 256	> 256	256	256	> 256	> 256
K2	128	128	> 256	> 256	256	256	> 256	> 256
S1	16	16	64	64	64	256	> 256	> 256
S2	128	128	128	128	> 256	> 256	> 256	> 256

ID = Identity; E1& E2 = *Escherichia coli*, S1& S2 = *Staphylococcus aureus;* Ps1& Ps2 = *Pseudomonas aeruginosa*; Pr1& Pr2 = *Proteus mirabilis*; K1& K2 = *Klebsiella pneumoniae*, CLSI BP = Clinical Laboratory Standard Institute Breakpoint.

DISCUSSION

Antibiotic resistance is a growing problem in the treatment of infections which has led to the narrowing of antibiotic options needed to treat

bacterial infections thus making this problem a global concern and thus requiring global solution (2). Although the natural phenomenon by which resistance emerges is accelerated and amplified by a variety of factors, the most important cause is the inappropriate use of antimicrobial agents (15). With reference to the Clinical and Laboratory Standard Institute (CLSI), 2011 guidelines (19), the results obtained from the sensitivity test using standard antibiotic disc showed that all the bacterial isolates used in this study are multidrug resistant strains. This confirms the earlier report by Dada and Muili (2010) of wide spread of resistant uropathogens among patients with UTI in Ibadan, Southwest Nigeria (20).

Variation observed in the potency of some brands of the parenteral antibiotics used in this study against the same organism could be as a result of differences in the formulation methods and excipients used which may affect the penetration of the antibiotics into the bacteria cell. Also, it could be as a result of the condition of storage which could affect product potency, efficacy and overall quality. The 80% susceptibility of the isolates to the standard ciprofloxacin disc and the 100% resistance of the isolates to the two brands of ciprofloxacin injection used in this study suggest that the potency and efficacy of the parenteral antibiotic preparations have been altered either during formulation or on shelf. Therefore, stringent regulations must be put in place to ensure that formulation and storage of antibiotics is done appropriately according to standard. In the preparation of parenteral antibiotics, care must be taken to ensure that current good manufacturing practices (CGMPs) are followed so as to produce drugs of high standard that will be physically and chemically stable throughout their intended shelf life. Packaging of drugs must maintain the products' integrity throughout the shelf life and during administration. Agencies involved in the regulation of sales of drugs should make sure that pharmacies and chemists display and store their drug items under correct storage conditions without any compromise as poorly stored drugs can loss their efficacy and potency, especially antibiotics that require strict storage conditions.

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