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PREVALENCE AND IMMUNE STATUS OF HIV/HBV CO-INFECTED PREGNANT WOMEN

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ABSTRACT
HIV/HBV co-infection places patients at high risk of liver-related morbidity and mortality and the interaction of the two viruses can further complicate treatment. Pregnant women are especially at high risk for increased morbidity and mortality due to infection, and information about HIV/HBV co-infection in pregnant women is scanty. This study examined the occurrence of HBV antibodies in HIV-1 positive pregnant women and the relationship to Ante-retroviral therapy (ART) and other demographic characteristics. Blood samples were collected from 135 HIV pregnant positive women who were either on ART or Not, from May – June, 2008 at the Jos University Teaching Hospital (JUTH) and the Plateau State Specialist Hospital (PSSH). Presence of hepatitis B surface (HBsAg) antigen in serum was determined using Antec strips (Antec diagnostics UK) and their immunologic status were determined by measuring the CD_4^+ counts using SL_3 cyFlow counter (Partec, Germany) . Sixteen 16 (11.8%) of the women examined were seropositive for Hepatitis B virus. Occupation was significantly associated with the prevalence of the hepatitis co-infection in the population examined (8.8% of house wives and 5.5% of business women had co-infection, $p<0.05$). The immunologic status (CD_4^+ of most of the HIV/HBV co-infected pregnant women (81.5%) was low (below 300 cells/mm^3) although all were on Anti retroviral therapy. The 11.8% prevalence rate reported in this study confirms the endemicity of HBV /HIV co-infection in Nigeria, and this supports the calls for screening for Hepatitis B as a routine in antenatal care.

Keywords: HIV, Hepatitis, Co-infection, CD_4, Pregnant women

INTRODUCTION

Of the 33.3 million people currently living with HIV/AIDS, 68% reside in sub-Saharan Africa ([1]). HIV/AIDS weakens a person's ability to fight infections; HIV-infected people are three to six times more likely to develop a chronic or long-term hepatitis B infection because of their weakened immune systems than individuals without HIV (2). Approximately 5%–10% of HIV-infected persons also have chronic HBV infection, defined as testing positive for HBsAg for more than 6 months.

An estimated 400 million people are infected with Hepatitis B virus (HBV) with the majority of cases occurring in regions of Asia and Africa where the virus is endemic. In these continents up to 70% of adults show serologic evidence of current or prior infection and 8 to 15% have chronic HBV infection (2). Hepatitis B virus (HBV) is the most common cause of serious liver infection; it causes transient and chronic infections of the liver. Clinical presentation of Hepatitis B ranges from subclinical to symptomatic hepatitis and, in rare instances, fulminant hepatitis ([3]). HBV and HIV have common characteristics such as transmission routes (vertical, parenteral, and sexual), and the propensity to establish chronic infections which are often difficult to treat with currently available anti-viral agents ([4],5,6). HIV/HBV co- infection is associated with increased liver related morbidity and mortality. The pathogenesis and clinical manifestations of both are due to the interaction of the virus and the host immune system ([7]). The immune system attacks hepatitis B virus and causes liver injury. Activated CD4+ and CD8+ lymphocytes recognize various HBV-derived peptides located on the surface of the hepatocytes, and an immunologic reaction occurs. Impaired immune reactions, (such as cytokine release, antibody production) or relatively tolerant immune status results in chronic hepatitis (8). The immune suppression caused by HIV infection accelerates the course of liver disease caused by chronic HBV infection and results in increased mortality compared to HIV monoinfected patients (9).

Interferon does not appear to adversely affect the embryo or fetus. However, the data is limited, and the potential benefits of interferon use during pregnancy should clearly outweigh possible hazards (10, 11). Lamivudine used in combination with zidovudine caused pronounced and sustained increases in CD4-cell counts and reductions in HIV-1 viral load in four separate studies, done in both previously antiretroviral untreated and pretreated HIV-1-positive patient Interestingly, lamivudine is also used to treat hepatitis B infections (12, 13). HBV replication has been proven to be inhibited by

lamivudine in 86.4% (95% CI, 75.7–93.6) of HIV-HBV–coinfected patients (14, 15). Chronic HBV does not substantially alter the progression of HIV infection and does not influence HIV suppression or CD4+ cell responses following ART initiation (16,17). Initial data do not suggest that Lamivudine is teratogenic (18). Lamivudine has been used in the latter half of pregnancy in attempt to prevent perinatal transmission of hepatitis B virus infection with mixed success (19). Mother to child transmission of HIV and HBV occurs often either in utero or through exposure to blood or blood contaminated fluids at or around birth. Such perinatal transmission is believed to account for 35-50% of carriers (18). Perinatal childhood infection of HBV is characterised by symptomlessness, but places carriers at a high risk of developing chronic disease (3). It has also been shown that vertical transmission of hepatitis B virus occurs in about 10% of neonates when the infection occur in the first trimester and in 60% to 90% of babies in the third trimester (20). Screening for HBsAg is routine in pregnancy in most developed countries, 122 Colleges of Obstetrics and Gynecology worldwide screen pregnant women for Hepatitis (21, 22). In Nigeria, HIV is routinely tested for pregnant women but not HBV despite their overlapping routes of transmission. Sero-prevalence studies on HBsAg in Nigeria show that the prevalence of the infection in pregnant women range from 2-15% (23, 24, 25).

The prevalence of hepatitis B virus (HBV) infections in Nigeria have been reported as 17.1% among female sex workers in Nigeria and 11.9% in HIV infected persons (26, 27). Imade *et al.*, had done a study in 2004 and reported a prevalence of 11.5% but did not check the immune status of the women in Jos and Oladokun *et al.*, reported a prevalence rate of 8.9% co-infection rate among pregnant women in Ibadan (28, 29). This study was carried out to determine the prevalence of hepatitis B among HIV infected pregnant women in Jos and also to establish the effect of co-infection on their immunological status.

MATERIALS AND METHODS

Study Design

A cross sectional study was carried out among HIV positive pregnant women at the Jos University Teaching Hospital (JUTH) and Plateau State Specialist Hospital (PSSH) between the months of May and June 2008. Blood samples were collected from one hundred and thirty five (135) of the subjects and Ethical clearance for this study was obtained from both the JUTH Institutional Health Research Ethical Committee and PSSH health research ethical committee. Patients were approached with a letter introducing the subject and their consent was obtained by the subject's signature on the questionnaire given to them. Laboratory numbers was indicated on the questionnaires and same number on the sample bottle to protect patients' identity. The outcome of this finding was referred to the clinician for extra attention and care. The samples were then tested for HBV surface antigens and CD_4+ counts.

Sample Collection

A total of 135 blood samples (The sample size was derived using the formula for sample size calculation: $N = z^2pq/d^2$ (where z = Standard normal deviation at 1.96 (which corresponds to 95% confidence interval; p = Prevalence of Hepatitis B surface antigen in ante-natal women in Nigeria from previous studies; q = 1-p; and d = degree of accuracy/ precision expected = 0.05). were collected from HIV positive pregnant women by venipuncture. 5mls of blood was collected aseptically and emptied into sterile vacutainer tubes and labelled. The uncoagulated blood was allowed to separate into serum at room temperature.

Detection of HbsAG Using Antec Strips

The serum was collected into clean containers and used to test for hepatitis B surface antigen HbsAg using the one step disposable Hepatitis B surface antigen test strips (Antec diagnostics; United Kingdom). The test procedure was carried out following manufacturer's instructions and the results interpreted and recorded accordingly.

Determination of Absolute CD4+ count

The blood was placed on a mixer in order to get a homogenous mixture. All the test tubes were labeled accordingly. Monoclonal antibody PE, 20µl was pipetted into a tube; 20µl of the patient's blood sample was added. The mixture was thoroughly mixed and incubated in the dark for 15 minutes. No-lyse phosphate buffered saline (PBS) dilution buffer, 800µl was added and the mixture connected to a cyflow counter (Partec, Germany) for analysis.

CD4+ % Enumeration

Monoclonal antibody CD_4+ (CD4 MAb PE) and CD_{45}+ (CD4 MAb PE), 10µl was pipette into rhoren tubes, 20µl of the patient's blood samples was added. The mixture was thoroughly mixed and incubated in the dark for 15 minutes at room temperature. No-lyse dilution buffer 1 and 2, 400µl was added just before reading and connected to SL_3 cyflow (Partec, Germany) and analysed.

Interpretation of Immunologic Status Result

The CD4+count of normal patients (healthy immune competent, HIV negative persons in Nigeria) lie between 547-1327 cells/ mm^3. Any CD_4+ Count within the range 200 – 500 cells/mm^3 suggests that the person is immune compromised (some damage has been done to the immune system) (30).

RESULTS

Prevalence Rates

Out of the 135 sera collected from HIV infected pregnant women examined, 16 of them were seropositive for Hepatitis B representing 11.8% of the total (Table 1). Women between the age ranges 36-40 had the highest rate of co-infection, this is not statistically significant (χ^2= 0.074, P=0.05 df=40.05), while women between the age range 16-20 had no co-infection (Table 1).

Association of HIV/HBV co-infection in Pregnant Women and Treatment, Occupational and Educational Characteristics

The occupation of the women examined showed that housewives and business women had higher rates of HIV/HBV co-infection (8.8% and 5.5% respectively), occupation is significantly associated with co-infection (χ^2=0.035 P=0.05 df=4) With respect to educational level, pregnant women who were educated only to secondary level had the highest prevalence of co-infection (4.4%), those with no formal education had the lowest prevalence rates (0.7%) but the difference in the prevalence rates was not statistically significant in women with secondary education compared to the rest (χ^2 = 0.545 P= 0.05 df=3) (Table 2). Among the 135 women examined 18.5% of those who had HIV alone were on antiretroviral therapy (ART) while 6.7% of those with HIV/HBV co-infection were on ART.

TABLE 1; AGE RANGE AND PREVALENCE DISTRIBUTION OF HIV/HBV CO-INFECTED IN HIV POSITIVE PREGNANT WOMEN

AGE RANGE	NUMBER EXAMINED*	NUMBER HBsAg Positiveβ	% POSITIVE FOR HIV/HBV
16-20	10	0	0.0
21-25	31	1	0.7
26-30	48	5	3.7
31-35	28	4	3.0
36-40	18	6	4.4
TOTAL	135	16	11.8

%= Percentage ; *= Number of pregnant women examined; β= Number of pregnant women positive for HBsAg; χ^2 =0.074 P=0.05 df=4

Immunologic Status

The CD4+ counts of the women with HIV/HBV co-infection were lower than those with HIV alone but there was no association between co-infection and the immunologic status of the pregnant women (χ^2= 0.297 P =0.05 df=5). But 81.2% of the women with HIV/HBV co-infection had CD4+ values below 300 cells/mm^3 (Table 3).

TABLE. 2; TREATMENT, OCCUPATIONAL AND EDUCATIONAL CHARACTERISTICS OF HIV/HBV CO-INFECTED PREGNANT WOMEN IN JOS

CHARACTERISTICS	HIV (%)	HIV/HBV (%)
Treatment Characteristics		
*ART	25 (18.5)	9 (6.7)
NON-ART	94 (69.6)	7 (5.2)
n=135		
Occupational Characteristics		
BUSINESS	7 (7.52)	5 (5.5)
HOUSE WIFE	30 (33.0)	8 (8.8)
SELF EMPLOYMENT	17 (18.7)	1 (1.1)
CIVIL SERVANT	12 (13.1)	1 (1.1)
PRIVATE ESTABLISHMENT	10 (11.0)	0 (0.0)
n=91 χ^2 =0.035 P=0.05 df=4		
Educational Characteristics		
PRIMARY	24 (24.0)	5 (5.0)
SECONDARY	30 (30.0)	6 (6.0)
TERTIARY	29 (29.0)	4 (4.0)
NO FORMAL EDUCATION	1 (1.0)	1 (1.0)
n= 100 χ^2 =0.545 P=0.05 df=3		

*ART = Patients on Antiretroviral Therapy; n= Number of respondents; χ^2 = calculated chi square value; % =Percentage

TABLE 3: IMMUNOLOGIC CHARACTERISTICS OF HIV/HBV CO-INFECTED PREGNANT WOMEN

CD_4 Count Range (cells/mm^3)	Number Subjects Examined	HIV Positive	HIV/HBV Co-infected
0-100	16	11	5
101-200	9	5	4
201-300	13	9	4
301-400	13	12	1
401-500	4	3	1
501-600	0	0	0
601-700	1	0	1
TOTAL	56*	40	16

χ^2 =0.297 P=0.05 df=0.05; n= 56; *=only 56 out of the total subjects sampled had their CD_4^+ counts determined

DISCUSSION

HBV infection in pregnancy is emerging as an increasingly important issue, although more attention has been given to HCV co-infection because of the higher possibility of developing chronic disease, HBV prevalence rates have been reported to be higher than HCV rates in Nigeria (31,32). In this study, 11.8% of the pregnant women population examined was found positive for HIV/HBV co-infection. In 2004 the rates of HIV/HBV co-infection among pregnant women in Jos was reported as 11.5%, this rate is almost similar to that reported by this study, suggesting that the situation with HIV/HBV co-infection in our environment has not changed (33).

Other Nigerian studies have reported different prevalence rates. In a study in the Federal Capital Teritory (FCT), Abuja, Nigeria the prevalence rate of HIV/HBV co-infection was reported as 7.1% , in Yola North East Nigeria, a prevalence rate of 8.2% was reported among pregnant women. Adesina et al., reported a prevalence rate of 8.9% among HIV infected pregnant women (34, 35, 36). Similarly, in other parts of Africa, varying prevalence rates have been reported; Chasela reported a prevalence rate of 10.4% among pregnant women in Malawi, in Uganda 4.1% was reported among HIV positive pregnant women and a rate of 2.4% in Rwanda also among HIV infected pregnant women (37,38). In Europe, a prevalence rate of 4.9% has been reported among HIV infected pregnant women and a low co-infection rate of 1.5% was reported among pregnant women in America (39,40). Jos is thus hyper-endemic area for Hepatitis B virus infection, according to WHO classification for Hepatitis B endemicity (41,42).

Age is not an important factor related to the prevalence of hepatitis B infection in HIV infected women as revealed in this study. The difference in HIV/HBV co-infection among the various age groups shows that women within the age range 36-40 had higher prevalence rates of HIV/HBV co-infection than women within other age brackets. Eke et al., and Habiba et al., however, reported higher prevalence rates in pregnant women within the age brackets of 20-24 and 25-35 years respectively (43,44). This deviation may have been due to the size of the population studied, a higher population size could change the dynamics.

Occupation was found to be associated with the prevalence of co-infection, among house wives and business women. This is similar to the report of Mohammadi et al., who reported significant association between HIV/HBV co-infection among house wives compared to other occupations in Iran (45).

Educational background was not associated with co-infection rates in our study , (P>0.05), and this was the observation of Eke et al. also, who reported no association between HBV infection and educational level among individuals in low resource settings in Nigeria (43). Ezegbudo et al., however found an association between educational background and HIV/HBV infection in pregnant women in Anambra state Nigeria (46).

With respect to the immune status of the study subjects, although the CD_4+ counts of the women with co-infection were below 300 cells /mm3 there was no statistically significant association between HIV/HBV co-infection and the immune status of the pregnant women. Most studies have reported reduced CD4+ count in HIV/HBV co-infected women when compared with monoinfected women. Otegbayo et al., observed lower CD4+ counts in HIV/HBV co-infected pregnant women in Ibadan, Nigeria (27). Forbi et al., also reported a lower CD4+ count in patients with HIV/HBV co-infection as compared with patients with HIV monoinfection, another study by Idoko et al., reported same in Jos (47,48). However Hoffman et al., (49) reported that HBV does not affect CD4+ cell count in people infected with HIV.

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RISK FACTORS FOR HEPATITIS C VIRUS ANTIBODY SEROPOSITIVITY AMONG CHILDREN WITH SICKLE CELL ANAEMIA IN ILORIN, NIGERIA.

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ABSTRACT

Background: Hepatitis C is an infectious disease of the liver caused by the hepatitis C virus (HCV) resulting to a chronic hepatitis. Chronic HCV infection constitutes a serious public health challenge in Nigeria where donor blood is not routinely screened for HCV. Patients with sickle cell anaemia (SCA) are considered a subset of the population at higher risk of acquiring the virus, due to their frequent needs for transfusion of blood and its products. Other risk factors like scarification markings, tattooing, and circumcision also predispose children to acquiring this infection. However, the magnitude of HCV infection has not been adequately measured in our general population and specific data on HCV in SCA patients are scanty, hence a prospective case controlled study to determine the risk factors that predispose to the acquisition of hepatitis C Virus infection.

Objective: To determine the risk factors for Hepatitis C Virus Antibody Seropositivity among transfused children with SCA in Ilorin.

Subjects and Method: Eighty two transfused SCA children aged 6 months to 14 years were recruited consecutively from February 2008 to January 2009 while eighty four non transfused SCA children of the same age range recruited over the same period served as controls. Hepatitis C virus Antibody screening was done using a second generation ELISA method. Information on the study population were collected by use of a pretested questionnaire by the investigator.

Results: There was a positive correlation between numbers of units of blood transfused and seropositivity. Those who had three or more units of blood had a prevalence rate of more than 50%. There was a strong correlation between seropositivity and scarification marks in both subjects and controls (p=0.001 and 0.02 respectively). Other plausible risk factors for hepatitis C infection tested in this study included circumcision and sharing of clippers which showed no statistically significant difference. No cases of tattooing, drug abuse, needle sharing or sexual activities were seen in this study.

Conclusion: Transfused SCA patients belong to a high risk group for hepatitis C virus infection compared to the non-transfused population. The risk of acquisition increases with higher number of transfusions and scarifications marks.

Key words: Hepatitis C virus, Sickle cell anaemia, Risk factors, Blood transfusion.

INTRODUCTION

The Hepatitis C virus (HCV) has become an important cause of chronic liver disease and liver cancer worldwide(2). Hepatitis C Virus infection is highly prevalent in Africa, however the epidemiology of this infection is yet to be well defined (1). Hepatitis C virus infection has been found to lead to chronicity in 70-85% of cases(3), which is similar to hepatitis B virus infection (HBV) which becomes chronic in about 75% of individuals infected (4). It is estimated that about 170million people worldwide are chronically infected with HCV and majority are in developing countries.[5] About 30% of those infected with HCV will progress to liver cirrhosis and ultimately to end stage liver failure and hepatic carcinoma (3,4,6). It is one of the agents transmissible by blood transfusion and is now known to be the major cause of non-A, non-B post transfusion hepatitis (NANBH) (7). The risk factors that are associated with transmission of both HCV and HBV infections are transfusion of blood and blood products, tattooing, scarification with re-usable instruments, body piercing, injection/illicit drug use, perinatal transmission and multiple sex partners (5,8). Since HCV was first identified (9,10), one of the best known and most extensively studied routes of its transmission has been blood and blood derivative transfusion (11,12), therefore patients at risk for HCV infection include transfusion dependent Haemophiliacs, homozygous β-Thalassaemia and Sickle cell anaemia (SCA) patients (13).

The commonest condition in Nigeria for which patients receive repeated blood transfusion is SCA (14)., which has a prevalence of 1.6-3% in Nigeria (15).

In Japan, unsafe injections as in the use and re-use of unsterilized needles in the practice of acupuncture and related techniques have been documented as significant routes of HCV transmission (2). Kaine *et al* (16) estimated that 2.3 to 4.7 million HCV infections annually might be caused by similar unsafe injections in developing countries.

In Nigeria, patients with Sickle Cell Anaemia (SCA) represent the group most likely to receive multiple blood transfusions. Severe anaemia crisis often complicated by malaria parasitaemia and other

infections constitute the major indications for blood transfusion (2). The risk of transfusion acquired HCV infection and its potentially devastating consequences are clearly greater in this environment where re-donor HCV screening is not standard practice (2).
In the present study, we hope to estimate the role of blood transfusion and other risk factors in the transmission of HCV infection.

SUBJECTS AND METHODS

The study was carried out at the University of Ilorin Teaching Hospital (UITH) Ilorin, Nigeria. The hospital has a well established sickle cell clinic that serves the north-central and south-west Nigeria. The University of Ilorin Teaching Hospital (U.I.T.H.) is a tertiary health facility that serves as a referral centre for Kwara, Kogi, Niger, Osun and Ekiti States of Nigeria and also offers secondary health services to the public. It runs a well-established Sickle Cell Disease Clinic for patients below the age of 14 years. An average of 30 mainly old and few new patients are seen in the clinic every Monday. On attaining the age of 14 years such individuals are transferred to the adult Sickle Cell Disease Clinic of the same hospital. The hospital provides a blood transfusion service and laboratory that screens blood for antibodies to HCV.

Eighty-two transfused SCA children aged 6 months to 168 months were recruited consecutively from February 2008 to January 2009 while 84 non transfused SCA children of the same age range recruited over the same period served as controls. Antibodies to HCV screening were done using a second generation ELISA method. The age of the subjects in this study were taken as their completed months at recruitment. The subjects were transfused at least 6 months before presentation to be eligible for the study.

Haemoglobin (Hb) electrophoresis was carried out using electrophoretic tank (Volkman SAE 2761) with cellulose acetate paper at pH 8.4 to confirm their status in the Haematology department of U.I.T.H Ilorin. A pretested questionnaire was used to obtain information on age, sex, history of blood transfusion, parents' educational level as well as occupation to obtain their social class.

Ethical clearance was obtained from the Ethical Review Committee of the hospital and official permission obtained from the head of the Haematology Department. After a clear explanation of the project to them, informed consent was also obtained from either or both parents/guardian and the children before subject enrolment.

Five milliliters of venous blood was collected fromeach subject after a verbal consent and then transported to the laboratory where the serum was separated and assayed for antibodies to HCV immediately using a 2nd generation HCV one step hepatitis C virus test strip manufactured by Acumen diagnostic incorporated, USA[112] which is a Rapid Chromatographic Immunoassay for the qualitative detection of antibody to HCV in serum was used for HCV analysis. The sensitivity and specificity of the test strip were 99.6% and 99.5% respectively.

Statistical analysis was done using SPSS statistical package. The chi-square test was used to assess the significance of the difference amongst the groups and a p-value of <0.05 was considered significant.

RESULTS

A total of 82 transfused sickle cell anaemia patients and 84 non-transfused sickle cell anaemia patients were recruited into the study. The male to female ratio was 1.6:1 in the subjects and 1.1:1 in the controls. The mean age of the subjects and the controls were 95.8+ 48.7 months and 93.5 + 54.0 months respectively and are comparable ($p = 0.77$). Table I. Table II shows that five of the children had detectable antibodies to HCV, constituting 3% of the total population studied, giving an overall hospital-based prevalence of Hepatitis C infection in sickle cell anaemia (SCA) to be 3%. The prevalence of anti-HCV in both the subjects and the controls were comparable ($p = 0.68$), as shown in Table II below.

Table III shows that the subjects and controls were comparable in terms of social stratification in classes I, IV and V, but more controls were in social classes II and more subjects in social class III in the subjects ($p = 0.003$ and 0.002 for classes II and III respectively). There is no significant difference in the seroprevalence of HCV infection in the low and high social classes, (Table IV) both in the subjects and controls.

Table V shows that among the subjects, anti-HCV seropositivity increased with the number of units of blood transfused. This increase was such that 2.2% of recipients of 1-2 units and 6.9% of recipients of 3-4 units were anti-HCV positive.
All the subjects received blood transfusion at one time or the other with the number of transfusions ranging from 1 to 13 units with a mean of 2.63 + 1.67 units per subject. A patient in the age group 121 to 168 months received greater than eight units of blood

Table VI shows other risk factors studied and shows that;
Circumcision: Two (66.7%) of the anti-HCV positive subjects were not circumcised while 1(33.3%) was

circumcised but the difference is not significant (p = 0.3).

Sharing of clippers: Sixty six percent of the anti-HCV positive subjects shared clippers, while 1(33.3%) never shared clippers. All the controls that are anti-HCV positive shared clippers. There was no significant difference whether the patient shared clippers or not in both controls and subjects (p = 0.12 for subjects and 0.41 for controls).

Scarification marks: All the three subjects and the two control patients that are positive for anti-HCV had scarification marks (p = 0.001 for subjects and 0.02 for controls). Other risk factors looked into were not found in the patients recruited into the study as depicted in table VI.

TABLE I: SEX AND AGE DISTRIBUTION OF THE TOTAL POPULATION STUDIED.

	Total	Subjects	Controls
	n (%)	*n (%)*	*n (%)*
Sex			
Male	**93(56)**	**50(61)**	**43(51.2)**
Female	**73(44)**	**32(39)**	**41(48.8)**
Total	**166(100)**	**82(100)**	**84(100)**
Age group in months			
6 – 60	**60(36.2)**	**29(35.4)**	**31(36.9)**
61 – 120	**52(31.3)**	**27(32.9)**	**25(29.8)**
121 – 168	**54(32.5)**	**26(31.7)**	**28(33.3)**
Mean age		**95.8± 48.7**	**93.5± 54.0****

***p*=0.77**

TABLE II: THE ANTI- HCV SEROPOSITIVITY IN THE SUBJECTS AND CONTROLS.

	Anti-HCV positive	Anti-HCV negative	Total		
	n(%)	n(%)	n(%)	χ^2	p
Subjects	**3(3.7)**	**79(96.3)**	**82(100)**		
Controls	**2(2.4)**	**82(97.6)**	**84(100)**	**0.23**	**0.68**

TABLE III: THE SOCIOECONOMIC CLASSIFICATION OF THE SUBJECTS AND CONTROLS.

Social Class		Subjects	Controls		
	Total	*n (%)*	*n (%)*	χ^2	p
I	**11**	**5(45.5)**	**6(54.5)**	**0.18**	**0.67**
II	**60**	**22(36.7)**	**38(63.3)**	**8.53**	**0.003**
III	**70**	**44(62.9)**	**26(37.1)**	**9.26**	**0.002**
IV	**22**	**10(45.5)**	**12(54.5)**	**0.36**	**0.54**
V	**3**	**1(66.7)**	**2(33.3)**	**0.00**	**1.00**

TABLE IV: ANTI-HCV POSITIVITY IN SUBJECTS AND CONTROLS ACCORDING TO SOCIAL CLASS

	Subjects			Controls		
Social class	**Anti-HCV positive** *n (%)*	**Anti-HCV negative** *n (%)*	**Total** *N*	**Anti-HCV positive** *n (%)*	**Anti-HCV negative** *n (%)*	**Total** *N*
I	**0**	**5(100)**	**5**	**0**	**6(100)**	**6**
II	**1(4.5)**	**21(95.5)**	**22**	**1(2.6)**	**37(97.4)**	**38**
III	**1(2.3)**	**43(97.7)**	**44**	**1(3.8)**	**25(96.2)**	**26**
IV	**0**	**10(100)**	**10**	**0**	**12(100)**	**12**
V	**1(100)**	**0**	**1**	**0**	**2(100)**	**2**
Categorised social class						
Low(III-V)	**2(3.6)**	**53(96.4)**	**55**	**1(2.5)**	**39(97.5)**	**40**
High(I-II)	**1(3.7)**	**26(96.3)**	**27**	**1(2.3)**	**43(97.7)**	**44**

χ^2 = 0.37, p = 1.00 (Subjects) χ^2 = 0.42, p = 1.00 (Controls)

TABLE V: CORRELATION BETWEEN ANTI–HCV SEROPOSITIVITY AND THE UNITS OF BLOOD TRANSFUSED.

Units of blood transfused	Anti-HCV positive *n (%)*	Anti-HCV negative *n (%)*	Total *N*	*OR	**CI	p
1-2	1(2.2)	45(97.8)	46			
3-4	2(6.9)	27(93.1)	29	0.30	0.01-4.54	0.33
5-8	0	61(100)	61	-	-	-
>8	0	1(100)	1			

*=Odds ratio. **=95% Confidence interval.

TABLE VI: THE RISK FACTORS FOR ANTI- HCV POSITIVITY IN THE SUBJECTS AND CONTROLS.

	Subjects					Controls				
Risk factors	Positive *n (%)*	Negative *n (%)*	Total *n (%)*	χ^2	P	Positive *n (%)*	Negative *n (%)*	Total *n (%)*	χ^2	p
<u>Circumcision</u>										
Circumcised	1(2.2)	45(97.8)	46(100)			2(4.2)	46(95.8)	48(100)		
Not circumcised	2(5.6)	34(94.4)	36(100)	0.66	0.41	0	36(100)	36(100)	0.27	0.3
<u>Sharing of clippers</u>										
Shared clipper	2(5.6)	34(94.4)	36(100)			2(6.7)	28(93.3)	30(100)		
Not shared clipper	1(2.2)	45(97.8)	46(100)	0.66	0.41	0	54(100)	54(100)	1.38	0.12
<u>Scarification marks</u>										
Scarification	3(30)	7(70)	10(100)			2(16.7)	10(83.3)	12(100)		
No scarification	0	72(100)	72(100)	14.72	0.001	0	72(100)	72(100)	6.17	0.02
<u>Blood transfusion</u>										
Transfused	3(3.7)	79(96.3)	82(100)							
Not transfused	2(2.4)	82(97.6)	84(100)	0.23	0.68					
Tattoo marks	0	0				0	0			
Abuse of drug	0	0				0	0			
Indulging in sexual activity	0	0				0	0			
Needle sharing	0	0				0	0			

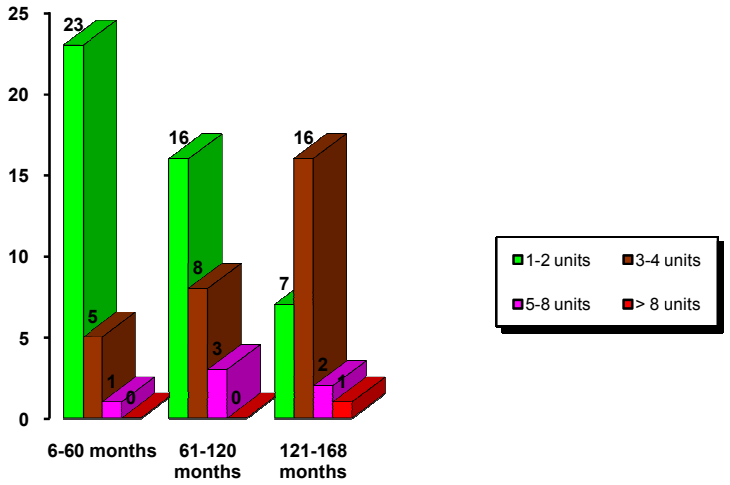

FIGURE 1: BAR CHART SHOWING THE UNITS OF BLOOD TRANSFUSED IN THE DIFFERENT AGE GROUPS AMONGST THE SUBJECTS.

DISCUSSION

In this study, the seroprevalence rate of anti-HCV is 3.7% in the transfused sickle cell anaemia (SCA) patients and 2.4% in the controls that were not transfused, a finding that is similar to previous reports.[2,4]

An overall prevalence of 3% was detected in sickle cell anaemia patients generally, with a higher prevalence of 3.7% in transfused patients, and 2.4% in non-transfused sickle cell anaemia patients. These values parallels the frequency of HCV infection in the world population in general estimated at 3%[4], while the frequency among SCA patients submitted to transfusion of blood or blood derivatives reported in the literature ranges from 2–30%. [2,5-9,15] In the present study, the ages of children screened for hepatitis C virus antibody falls between 6 months and one hundred and sixty-eight months with children within the age range six to sixty months having the highest percentage of seropositivity of 36.2% whilst children between the age of sixty one to a hundred and twenty having the lowest percentage of 31.3%. Sickle cell anaemia patients who received multiple blood transfusions did not appear to be at a greater risk of acquiring hepatitis C virus infection since the prevalence is comparable in the transfused cases and non transfused controls suggesting that blood transfusion may not be the only or major route of transmission.

The patients were evenly distributed in the five social classes and the subjects and controls were comparable except in social classes two and three where the differences in the subjects and controls were statistically significant. Over two-thirds of those that are anti-HCV positive were in the low socioeconomic class, a finding that is similar to other reports from the Niger delta[17] area of Nigeria and in the USA.[18] This can be attributed to the poverty and resultant ignorance and poor health seeking behaviour of this group of people.

There was also no correlation in prevalence between multi-transfused and non-transfused sickle cell anaemia patients, a finding that is similar to earlier reports[2,14] but at variance with others,[1,18] that reported a higher prevalence with multi-transfused SCA patients. Since multi-transfused SCA patients did not appear to be at a greater risk, blood transfusion may not be the only or major route of transmission. There was a linear correlation between the prevalence of anti-HCV and increasing number of units of blood transfused and this is similar to the trend in other studies.[1,19-21] The highest prevalence was in those who had more than 3 units of blood. This is comparatively higher than those that received more than 10 units of blood in both US studies with seroprevalence of 23% and 30%.[16,20]

The higher magnitude of the risk of positivity with each transfusion in the Nigerian studies may suggest that more contaminated blood is being transfused. This is not surprising considering that blood is not usually screened for HCV antibodies, it is therefore pertinent that blood be given only when necessary and blood for transfusion should also be screened for Hepatitis C virus antibodies.

Tribal marks, male and female circumcision, medicinal and other scarifications, all of which are common practices in this environment correlates well with a high seropositivity. Of the other risk factors considered none of the patients had tattoo marks, abused drugs, indulged in sexual activities

or shared needles. Some of the patients that were positive for anti-HCV were circumcised, shared clippers but these were not statistically significant.

It is interesting to note that all the 3 subjects and 2 control patients that were positive for anti-HCV had scarification marks for various reasons ranging from family culture to therapeutic indications. Most of the scarifications and sociocultural practices were done by Herbalists using crude methods involving sharp reusable and scientifically unsterilized devices.

It is plausible to suggest that engagement in these activities as verified in the cases that were positive for HCV antibodies could have exposed these patients to infection with Hepatitis C virus. This finding is in consonance with an earlier work in adults and blood donors carried out in Ilorin.[22] There is therefore the need for a larger survey in children to ascertain if this is a possible major route of transmission. Despite the high prevalence of other potential risk factors, only previous scarification marks and previous history of blood transfusion was associated with HCV antibody positivity in this study. Whatever the source of infection, screening of blood and blood products alone may not prevent the transmission of HCV infection effectively in this environment. Increasing awareness of the disease and its route of transmission, adequate enlightenment of the general population as well as health care workers on safe medical practices could make a significant impact on the spread of HCV infection. Furthermore, premarital antenatal counseling and screening for sickle cell disease will contribute to a decrease in prevalence through a corresponding decrease in the sickle cell gene frequency.

Transmission of HCV infection resulting from unsafe injections is being increasingly described and recent epidemiology in developing countries suggests that this and other sporadic non transfusion related routes which had previously been grossly underestimated, maybe the important routes of HCV transmission[2] In Japan, tattooing, scarification marks and the use of unsterilized needles in the practice of folk medicine including acupuncture and related techniques has been documented as significant routes of transmission. All these are in agreement with the findings of this study.

Larger studies are necessary to address the role of other risk factors like scarification marking, unsafe injections, use of public clippers as well as blood transfusion in the transmission of HCV infection.

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PREVALENCE OF RUBELLA VIRUS-SPECIFIC IMMUNOGLOBULIN-G AND -M IN PREGNANT WOMEN ATTENDING TWO TERTIARY HOSPITALS IN SOUTHWESTERN NIGERIA

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ABSTRACT
Background: Rubella is a self-limiting disease that causes congenital rubella syndrome (CRS) when rubella virus (RV) infects women in the first trimester of pregnancy.
Objective: To assess a population of pregnant women attending antenatal clinics in two tertiary hospitals in southwestern Nigeria for anti-RV IgG and IgM in order to determine the proportions susceptible to the viral infection and infectious to their fetuses.
Method: Ninety consenting pregnant women were consecutively recruited and aseptically bled for the study. The sera were screened with commercial ELISA kits for anti-rubella virus IgG and IgM. Due to the controls and calibrators included in the tests, the IgG test was performed on 89 sera while all 90 samples were screened for IgM.
Results: Of the 89 pregnant women tested for anti-RV IgG, 86 (96.6%) were positive with protective serum concentrations of the antibody. Only six (6.7%, 95% CI: 1.5-11.8%, n = 90) women were positive for anti-RV IgM with 5 (5.6%, 95% CI: 0.8-10.3%) having both anti-RV IgG and IgM. Overall, three (3.4%) of the women were susceptible to RV infection, one (1.1%) of these in the first trimester of gestation was probably infectious.
Conclusion: Majority of the pregnant women had protective levels of anti-RV IgG antibody although susceptibility to rubella as well as level of infectiousness were low. Intensification of rubella immunization of all females of child-bearing age in Osun and Oyo States is advocated.

Keywords: Prevalence, Rubella, Antibodies, Pregnant women, Southwestern Nigeria.

INTRODUCTION

Rubella, a vaccine-preventable viral disease, is predominantly a human disease that occurs between infancy and puberty (1). As a mild exanthematous disease, it is associated with low-grade fever, lymphadenopathy, headache, malaise, mild coryza, and conjunctivitis with a short-lived (acute) maculopapular rash (2, 3, 4). The etiologic agent - rubella virus (RV) - is an enveloped, positive-sense, ssRNA virus in the genus *Rubivirus* of the family *Togaviridae*. Individuals infected with RV are most infectious when the rash is erupting, but they may shed virus from 7 days before to 14 days after the onset of rash. Following exposure, the incubation period before onset of symptoms is usually 14–18 days (range 12–23 days) (5).

Prior to the introduction of immunization programs, rubella was endemic worldwide and peak infection occurred in the 5-9 year-old age group. After vaccine implementation, the disease shifted from children to young adults until its virtual elimination from North America and Europe in recent years (6, 7). In tropical countries however, epidemics still occur but the lack of effective monitoring programs coupled with the absence of serious clinical symptoms in affected children, make the epidemics difficult to assess (8). In countries that have not implemented vaccination programs, infection at an early age is still the norm, with high seroconversion rates found in both preschool populations and in the 5 to 9 year-old age group (9). However, a substantial number of women of childbearing age (10-25%) were reportedly susceptible to rubella (10).

Cell-mediated and humoral immunity develop in humans following natural RV infection and with rubella immunization (11). Antibodies produced against RV comprise the primary immunoglobulin M (IgM) and later IgG which are the markers of current RV infection and past infection or protective immunity respectively. With natural infection, RV-specific IgM antibodies become detectable within 3–4 days and IgG antibodies within one week of the onset of rash while following rubella vaccination, the appearance of RV-specific IgM and IgG antibodies is somewhat delayed and peak levels are lower compared to natural infection. Rubella-specific IgM can often be detected in individuals up to two months after illness and in a decreasing percentage of

individuals up to six to seven months after natural infection, vaccination and re-infection (11). Vaccination of children and mothers with RV vaccine is capable of conferring herd immunity that interrupts RV transmission. Vaccinated or naturally-infected mothers with subsequent seroconversion pass protective anti-RV IgG to their newborns (3, 12). It is noteworthy however that false-positive IgM test results may occur due to cross-reacting IgM antibodies to Epstein Barr virus, human parvovirus B19, rheumatoid factor or other auto-antibodies.

Though males and females are susceptible, RV infection becomes more important when the fetuses of pregnant women are infected during the first trimester of pregnancy. This usually results in serious consequences such as miscarriages, stillbirths and a constellation of severe birth defects known as congenital rubella syndrome (CRS). Ninety per cent (90%) of mothers infected during the first 11 or 12 weeks of gestation will deliver an infant with CRS and the most common congenital defects of CRS are cataracts, heart defects and hearing impairment, mental/physical retardation *et cetera* (4, 13). The primary objective of the rubella immunization program therefore is to prevent CRS.

Immunity to RV is most frequently ascertained by detection of specific antibodies using enzyme-linked immunosorbent assay (ELISA) or other immunoassay methods (5, 14, 15). In Nigeria, Odelola *et al*. (16) in a multicenter study involving Northern, Eastern and Western Nigeria, reported that an average of 68% of the Nigerian population possessed rubella virus antibodies while Pennap *et al*. (17) reported a 3.9% prevalence of RV IgM among pregnant women in Makurdi, Benue State. Other studies among pregnant women detected rubella IgG antibodies of 68.5% in Ibadan (18), 54.1% in Maiduguri (19) and 76% in Lagos (20). The present study was conducted to determine the proportion of pregnant women with anti-rubella virus IgG and IgM in two tertiary hospitals in southwestern Nigeria; to assess the rate of susceptibility to rubella virus infection and determine the possibility of vertical transmission of the virus.

MATERIALS AND METHODS

Study area and population

This study was carried out in Wesley Guild Hospital, Ilesa, Osun State and Jericho Nursing Home, Ibadan, Oyo State between October, 2011 and May, 2012. Wesley Guild Hospital is located in Ilesa West local government area (LGA) of Osun State while Jericho Nursing Home is in Ido LGA, Oyo State. Figure 1 shows the demographics of the women.

Study design

For this study, a cross-sectional, hospital-based design was employed following ethical approval by the Health Research Ethical Committee of the College of Health Sciences, Osun State University, Osogbo. A designated physician (Obstetrician) in the two hospitals discussed the objectives and procedures of this study with pregnant women visiting the antenatal clinic of both hospitals. Ninety pregnant women that consented to participate in the study were consecutively recruited. Each pregnant woman provided pertinent demographic data that were obtained through interviewer-administered questionnaires. These data included age, report of MMR vaccination, educational status, marital status, trimester of pregnancy, number of pregnancy (parity), and present/past experience of skin rash, history of family member with skin rash and knowledge of rubella.

Blood sample collection/serum preparation

About 5 ml blood sample was aseptically collected by venipuncture from each pregnant woman. Each blood sample was dispensed into appropriately labeled anticoagulant-free sample tubes, screw-capped and left at room temperature for about 45 minutes to 1 hour, after which it was spun at 3,000 revolutions per minute for 10 minutes to separate serum from blood clot. The serum was dispensed into correspondingly labeled Eppendorf tube and stored at -20^{o}C until tested.

Serological analysis of samples

The sera of the pregnant women were tested for anti-RV IgG and IgM using commercial ELISA kits - RUB IgG ELISA for the quantitative/qualitative determination of IgG antibodies and RUB IgM "Capture" for the determination of IgM antibodies to rubella virus in human serum and plasma (DIA.PRO, Diagnostic Bioprobes Srl, Sesto San Giovanni, Milano, Italy). The serologic tests and interpretation of results were done in accordance with the manufacturer's instructions while optical signals generated were read at 450nm with ELISA plate reader (Optic Ivymen® System, Model 2100C). Due to the controls and calibrators included in the tests, the IgG test was performed on 89 sera while all 90 samples were screened for IgM.

Interpretation of ELISA results

According to the IgG ELISA kit protocol, serum samples with anti-RV IgG concentrations < 10 WHO IU/ml were considered negative for anti-RV IgG antibody while those with concentrations ≥ 10 WHO IU/ml were considered positive. The latter titer is considered the lowest concentration of IgG that provides an effective immunological protection against a second infection of RV. Therefore, for the purpose of determining the seropositivity and

corresponding concentration of anti-RV IgG in each serum sample, the lower limit of the serum control (i.e. 18 IU/ml of anti-rubella virus IgG equivalent to OD of 0.75) was used to estimate the IgG concentration. For instance, serum sample 1 recorded OD of 1.304 which is equivalent to 31.296 IU/ml of anti-RV IgG. The pregnant woman having this sample was hence considered seropositive with protective level of anti-RV IgG. This estimation was done for each of the 89 serum samples.

For the IgM ELISA, serum samples with Sample to Cut-off (S/Co) ratio > 1.2 were considered positive for anti-RV IgM antibodies while those with S/Co ratio < 1.0 were considered negative. Samples with S/Co ratio between 1.0 and 1.2 were considered equivocal as recommended by the kit manufacturer.

Data analysis

The results obtained were analyzed using descriptive statistics i.e. mean and percentages with 95% confidence interval (CI) but in order to prevent report of negative values, CI was not estimated for proportions close to 0.0% and 100.0%. For statistical comparison of the mean age and educational status of the women from Ilesa and Ibadan, independent t-test and Chi-square test were used while to establish significant differences between groups, we used independent t-test (for two groups) and ANOVA (for more than 2 groups) for average values of serum concentration of anti-RV IgG. For IgM seropositivity, Chi-square test was used and p-values < 0.05 served as indicator of statistical significance. The data analysis was done with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

The overall age of the study participants ranged from 19-44 years (yrs) (mean age: 30.8 [95% CI: 29.9-31.7] yrs). The demographic characteristics of the pregnant women involved in the study are shown in Figure 1. It was observed that the pregnant women from Ilesa were statistically comparable in age (P = 0.31) and educational status (P = 0.18) with those from Ibadan.

Five pregnant women (3 from Ilesa and 2 from Ibadan) were positive for both anti-RV IgG and IgM antibodies giving an overall prevalence rate of 5.6% (95% CI: 0.8-10.3, n = 90).

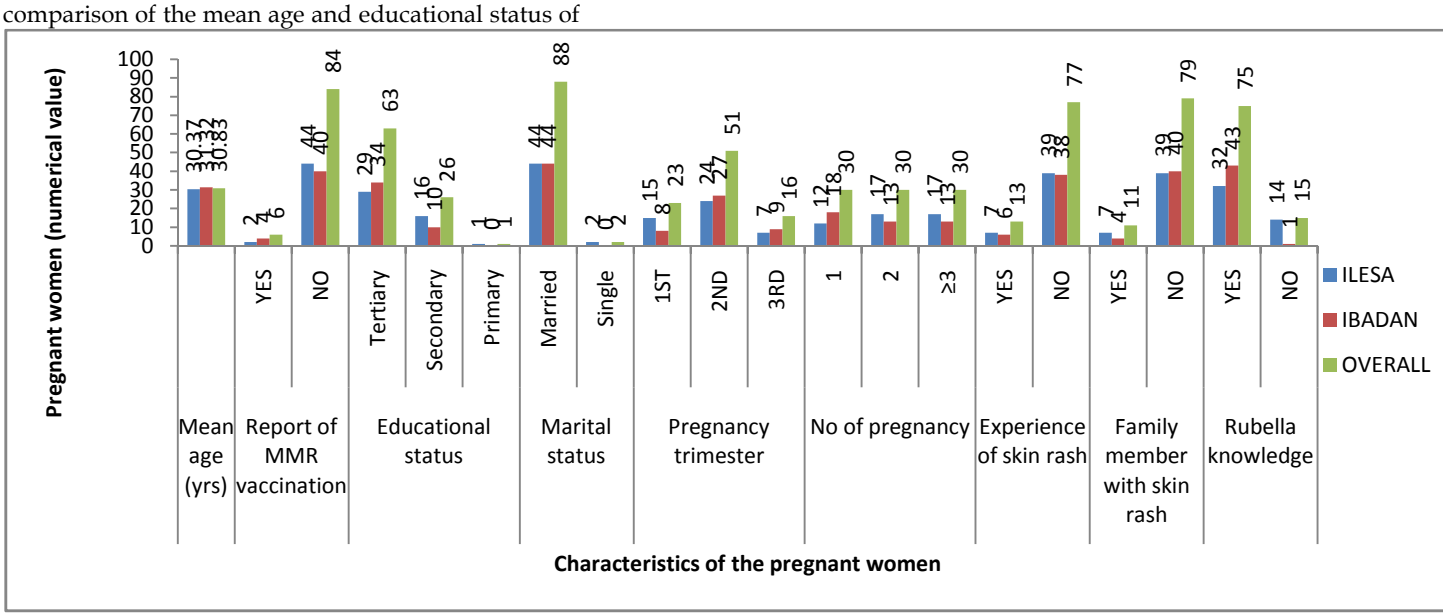

FIGURE 1: DEMOGRAPHIC CHARACTERISTICS OF PREGNANT WOMEN INVOLVED IN THE STUDY

Three pregnant women (two from Ilesa, one from Ibadan) were negative for anti-RV IgG; two of the three were negative for both anti-RV IgG and IgM antibodies.

These three were considered susceptible to RV infection, giving a rubella susceptibility rate of 3.4% (n=89).The third pregnant woman (from Ilesa) (1.1%, n = 90) who was positive for IgM was probably in the acute phase of infection at the time of sample collection. She could therefore be a source of vertical transmission of the virus to the fetus and to other susceptible contacts.

Eighty-six (96.6%, n = 89) of the pregnant women were positive for anti-RV IgG antibody while six (6.7%) (95% CI: 1.5-11.8, n = 90) were positive for anti-RV IgM antibody. The proportions of the pregnant women from Ilesa and Ibadan with respect to results of serologic tests are shown in Figure 2. Sera from three pregnant women (two from Ilesa and one from Ibadan) yielded equivocal IgM results.

Comparison of anti-RV IgG and IgM prevalence rates between pregnant women from Ilesa and Ibadan revealed statistically insignificant results due to very high IgG positivity and very low IgM positivity. When the optical density (OD) of IgG was expressed in WHO IU/ml equivalent and the mean value of anti-RV IgG of the women from Ilesa was compared with that of the women from Ibadan, no significant difference was observed (P = 0.06, n = 89).

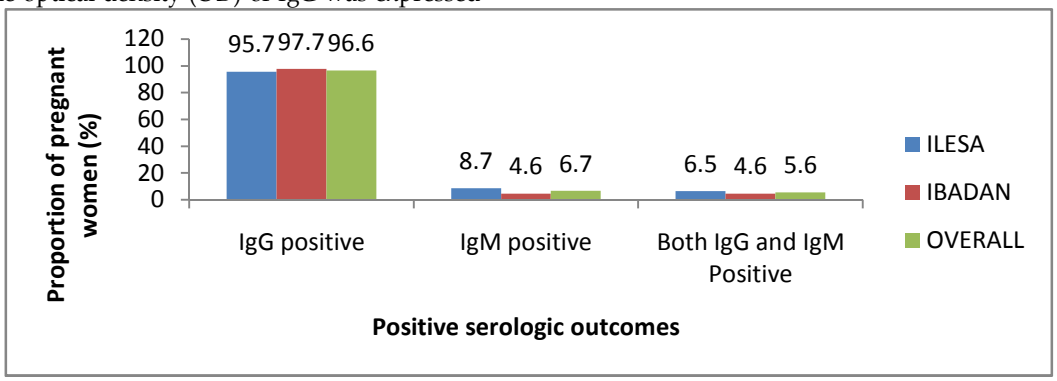

FIGURE 2: DETECTION OF ANTI-RUBELLA VIRUS IgG AND IgM IN PREGNANT WOMEN IN ILESA AND IBADAN

Comparison of the mean values of anti-RV IgG concentration of the pregnant women with respect to trimester of pregnancy and parity revealed that they were statistically comparable at P = 0.62 (n = 89) and P = 0.38 (n = 89) respectively. Anti-RV IgG prevalence rate among pregnant women in the first and second trimester was 95.9% (n = 73) and corresponding IgM prevalence rate was 8.1% (n = 74). Out of the 86 pregnant women positive for anti-RV IgG antibody, 6 (7.0%) had received rubella vaccination. Comparison of the mean serum anti-RV IgG concentration of these 6 women (28.0 IU/ml, 95% CI: 17.11-38.87) with that of the remaining 80 women (28.3 IU/ml, 95% CI: 26.49-30.05) showed that they were statistically comparable (P = 0.96).

DISCUSSION

In this study, we aimed at determining the proportion of pregnant women attending two tertiary hospitals in southwestern Nigeria with anti-RV IgG and IgM, the proportion susceptible to RV infection and those infectious to their unborn children. Our findings revealed that a high proportion (96.6%) of the women at both study sites were positive for anti-RV IgG (Figure 2). While a high anti-RV IgG prevalence rate of 95.7% was detected in Ilesa where, to the best of our knowledge, no such study had been done before, the detection of 97.7% prevalence of anti-RV IgG in Ibadan is consistent with similar high prevalence of 94.2% obtained by Adesina *et al*. (21) from pregnant women in Ibadan. Anti-RV IgG is valuable for all pregnant women and women of child-bearing age as it protects them and their fetuses against RV infection (22). In this study, the recorded responses of the 90 women tested showed that only six of them had received MMR vaccination which indicates that majority of those that were anti-RV IgG-positive had natural exposure to the virus and had seroconverted with protective levels of anti-RV IgG. This observation shows that Ilesa and Ibadan could be described as endemic for rubella. Comparison of mean serum IgG concentrations of women from both study sites revealed no significant difference (P = 0.06), a further indication of comparable endemicity of rubella in both locations.

It is noteworthy that the pregnant women seropositive for anti-RV IgG and IgM were in the first and second trimesters of pregnancy while all the 16 women in the third trimester were positive only for IgG. Anti-RV IgG and IgM prevalence rates among women in their first and second trimesters were 95.9% (n=73) and 8.1% (n=74) respectively. This is contrary to the findings of Agbede *et al*. (23) who reported low anti-RV IgG and IgM prevalence rates of 7.0% and 1.1% respectively among 92 pregnant women in their first and second trimesters at University of Ilorin Teaching Hospital, Ilorin, Kwara State located in north-central Nigeria. This might indicate a higher susceptibility of pregnant women in their first and second trimesters of pregnancy to RV infection in north-central compared to the southwestern Nigeria. Probably, regional differences in endemicity of rubella accounted for the observed variations. Of the six pregnant women (4 from Ilesa and 2 from Ibadan) positive for anti-RV IgM, five had protective anti-RV IgG. Since these six, reportedly, did not receive rubella vaccination, it can be inferred that the five women were naturally exposed to the virus.

The prevalence rate of anti-RV IgM was low among the women in both study sites (Figure 2). This indicated low rate of rubella virus infection among the women as at the time of blood sample collection which could be due to high level of immunity to the virus (or RV endemicity) as shown by very high IgG prevalence rate. Additionally, only one of these six women reported having skin rash while two of them

reported family members having skin rash in the past. It could not be confirmed whether the skin rash was linked to RV infection or not as five of the women had protective levels of serum IgG. Moreover, it was observed that these five pregnant women had IgG concentrations > 10 IU/ml (i.e. protective levels of anti-RV IgG) and were all in the first and second trimesters of gestation. This implies that, although they might have detectable serum anti-RV IgM concurrently with IgG, they were not possibly infectious to their fetuses or to their susceptible contacts.

Furthermore, two of the pregnant women studied were seronegative for both anti-RV IgG and IgM while another one was seropositive for only IgM. The former were considered susceptible to RV infection as they had no report of rubella vaccination and were in the first and second trimesters of pregnancy. We could, however, not state whether or not they were infected with the virus (they probably had undetectable levels of anti-RV IgM) as at the time of blood sample collection. The third woman, who was negative for anti-RV IgG but positive for IgM, was a primigravida in the first trimester of gestation and had no history of rubella vaccination. This woman was most likely infected with RV as at the time of blood collection and was probably infectious to her fetus and other susceptible contacts. The fetus of this woman thus had a high risk of contracting RV from the mother with corresponding high risk of the child developing CRS (5).

Also, we observed that six pregnant women (2 from Ilesa and 4 from Ibadan) had history of rubella vaccination and were expectedly negative for anti-RV IgM with protective levels of anti-RV IgG, the mean of which was statistically comparable (P = 0.96) to that of the remaining 80 pregnant women having protective levels of IgG. While five of these women were multiparous, only one was a primigravida. The test used in this study could however not detect whether the IgG in the sera of these women was induced by rubella vaccine or natural RV infection. We also observed that overall, parity did not influence IgG concentration (P = 0.38) among the pregnant women.

In this study, we observed that the educational status of the pregnant women and their knowledge of rubella were generally high but the level of receipt of MMR vaccination was very low. This could be attributed to the non-inclusion of rubella vaccination in routine national immunization programme in Nigeria, probably due to endemicity of rubella in the country. It has been estimated that over 100,000 infants are born with CRS each year, mostly in developing countries that are yet to introduce rubella vaccines (24, 25). In this study therefore, there exists a likelihood of infecting one unborn child since the only infectious mother detected happened to be in the first trimester of gestation when up to 90% fetuses of RV-infected pregnant women may develop CRS (26, 27).

In conclusion, high prevalence of anti-rubella virus IgG with protective levels of the antibody was reported in pregnant women in this study. Although the rate of susceptibility to rubella as well as possibility of vertical transmission of the virus was low among the women, there was a chance of an expectant mother infecting her fetus with the virus with the possibility of RV-induced congenital abnormality. It has previously been reported by WHO (28) that a low level of susceptibility to RV cannot be taken to mean no risk of CRS. Therefore, based on the findings of this study, we advocate that RV immunization of all females of child-bearing age in Osun and Oyo States, and indeed throughout Nigeria, be intensified so that the likelihood of infecting their fetuses and their contacts during pregnancy can be considerably reduced.

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ORIGINAL ARTICLE

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IN-VITRO ANTIFUNGAL EFFECT OF *GARCINIA KOLA* AND GARLIC (*ALLIUMS SATIVUM*) ON VAGINAL ISOLATES OF *CANDIDA*

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ABSTRACT

Background/Objectives:

Within the last decade there has been an emergence of antifungal drug resistance. *Alliums sativum* and *Garcinia kola* seeds were tested for their anticandidal properties in comparison with fluconazole and miconazole.

Methods: High Vaginal swab samples from patients with vulvovaginal candidiasis were processed and identified to the species level by germ tube method, morphology on corn meal agar and sugar fermentation reactions. Methanol and aqueous extracts of *Garcinia kola* and *Alliums sativum*, as well as fluconazole and miconazole were tested in-vitro using the agar dilution method.

Results: One hundred and twenty six women with symptoms of vulvovaginal candidiasis were sampled and *Candida species* were isolated from 25 of them. *Candida spp.* identified were *C. albicans* (44%), *C. tropicalis* (28%), *C. glabrata* (16%) and *C. parapsilosis* (12%). All species except *C. glabrata* were inhibited by fluconazole and miconazole, all isolates of the same species having same minimum inhibitory concentrations (MICs). The highest MICs (25 mg/ml) with the alcoholic extracts were shown by *C. albicans* and *C. glabrata* and the lowest MICs (12.5 mg/ml) were shown by *C parapsilosis* and *C tropicalis*. All the isolates tested with *Garcinia kola* aqueous extract had a uniform MIC of 50 mg/ml, those tested with garlic aqueous extract had an MIC of 200 mg/ml. *C. albicans* and *C. glabrata* had MIC of 200 mg/ml of the alcoholic extract but *C. tropicalis* was inhibited at 25 mg/ml.

Conclusion: We found that *Garcinia kola* and *Alliums ativum* have activity against the vaginal *Candida* species isolated thus showing promise as alternative therapy for vaginal candidiasis.

Keywords: *Alliums ativum, Candida spp, Garcinia kola*, Minimum inhibitory concentrations

INTRODUCTION

Candida vaginosis is one of the most frequent infections of the female genital tract. At least 75% women suffer once in their life from one episode of a candida infection (1-3). Although *Candida albicans* is the pathogen identified in most patients with vulvovaginal candidiasis, other possible pathogens include *Candida tropicalis, Candida glabrata, Candida parapsilosis* amongst others, which are responsible for up to 33 percent of recurrent infections (4-6). *Candida tropicalis* and *glabrata* are the most important of the *non-C albicans* infections (7, 8). *Candida species* other than *albicans* have been found to cause yeast vaginitis (8). Relatively higher antifungal resistance rate of non-*C albicans species* may contribute to higher rates of recurrent infections.

Imidazole is the first-line treatment for *C. albicans* infections. *In vitro* studies have shown that imidazole antifungal agents such as miconazole and clotrimazole are not as effective against non-*C. albicans* fungi as against *C. albicans* fungi. *C. tropicalis* and *C. glabrata* are 10 times less sensitive to miconazole than is *C. albicans* (9, 10). The recognition of yeast speciation and the need for use of a broad-spectrum antifungal preparation that covers these organisms is now apparent (11-14). However many of the commonly used antifungal drugs are of limited use due to their toxicity and side effects which includes dangerous drug interactions, liver damage, and heart failure (15). Within the last decade there has been an emergence of antifungal drug resistance, which was uncommon in the past (11-14)

Recently in developing countries the antimicrobial effects of plant extracts have been reported and several attempts made to destroy bacteria and their spores by the application of these extracts (16 -21). In addition, plants extracts promote good human health and several plant extracts are effective against a number of human pathogens including *Candida albicans* (21, 22). Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations.

Medically important strains of fungi have been reported to have multiple drug resistance and this has led to development of more potent synthetic antifungal drugs (23-26). These new antifungal drugs are not readily available in our environment and when available is expensive thus making compliance an issue. The alcoholic extract of *Garcinia kola* has been reported to exhibit significant sensitivity and inhibitory activities against fungi and bacteria (27, 22). *Alliums sativum* has also been reported to have antifungal activity (28).

This work aimed to investigate the antifungal activity of *Garcinia kola* and *Alliums sativum* on various *species* of *Candida* isolated from the vagina with a view of possibly recommending their incorporation into formulations of efficacious drugs for the treatment of vaginal candidiasis in future.

MATERIALS AND METHODS

Study Design: Clinical isolates of *Candida* causing vulvovaginitis in women attending two separate centers of a community clinic in Lagos were exposed tofluconazole and micaconazole and some plants on trial. The study was conducted between May and September 2007 in the Department Medical Microbiology and Parasitology of the College of Medicine, Idi-Araba, Lagos. It was approved by the Research and Ethics Committee of the LUTH and informed consent was obtained from the study participants.

Study Population

High vaginal swab samples were collected from women attending the Lekki and Idi-Araba branches of a community healthcare facility for women and children in Lagos. Candidiasis was diagnosed in the women if they complained of vaginal discharge and pruritus, and *candida spp*. were seen on gram staining or culture of their vaginal discharge. Specimens were cultured on Sabouraud dextrose agar (Oxoid) incubated at 37^0C. Isolates were identified to the species level by germ tube method, morphology on cornmeal agar and sugar fermentation reactions. Identified isolates were stored on nutrient agar slant at room temperature for subsequent susceptibility testing.

Preparation of Drug Concentrations

In this study, isolates from patients were subjected to antimicrobial susceptibility testing according to the method recommended by Clinical Laboratory Standard Institute (CLSI, 2007). Antimicrobial agents used were as follows Fluconazole: (Merck Inc., West Point, PA, USA); and Miconazole (Rodhia Farma Ltd, Sao Paulo, SP, USA); Antifungal agents were reconstituted according to the manufacturers' instructions and serial two-fold dilutions (ranging from 0.06 µg/ml to 64 µg/ml) were prepared on the day of the test and added to Mueller Hinton Agar. Plates were inoculated with 105 cfu/ml of isolates. Control plates without antimicrobial agents were inoculated before and after each set of drug-containing plates. Plates were then incubated aerobically for 24 at 37^oC. A reference strain of *C. albicans* ATCC 25285 was included as control. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that yielded no bacterial growth.

Preparation of Plant Extracts

The Seeds of *Garcinia kola* were purchased from Mushin market (Lagos, Nigeria) and identified in the Pharmacognosy department of University of Lagos; Idi-Araba. The seeds were air dried at room temperature (29) grinded into powder form, the powdered plant was loaded into a soxhlex extractor and extracted using methanol and sterile water.

Cloves of Garlic *Alliums sativum* were purchased from same market and identified in the Pharmacognosy department of University of Lagos, Idi-Araba .The outer coat were removed, the cloves were air dried at room temperature then grounded into powder form. The powdered plant was loaded into a soxhlet extractor and extracted using methanol and water.

Agar Preparation for Plant Extract Sensitivity

The MIC was determined using the agar dilution method. Each of the plant extracts were incorporated into Mueller Hinton agar at different concentrations obtained by weighing the desired concentration of each of the plants into appropriate volume of Mueller Hinton agar.

Each of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations was prepared for both the *Garcinia kola* aqueous and alcoholic extracts. Weighing 5g, 2.5g, 1.25g and 0.625g of each of the extract into a 100ml of Mueller Hinton agar (Oxoid, UK) and mixing vigorously to obtain a homogenous mixture achieved these concentrations.

For the Garlic (*alliums sativum*), each of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml concentrations were prepared for both the aqueous and alcoholic extracts. Weighing 20g, 10g, 5g, 2.5g, 1.25g and 0.625g of each of the extract into a 100ml of Mueller Hinton agar and mixing vigorously achieved these concentrations.

Inoculum Preparation

Three to five isolated colonies of similar colony morphology were picked from positive plates and subcultured onto SDA. The plate was incubated and used for the initial inoculums preparation.

Using the tip of a sterile applicator stick, five isolated colonies of similar colony morphology at least 1mm in diameter were picked, and added to 5ml of sterile

0.85% NaCl, mixed for 15 to 20s. The suspension was adjusted to 0.5 McFarland standards.
Each of the prepared plates with the antimicrobial agents was inoculated with 0.1ml of the prepared inoculums. The plates were incubated at 37◦C for 24 to 48 hours and then observed thereafter, plates with growth were interpreted as positive while those without growth were said to be negative.
For each of the antifungal agents and plant extracts concentrations prepared, control plates were also incubated along without inoculums.

RESULTS

One hundred and twenty six vaginal swabs were collected from women attending both clinics and 25 (19.8%) of the women had vulvovaginal candidiasis. Four species of *C.* were isolated; *Candida albicans* 11 (44%) was the most commonly isolated followed by *C. tropicalis* 7 (28%), *C.andida glabrata* 4 (16%) and *C parapsilosis* 3 (12%).

All species except *C. glabrata* were inhibited by fluconazole and miconazole, with isolates of the same species having the same minimum inhibitory concentrations (MIC) (Table 1 and 2). The effect of methanol and aqueous extract of *Garcinia Kola* was also tested on all the isolates. The different *species* of *candida* were inhibited by various concentrations to the *Garcinia kola* extract. (Tables 3 and 4) . The aqueous extracts of the herbs were less active than the alcoholic extracts. There were also variations in the MICs of *Garcinia kola* for different species of *Candida.* The highest MICs with the alcoholic extracts were shown by *C. albicans* and *C. glabrata*. They were inhibited at a concentration of 25 mg/ml and the lowest MICs were shown by *C. parapsilosis* and *C. tropicalis* they were inhibited at 12.5 mg/ml (Tables 5 and 6). All the isolates tested with *G. kola* aqueous extract had a uniform MIC of 50 mg/ml.

The aqueous extract of Garlic was also less active than the alcoholic extract and the MICs varied for different species of *Candida*. *Candida albicans* and *C glabrata* which were inhibited only at a concentration of 200 mg/ml of the alcoholic extract but *C. tropicalis* was inhibited at 25 mg/ml. All the isolates tested with garlic aqueous extract had a uniform minimum inhibitory concentration of 200 mg/ml.

TABLE 1: SENSITIVITY OF *CANDIDA SPECIES* TO FLUCONAZOLE

Candida species	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml
C. glabrata	+	+	+	+
C. tropicalis	-	-	+	+
C. albicans	-	-	+	+
C. parapsilosis	-	-	+	+

TABLE 2: SENSITIVITY OF *CANDIDA SPECIES* TO MICONAZOLE

Candida species	16ug/ml	8ug/ml	4ug/ml	2ug/ml
C. glabrata	-	+	+	+
C. tropicalis	-	-	+	+
C. albicans	-	-	+	+
C. parapsilosis	-	-	+	+

TABLE 3: SENSITIVITY OF *CANDIDA SPECIES* TO ALCOHOLIC EXTRACT OF *GARCINIA KOLA.*

Candida species	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	-	+	+
C. tropicalis	-	-	-	+
C. glabrata	-	-	+	+
C. parapsilosis	-	-	-	+

TABLE 4: SENSITIVITY OF *CANDIDA SPECIES* TO AQUEOUS EXTRACT OF *GARCINIA KOLA.*

Candida species	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+
C. tropicalis	-	+	+	+
C. glabrata	-	+	+	+
C. parapsilosis	-	+	+	+

TABLE 5: SENSITIVITY OF *CANDIDA SPECIES* TO ALCOHOLIC EXTRACT OF *ALLIUMS SATIVUM*

Candida species	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+	+	+
C. tropicalis	-	-	-	-	+	+
C. glabrata	-	+	+	+	+	+
C. parapsilosis	-	-	+	+	+	+

TABLE 6: SENSITIVITY OF *CANDIDA SPECIES* TO AQUEOUS EXTRACT OF *ALLIUMS SATIVUM*.

Candida species	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
C. albicans	-	+	+	+	+	+
C. tropicalis	-	+	+	+	+	+
C. glabrata	-	+	+	+	+	+
C. parapsilosis	-	+	+	+	+	+

KEYS FOR TABLES 1-6
+: Growth -: No growth

DISCUSSION

Most cases of vaginitis in the study was caused by *C. albicans,* which accounted for 44% of all isolates while the non- *C. albicans* accounted for 56%. This is consistent with previous findings (30,31).This study demonstrated the inhibitory effects of local herbs in comparison with known antifungal agents on vaginal Candidasis, Fluconazole and miconazole are azole anticandidal agents known to be highly active against Candida and so their inhibitory effects in this study is not surprising. The herbs investigated also showed inhibitory effects, but their MICs were high, compared to 2.5-7.5mg/dl reported by Akerele *et al* 2008 (27); implying that a high systemic concentration would be required for therapeutic effects, which implies possibility of systemic toxicity usually associated with a high therapeutic dose. There is a need to carry out a toxicity study. In addition we suggest that these agents can serve as good topical agents if the results are generated and the results of all studies are corroborated in a larger study or clinical trials.

Both herbs are easily and locally available. As shown by the higher MIC of Garlic, *Garcinia kola* may have better antifungal properties *in vitro* against vulvovaginal candida. *Garcinia Kola* seeds are rich in phytonutrients such as flavonoids, phenolic compounds, tannins, saponialkaloids. The phenolic compounds are antimicrobial agents. Phenolic compounds have been extensively used in disinfection (18). The antifungal activity of *Garcinia kola* has been attributed to the presence of hydroxybiflavonoids (22, 32) and that of A*lliums sativum* to Allicin, *S*- allylcysteine and saponins (33-35). It would be interesting and beneficial to determine the time kill effect of these substances to investigate effective use as possible disinfectants.

A comparison of the results of both aqueous and methanolic extracts shows that the methanolic extract is a better antifungal agent than the aqueous extract and this is similar to previous findings (27,28). Methanol is an organic solvent and will dissolve organic compounds better and as such will liberate the active ingredients required for antimicrobial activity (27, 28). It therefore possibly shows that the solvent used in extraction affects the degree of microbial activity. It is already known that alcohol has antibacterial effect. It will be worthwhile to investigate its effect on Candida before concluding that alcohol itself has anti candidal effect.

This study shows that the extracts of *Garcinia kola* and *Alliums sativum* possess anticandidal activity and provide preliminary evidence of the presence of one or more soluble constituents with antifungal properties. The antifungal properties can be investigated further by purifying and characterizing the active agents and by determining toxicological effect if any on normal vaginal micro flora. There is need for more work on these plants extracts to usher in a cheap and readily available antifungal agent.

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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN SAUDI ARABIA: SYSTEMIC REVIEW AND META-ANALYSIS

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ABSTRACT

In recent years, methicillin-resistant *Staphylococcucs aureus* (MRSA) have become a truly global challenge. Systemic review and meta-analysis was performed to summarize the prevalence of MRSA in different regions of Kingdom of Saudi Arabia (KSA). A search of the PubMed, Google and Google Scholar databases for studies published during the period of 1 January 2002 through 31 December 2012 was conducted. We included studies that looked at the number and prevalence of MRSA among total *S. aureus*. Meta-analyst and comprehensive meta-analysis were used for statistical analysis. Twenty six studies were included in the review, representing five regions of KSA. Pooled estimation of 22,793 *S. aureus* strains showed 35.6% (95% Confidence interval (CI), 0.28 – 0.42; P < 0.01) of the strains were MRSA with significant heterogeneity. Prevalence of MRSA ranged from 5.97% to 94% in Dahran and Riyadh cities, respectively. MRSA proportion among KSA regions is slightly high and varied from one city to the other.
Key words: Saudi – methicillin-resistant *Staphylococcucs aureus* -systemic review.

INTRODUCTION

S. aureus is a versatile human pathogen that causes diseases ranging from mild infection of the skin to life threatening sepsis (1). After introduction of penicillin in 1940, penicillinase-producing *S.aureus* were detected, leading to development of penicillinase-resistant semi-synthetic penicillins as methicillin and oxacillin. Within a year from this, MRSA were reported in United Kingdom (2). The emergence of strains resistant to methicillin and other antimicrobial agents has become major concern because of the higher mortality due to systemic MRSA infections (3).

Although the rates of isolation of MRSA have been increasing throughout the world for the last decades and in some areas the rates reached > 50%, there are considerable variations in the prevalence of MRSA according of geographic area (4,5,6).

KSA covers over 2 million Km^2 area and estimated population over 27 million and considered potentially hot spot for the collection of MRSA because up to 6 million of their populations are expatriates from many countries. In addition, KSA hosts about four million Muslim pilgrims from all over the world (7). KSA studies in the early 1990s consistently found low MRSA prevalence (5% and 7.5%) which increased dramatically up to 91% after 1995 (8). This study aims to determine average MRSA prevalence in KSA hospitals by conducting a systemic review and meta-analysis.

MATERIALS AND METHODS

Data acquisition: literature search was conducted in the PubMed and Google Scholar database for the time period spanning January 2002 to December 2012. The search strategy used keywords (and combinations thereof) "*Staphylococcus aureus*", "*S. aureus*", "Prevalence", "Methecillin"," Methecillin-resistant *Staphylococcus aureus*", "MRSA" and "Saudi Arabia". Additionally, abstract book of International congress on infectious Diseases was explored.

Inclusion and exclusion criteria:

The following features were included in the study:

1- *S. aureus* samples were collected from Saudi hospitals.

2- . Prevalence of infection or colonization with MRSA in patients or residents in clinical and nursing healthcare settings was reported.

Studies were excluded for the following reasons:

1- There were ≤ 2 cases of MRSA bacteremia reported.

2- The study described results that used previously published data with > 1 year of overlap or 75% time overlap between studies (in these cases, one representative study was chosen).

3- . Samples were partially/totally selected from MRSA culture collections. 4. Studies failed to focus on MRSA prevalence (these included studies those mixed results of " coagulase-negative Staphylococcus and *S. aureus* or healthy people and patients, estimating MRSA transmission, or The therapeutic efficacy

Data extraction

A data extraction form was developed to collect information from MRSA prevalence studies. This included study location, study period, specimens and/or sites, number of patients or specimens, and results present in form of proportions (express as the percentage of MRSA cases among isolates of *S.aureus*.

Statistical analysis

Statistical analysis was performed by the use of Meta-analyst (version 3.13 Beta) and Comprehensive Meta-Analysis (version 2.0) software. By using Meta-Analyst, overall proportion of MRSA in Saudi was pooled by forest plot with 95% confidence interval (95% CI). The Q statistic for homogeneity and the I^2 test were calculated to assess whether results varied no more than might have been expected by the play of chance

(random sampling). A significant heterogeneity was considered for P < 0.10 and I^2> 50% (9). The small study bias was measured by Begg's funnel plot and by the Egger test (10,11).

RESULTS

From January 2002 through December 2012, Twenty six published articles that matched inclusion criteria were selected for meta-analysis (12 - 37). Among these publications which reported the prevalence of MRSA in KSA, twenty five studies were represented five regions only (from thirteen total regions of KSA) and one study did not reported location (submitted as KSA) (Table 1). Study duration varied between 4 and 96 months whereas one and five days study period were reported in two publications (23,30). Most studies, screening was performed as multi-site swabbing, most often by additional swabs taken from skin lesions, nasal or wounds. While three studies did not mentioned screening sites only clinical specimen or pediatric and adult population reported (15,16,19).

As shown in Forest plot (Figure,1) sample size and 95% CI of each study was reported. Additionally, pooled estimation (Overall) of 22793 *S.aureus* showed 35.6% (95% CI, 0.28 – 0.42; P < 0.01) of strain to be MRSA. There was a high level of heterogeneity, random model methods (I^2= 100%, P <0.001). According to Begg and Mazumdar rank correlation test, a significant correlation suggests that bias exists but does not directly address the implications of this bias (Kindall s tau= -0.11) with a 1-tailed P-value = 0.20.

MRSA proportions variation ranged from 5.97% to 94.59% in Dahran and Riyadh cities, respectively (14,33). Average proportion of MRSA in different KSA regions reported that, Al-Gouf recorded <15% in contrast to highest prevalence in Assir and Riyadh (40%-60%) respectively. Makkah was positioned in intermediate value (25%-40%) as MRSA prevalence (Figure, 2).

TABLE 1: SUMMARY OF STUDIES REPORTING METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN HOSPITAL OF SAUDI ARABIA.

Location		Study period, (months)	Specimens and/or sites	No. of patients (P) or specimens (S)	No. of *S.aurius*	No. of MRSA (%)	Reference (1st author)
Region	Hospital (City)						
Al-Gouf	A/Sidery H. (Al-Gouf)	2008-2009 (9)	Urine	930 (S)	100	13(13)	Al-Ruaily (2011)
Al-sharqia	1-KF- H. 2-TCC. (Al-Hasa)	1999-2004	Wound-blood-sputum- eye-catheter	-	6765	161(2.37)	Panhotra (2005)
	SAMSO. (Daharan)	1999-2003 (60)	Skin- soft tissue- blood	-	5162	308(5.97)	Al-Tawfiq (2006)
	KA-H. (Al-Ahsa)	2006-2008	Pediatric and adult population	-	1930	463 (23.98)	Ahmed (2009)
	KF- H. (Alkhobar)	2002-2005 (44)	Clinical specimens	5534(S)	4063	1918(47.20)	Akhtar (2009)
	SAMSO. (Daharan)	2002-2006 (60)	Blood	1103 (P)	122	27(22.13)	Al-Tawfiq (2009)
	KF- UH. (Al-Khobar)	2001-2008 (96)	Soft tissues and invasion infection	-	633	243(38.38)	Bukharie (2010)
Assir	Assir CH. (Abha)	2003-2004 (6)	Clinical specimens	-	85	39(45.88)	Al-Azraqi (2005)
	1-Asir CH. 2-Abha GH., (Aseer)	2004 (7)	Wounds - abscesses -vaginal discharges.	9631(S)	200	86(43)*	Hamid (2011)
	Aseer CH. (Aseer)	2011 (6)	Nasal swab	81 (P)	81	50 (61.72)	Abdalla (2012)
Makkah	KA-UH. (Jeddah)	1998 (12)	Sputum- blood	111(p)	292	111(38.01)	Madani (2002)
	THNG. (Jeddah)	2002 5-day	Nasal swabs	240 (P)	240	10(4.16)	Austin (2003)
	Al-Hada AFH. (Taif)	2004 (12)	Blood - surgical urinary and respiratory tract infection- wounds	1382(P)	252	146(57.93)	Abdel-Fattah (2005)
	1-Al-Nour H. 2-KA-H. 3-Hera H. 4-KFl-H. (Makkah)	2004-2005 (12)	Blood	1626 (S)	303	161 (53.13)	Ashgar (2006)
	1-Al-Nour H. 2-KA-H. 3-Hera H. 4-KFl-H. (Makkah)	2003-2004 (12)	Wound- eye- respiratory tract - blood- urine – ear	124-132 (S) per hospital	512	199(38.86)	Ashgar (2006)
	1-Al-Nour H. 2-KA-H. 3-Hera H. (Makkah)	2008-2009 (12)	Blood- wound- respiratory tract and urinary tract infection- septicemia- eye- ear	1087(P)	688	271(39.38)	Asghar (2011)

	UH, Western region, (Makkah)	2009-2010 (12)	Wound – skin – soft tissues – pneumonia - eye- joint- sinus- catheter	186 (P)	200	79 (39.5)	El Amin (2012)
Riyadh	KK-UH. (Riyadh)	2004 (12)	Blood stream	220(P)	21	3(14.28)	Babay (2005)
	KF- National Guard H. (Riyadh)	2003 (1day)	Blood -surgical-urinary tract infection - pneumonias	121(P)	12	5(41.66)	Balkhy (2006)
	AFH. (Al-Kharj)	2004-2007 (36)	Pus- wound - ear - aspirates- sputum	689 (S)	166	37(22.28)	Ahmad (2009)
	KK-UH. (Riyadh)	2005-2008 (39)	Skin - soft tissues	280 (P)	280	80 (28.57)	Bukhari, (2009)
	KK-UH. (Riyadh)	2007	skin – soft tissues - abscess - cellulites-ulcers	-	37	35(94.59)	Moussa (2009)
	Specialist H. (Al-Kharj)**	2007 (4)	Nasal swabs	352 (S)	112	9(8.03)	Ahmad (2010)
	KK-UH. (Riyadh)	2005-2008 (48)	Skin and soft tissues - invasive infections	285 (p)	285	85 (29.82)	Al-Otaibi (2010)
	H. and HC. (Riyadh)	2008-2009	Wound – skin – soft tissues –lung abscess - pneumonia –fracture bone –burn - blood	-	135	127(94)	Moussa (2011)
KSA	-	2001-2004	Blood- nasal swabs tracheal aspiration- catheter- wound, sputum	-	117	24(20.51)	Tayfour (2005)

H= Hospital; UH= University hospital; GH= General hospital; HC= Health centers; CH= Central hospital; SH= Specialist Hospital; TCH= Tertiary-care hospital; TCC=Tertiary care center; AFH= Armed force hospital; AFH=Armed Forces Hospital; SAMSO = Saudi Aramco Medical Services Organization;THNG= teaching Hospital of national guard; KF= King Fahd; King Abdul-Aziz= KA; K.Fl.= King Faisal; KKESH= King Khaled Eye Specialist Hospital; * = environmental isolates were excluded; * *= Hospital not mention and write the author city; KSA = Kingdom of Saudi Arabia.

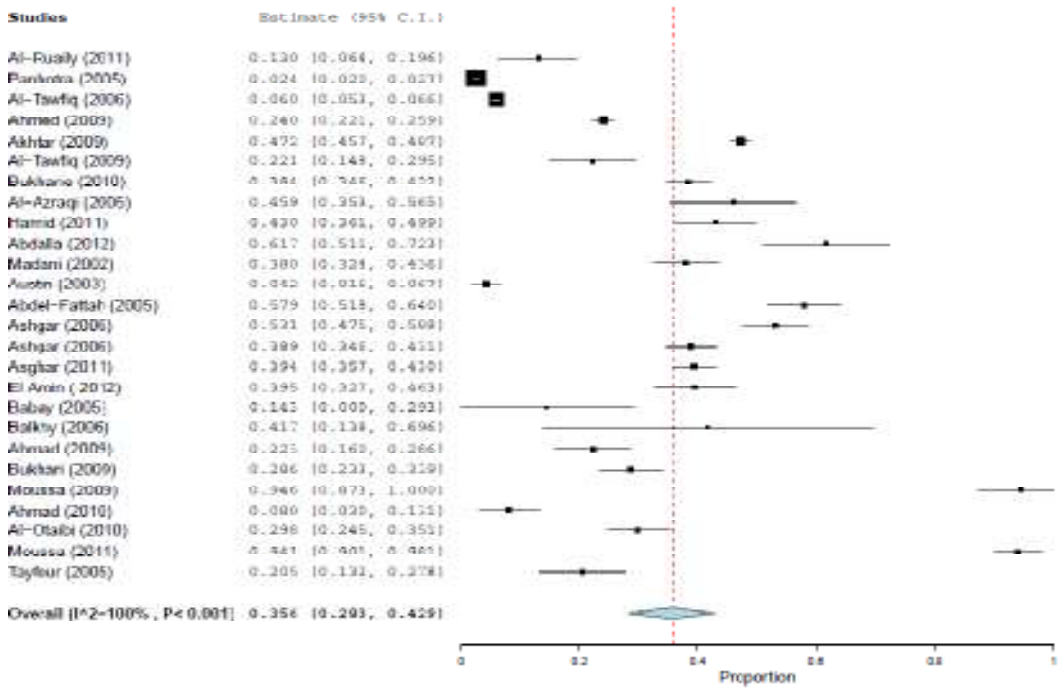

FIGURE 1: FOREST PLOT OF PROPORTION OF MRSA AMONG *STAPHYLOCOCCUS AUREUS* IN THE 26 SAUDI STUDIES, 2002-2012.

FIGURE 2: PROPORTIONS VARIATION OF MRSA IN SAUDI ARABIA, 2002-2012.

DISCUSSION

In this review, 26 studies on MRSA prevalence in different KSA regions were analyzed since 2002 to 2012. We summarized the cumulative prevalence of MRSA and supplied a map to explain the epidemiology of MRSA in Saudi.

According to our study, the overall estimation of MRSA prevalence in Saudi was 35.6% (95% CI, 0.28 – 0.42). whereas MRSA prevalence mean was different from region to another (Figure, 2). While, variation in MRSA proportion exists in several cities (5.97% to 94%). As we know, meta-analysis studies for MRSA prevalence in KSA are not reported but some studies mentioned MRSA average. MRSA proportion from KSA peer-reviewed studies between 1993 and 1997 is low (5-10%) (38). Recently, MRSA prevalence in Saudi is closely with our data where detected 29.9% from January 1990 through April 2011 (39). This finding indicates that temporal increases in MRSA prevalence in KSA. One major factor that could drive regional MRSA dissemination could be the ineffective of infection prevention. Although the reasons for variation of MRSA proportion in KSA cities are unknown, Van Belkum et al have looked at the clonal distribution of MRSA in various part of KSA and found that a single clone account 93% of examined isolates. Therefore it seems that difference in the incidence of MRSA reflects host or environmental factors (40). Additionally, high variation may be due to epidemiology of MRSA is in transition period and infection control rules may be most effective (41). In regional perspective, Saudi has a higher prevalence of MRSA than Bahrain, Kuwait, and Lebanon countries. In contrast, MRSA prevalence in Egypt, Oman, Iran and Jordan was reported more than 50% (39,42,43) . From an international stand, the mean incidence of MRSA across China was over 50% and in Shanghi, the prevalence was over 80% (44). In Spain, the prevalence of MRSA was 29.2% (45).

Limitations of the study

1- The studies we review contain data about different patient population (up to six million of whom are expatriates mainly from south and east Asia beside annually a host of Muslim pilgrims)
2- Only five KSA regions which cannot fully represent Saudi was pooled data
3- Due to limited access to in-press articles and theses, some studies might been missed beside low number of MRSA prevalence studies in KSA (26 studies).

Conclusion

Our study showed that MRSA proportion among KSA studies is slightly high and varied from one city to the other. Thus, to combat the MRSA dissemination, public health researchers and all health professionals must understand the role of hospital hygiene protocols and of antimicrobial drug policies, as well as mechanisms of regional spread of MRSA throughout hospitals. Future systematic reviews would also ideally be based on a greater number of studies that are of high quality.

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AEROBIC BACTERIAL ISOLATES FROM INFECTED WOUNDS

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ABSTRACT

Background: Wound infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stay, profound discomfort and significant increased in healthcare cost. Infection in a wound delays healing and may cause wound break down, herniation of the wound and complete wound dehiscence.Therefore the knowledge of the causative agents of wound infection will be helpful in the control of wound infection and selection of empiric antimicrobial therapy as an infection control measure. Methods:A total of 207 wound specimens collected from patients attending the University of Benin Teaching Hospital were used for this study. All specimens were collected using sterile swabs sticks. Specimens were processed using standard microbiological methods. Results:A total of 278 bacterial isolates were obtained from 207 wound specimens processed in this study. Positive growth were observed in 185 (89.4%) of the wound cultures and no bacterial isolates were obtained in 22 (21.1%) of the cultured materials. *Staphylococcus aureus* (26.9%) was the most predominant isolate followed by *Klebsiellapneumoniae* (17.6%), *Pseudomonas aeruginosa* (16.9%) and *Escherichia coli* (12.6%). All isolates were resistant to ampicillin, amoxyillin-clavulanate and tetracycline but show variable susceptibility to other antibacterial used. Majority of the isolates produced beta lactamase. Conclusion: A high proportion of the wounds were infected.The variety of microorganisms observed in this study support the need to obtain culture specimen from infected wounds for microbiological evaluation and antibiotic susceptibility determination, so that adapted chemotherapy can be prescribed.

Key words:wound infection, polymicrobial, immune status, host

INTRODUCTION

A wound is any physical injury involving a break in the skin (1). The exposed subcutaneous tissues provides a favourable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized and the host immune response is compromised, the conditions become optimal for microbial growth (2).This is because the host immune response plays a critical role in determining whether wound infection will arise (3).

Wound infection refers to the deposition and multiplication of bacteria in tissue with an associated host reaction (4). This may be characterized by the classic signs of redness, pain, swelling and fever (5).

The progression of a wound to an infected state is likely to involve a multitude of microbial or host factors including the type, site, and depth of wound, the extent of non viable exogenous contamination, the general health and immune status of the host, the microbial load, and the combined virulence expressed by the types of microorganisms involved (2).

Although the majority of wounds are polymicrobial involving both aerobes and anaerobes, aerobic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeroginosa*, and beta haemolytic *Streptococci* have been most frequently reported as the cause of delay wound healing (6-9, 3). However, Trengrove et al., (10) reported that no single microorganism or group of organisms was more detrimental to wound healing than any other.

The following organisms are commonly associated with wound infection;*Streptococcus pyogenes*,*Staphylococcus aureus*,*Pseudomonas aeruginosa*., *Escherichia coli*,*Klebsiella*species, *Proteus* species,*Clostridium* species and *Bacteroides*fragilis, *Candida* species and *Aspergillus* species (1-3).
Wound infections cause great distress in terms of associated mortality and morbidity; increased length of hospital stay, delayed wound healing, profound discomfort and significant increased in healthcare cost (11).

Numerous reports exist in the literature regarding wound infection (1-3, 12-17). However, a reassessment of the etiology and antimicrobial susceptibility pattern of wound infection is necessary

for current management of this infection. This study focused on determining the spectrum of aerobic bacterial associated with wound infection in Benin City and their susceptibility to various antibacterial agents.

MATERIALS AND METHODS.

Study Population

A total of 207 wound specimens collected from patients attending the University of Benin Teaching Hospital were used for this study.The Ethical Committee of University of Benin Teaching Hospital approved the protocol for this study.

Specimen Collection and Processing

All specimens were collected using sterile swabs sticks. Specimens were processed according to the method previously described (19). Briefly, the swabs were streaked on the surface of Blood agar, MacConkey agar and incubated aerobically at 37^0C for 24hrs. Smears were prepared on slides and stained by Gram technique, and examined using 40x and 100x objectives for pus cells and bacterial. Emergent colonies from culture were identified.

Identification of Isolates

All bacterial isolates were identified according to the criteria described by Cowan and Steel (20). The criteria include colonial appearance, morphological characteristics as seen by staining and biochemical tests.

Antibacterial Susceptibility Test

The disc diffusion susceptibility test was performed according to the modified Bauer-Kirby method (21-22).

Determination of Beta Lactamase Production.

Beta lactamase production were determined using the iodometric tube method previously described (23).

RESULTS.

The results obtained in this study are shown in table 1-4. Table 1 shows the infection rate from processed specimen. Gender has no effect on wound infection rate. 22 (21.1%) of the processed specimen yielded no bacterial isolate.

A total of 278 bacterial isolates were recovered from various infected wounds, majority of the isolates were from males. *S. aureus* seems to be the most common isolate while *E. feacalis* is the least (Table 2).

All isolates were resistant to Ampicillin, Amoxicillin-clavulanate and tetracycline, while they showed variable susceptibility to other antibacterial agents (Table 3).

A total of 258 (92.8%) out of 278 isolates produced beta lactamase. Majority of the bacterial isolates produced beta lactamase (Table 4).

TABLE 1: INFECTION RATE FROM PROCESSED SPECIMEN.

Gender	No. tested (%)	No. with growth (%)	No. with mixed growth (%)	No. without (%) growth
Male	120	107(89.2)	55(51.4)	13(10.08)
Female	87	78(89.1)	38(48.7)	9(10.3)
Total	207	185 (89.4)	93(50.2)	22(21.1)

DISCUSSION

Infection in a wound delays healing and may cause wound break down, herniation of the wound and complete wound dehiscence (24). Therefore the knowledge of the causative agents of wound infection will be helpful in the control of wound infection and selection of empiric antimicrobial therapy as an infection control measure in hospital and community settings. This study was carried out to generate data that will be useful in the formulation of policy that will aid in the aforemention areas.

The results obtained in this study reveal that 185 (89.4%) out of 207 wounds swabs yielded growth with 50.2% being polymicrobic. The prevalence of high rate of wound infection as well as polymicrobic infection had also been reported by Shittu *et al.,* (9). Gender had no effect on wound infection rate.

TABLE 2: BACTERIAL ISOLATES FROM PROCESSED S

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Organisms	Gender		Total (%)
	Male (%)	Female (%)	
Staphylococcus aureus	43 (26.5)	32 (27.5)	75 (26.9)
Coagulase negative *Staphylococci*	10 (6.2)	4 (3.5)	14 (5.0)
Enterococcus feacalis	5 (3.1)	2 (1.8)	7 (2.5)
Escherichia coli	19 (11.7)	16 (13.8)	35 (12.6)
Klebsiellapnuemoniae	31 (19.1)	18 (15.5)	49 (17.6)
Proteus vulgaris	11 (6.8)	9 (7.8)	20 (7.2)
Proteus mirabilis	13 (8.1)	11 (9.5)	24 (8.6)
Providenciarettegeri	4 (2.5)	3 (2.6)	7 (2.5)
Psuedomonasaeruginosa	26 (16.1)	21 (18.1)	47 (16.9)
Total	162 (58.3)	116 (41.7)	278 (100.0)

TABLE 3: SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES

Organisms	Amp. 10µg	Amx-cla 30µg	Amx 30µg	Cef 30µg	Tet 10µg	Gen 10µg	Cip 5µg	Ofl. 5µg
Staphylococcus aureus(75)	0	0	0	19(25.3)	0	22 (29.3)	28 (37.3)	47 (62.7)
Coagulase negative *Staphylococci* (14)	0	0	2 (14.2)	3 (21.4)	0	7 (50.0)	9 (64.2)	11 (78.5)
Enterococcus feacalis(7)	0	0	1 (14.2)	2 (28.5)	0	4 (57.1)	6 (85.7)	6 (85.7)
Escherichia coli (35)	0	0	0	9 (25.7)	0	10 (28.5)	22 (62.8)	30 (85.7)
Klebsiellapnuemoniae(49)	0	0	0	14(28.5)	0	18 (36.7)	27 (55.1)	32 (65.3)
Proteus vulgaris (20)	0	0	0	6 (30.0)	0	9 (45.0)	11 (55.0)	13 (65.0)
Proteus mirabilis (24)	0	0	0	7 (29.2)	0	10 (41.6)	12 (50.0)	16 (66.7)
Providenciarettegeri(7)	0	0	0	2 (28.6)	0	31 (42.9)	4 (57.1)	4 (57.1)
Psuedomonasaeruginosa(47)	0	0	0	12 (25.5)	0	14 (39.8)	23 (48.9)	29 (61.7)

Abbrevation: Amp- Ampicillin, Amx- Amoxicillin, Amx-cal – Amoxicillin-clavulanate, Cef- CefuroximeTet- Tetracycline, Gen- Gentamicin, Cip- Ciproloxacin, Ofl- Ofloxacin

A total of 278 clinical isolates were obtained from this study. *S. aureus* (26.9%) was the most predominant isolate in this study. This agrees with the reports of previous investigators (9, 15, 17, 25-26,). But does not agrees with the report of Thanni *et al* (18) who reported *S. aureus* as the second most common organism in their study. The other isolates in decresing order of prevalence were *K. pnuemoniae* (17.6%), *P. aeruginosa* (16.9%), *E. coli* (12.6%), *P. mirabilis* (8.6%), *P. vulgaris* (7.2%), coagulase negative *S. aureus* (5%), *E. feacalis* and *P. rettgeri* (2.5%) respectively. These isolates are common isolates found in wounds (9, 25). These isolates contribute to pathology of the wound infection, for example Streptococcal invasion of wound delays healing as well as results in deterioration of wounds (27). Pseudomonas spp, Enterococci spp, and Proteus spp are responsible for extensive tissue destruction with poor blood circulation to the affected site especially diabetic foot ulcer (28).

TABLE 4: NUMBER AND TYPE OF ISOLATES PRODUCING BETA LACTAMASE

ORGANISMS	NO TESTED	NO POSITIVE (%)
Staphylococcus aureus	75	69 (92.0)
Coagulase negative *Staphylococci*	14	13 (92.8)
Enterococcus feacalis	7	7 (100.0)
Escherichia coli	35	31 (88.6)
Klebsiellapnuemoniae	49	44 (89.8)
Proteus vulgaris	20	18 (90.0)
Proteus mirabilis	24	24 (100.0)
Providenciarettegeri	7	7 (100.0)
Psuedomonasaeruginosa	47	45 (95.8)
TOTAL	278	258 (92.8)

All isolates were resistant to ampicillin, amoxillin-clavulanate and tetracyline. The resistant observed for ampicillin and tetracyline could be due to their long period of use. But that of amoxicillin-clavulanate is surprising as the use of this drug is more recent than ampicillin and tetracycline. Susceptibility pattern of the bacterial isolates to other antibacterial agents varies.

Majority of the bacterial isolates in this study produced beta lactamase. This enzyme is used by microorganism to inactivate beta lactam antibacterials. This may explain the resistance observed for ampicillin, amoxicillina and amoxicillin-clavulanate. The fluroquinolones and gentamicin were more effective in this study. This agrees with the report of Mordi and Momoh, (15). These antibacterial should be use in the management of wound infection.

The variety of microorganisms observed in this study support the need to obtain culture specimen from infected wounds for microbiological evaluation and antibiotic susceptibility determination, so that adapted chemotherapy can be prescribed. This will not only facilitate successful wound management but also assist in the control of anatibiotic usage and hence stem the spread of antibiotic resistant bacterial. Continous dialogue between the microbiology department and wound care practitional is strongly advised.

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A PROFILE OF WOUND INFECTIONS IN NATIONAL HOSPITAL ABUJA

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ABSTRACT
Background: Wound Infections cause prolonged hospital stay, increased costs and also can result in increased patient morbidity and mortality. The current spread of multi -drug resistant bacteria has further heightened the need for regular bacteriological review of infected woundsand regular antibiotics surveillance studies so as to avoid the unguidedempirical treatment of wound infections which is quite common in this environment
Aim: To determine the distributionof theisolates from wound specimens submitted to the medical microbiology laboratory of National Hospital Abuja for processing.
Method: A reviewand analysis of 380 woundspecimens results from various wards in the hospital over a period of 10 months (1st Marto Dec 31st 2010) was conducted.
Result: A total of 314 isolates were recovered from the 380 wound specimens giving a yield of 83%. 240(76%) yielded single isolates of various pathogens, while 74(24%) were poly-microbial. Gram negative bacilli constituted 66% of all the pathogens with *Pseudomonas aeruginosa* (19%) and *Proteus species* (18%) as the most frequent, while gram positive isolates made up 33% with *S aureus* (27%) as most predominant and most frequently isolated bacteria from all the wound specimens. Two candida species comprised about 1% of the isolates.
Frequency of infection was highest in surgical wards (27%), gynaecology ward (14%) and accident and emergency unit (12%). The fluoroquinolones, aminoglycosides, and Beta-lactam antibiotics were the most effective drugs for most of the isolates. *Staphylococcus aureus* was most sensitive to amikacin (83%) and erythromycin (79%); *Pseudomonas aeruginosa* to imipenem (96%) and amikacin (83%) and *Proteus species* to amikacin (100%) and imipenem (78%).
Conclusion: *S aureus, Pseudomonas aeruginosa* and *Proteus species* were the predominant bacteria from wounds, with surgery and gynaecology wards having the highest prevalence. Resistance to commonly used antibiotics is high. There is need to institute antibiotic stewardship and effective and efficient infection control measures in the hospital
Keywords: Wound infections, National Hospital , Abuja

INTRODUCTION

A wound is a breach in the skin, and exposure of subcutaneous tissue following loss of skin integrity, thus providing a moist, warm and nutritive environment that is conducive for colonization and proliferation of opportunistic and pathogenic microorganisms (1). Colonization of wounds by microorganisms is seen commonly in both the hospital and community settings. Most times contaminating microbes are eliminated by the host immune system and do not persist, but species that grow and divide may become established, multiply causing wound colonization and infection.

Infection results in delayed healing and maycause wound breakdown or complete wound dehiscence (2). The severity of complications depends mainly on the infecting pathogen and site of infection (3,4). Wound infection is a concern to healthcare practitioners due to associated increased morbidity, mortality and increased cost of care (1). In a study done at Ile-Ife by Shittu *et al.*, an isolation rate of 95% from wound specimens was reported (5), while another study done in Ibadan on burn wound infections gave an isolation rate of 71.4% from wound swabs and 90.2% form wound biopsyspecimens (6). The current spread of multi-drug resistant bacteria has from clinical isolates has heightened the need for regular bacteriological review of wound infections so as to avoid the unguided empirical treatment which appears common in this environment (7)

In Abuja, the Federal capital of Nigeria and one of the fastest growing cities in Africa, there is paucity of research data on wound infections, thus justifying the need for this study, which is aimed at determiningthedistribution and antibioticsusceptibility pattern of bacterial isolates from wound specimens submitted to the National Hospital Abuja (NHA) Medical Microbiology laboratory with a view to providing guide for rational empirical antimicrobial choice in themanagement of wound infections.

METHODS

This is a retrospective study of the data of 380 woundspecimens from various wards submittedto the Medical Microbiology laboratory of NMA for processing over a period of10 months from Mar 1st 2010 to Dec 31st 2010. The hospital is a tertiary hospital serving the needs of FCT and surrounding states.

The patients' bio data and results of the processed specimens from various wardsand clinics were retrieved and analyzed for thepurpose of this study. Information such as age, sex, ward, culture result, and antibiotic sensitivity pattern were also extracted from the records.

In our centre,wound specimensare inoculated onto MacConkeyand blood agar and incubated aerobicallyat 35-37°C for 18-24hrs. The isolates are gram stained and identified usingstandard bacteriological procedures[8]. Antibiotic susceptibility is tested using theModified Kirby-Bauer disk diffusion method and the results are interpreted usingClinical and Laboratory Standards Institute (CLSI) recommendations (9).

RESULTS

Of the 380 wound specimens results reviewed, 314grew organisms giving a yield of 83%. 240 (76%) were pure single isolates and were considered pathogens while growth from 74 (24%) specimens were polymicrobial and were discarded as colonisation. 238(99.%)ofthe pathogens were bacteria, dominated by Staphylococcus aureus (27%), Pseudomonas aureus (19%), Proteus species (18%), Escherichia coli (14%) and Klebsiellapneumoniae (13%). Two (1%) Candida species were isolated [table 1] The 64 (27%), 34 (14%), 29 (12) and 22 (9%) of the isolates were from surgery wards, gynaecology ward, accident and emergency unit and surgery out-patient clinic [table 1].

TABLE 1. WARD AND CLINIC DISTRIBUTION OF WOUND ISOLATES.

Wards/Clinics	Isolates						
	S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Proteus spps*	*Others*	Total
Gynae Ward	10	4	4	7	5	4	34(14)
Surgery Ward	19	13	8	6	13	5	64(27)
Accid/Emergency	9	1	9	3	7		29(12)
Oncology Ward	2	3	-	3	4	2	14(6)
Medical Wards	6	6	2	2	5		21(9)
Intensive care unit	-	3	1	-	-	1	5(2)
SCBU/NICU/IPP	3	3	3	2	-	-	11(5)
Orthop OPD	3	2	3	-	2	1	11(5)
General OPD	5	5	3	1	-	3	17(7)
Surgery OPD	5	3	1	6	4	3	22(9)
Medical OPD	2	-	-	-	4	-	6(3)
Others	-	2				4	6(3)
TOTAL	64(27)	45(19)	34(14)	30(13)	44(18)	23(9)	240(100)

Other Wards/Clinics: Private wing; Special Treatment clinic;
SCBU –special care baby unit; NICU- neonatal intensive care unit; IPP- in-patient paediatric; OPD- out-patient department
Other isolates: Enterococci spp -12; Coagulase negative staphylococcus – 4; Citrobacterfreundii – 3; Providentiaspp – 3; Candida spp – 2

Staphylococcus aureus was most sensitive to amikacin (83%) and erythromycin (79%) and least sensitive to amoxicillin (38%), clindamycin (55%) and cefuroxime (55%);
Pseudomonas aeruginosa was most sensitive to imipenem (96%) and amikacin (83%) and least to gentamicin (59%), ceftazidime (60%) and ofloxacin (61%); Proteus species most sensitive to amikacin (100%) and imipenem (78%) and least to amoxicillin/clavulanate (54%) and cefuroxime (63%) [table2]. Escherichia coli and Klebsiella pneumonia 100% showed sensitive to imipenem,, but highly resistant to gentamicin 26% and 43% respectively.

TABLE 2.ANTIBIOTIC SENSITIVITY PATTERN OF THE FIVE COMMONEST ISOLATES

Antibiotics	Isolates									
	E. coli		K. pneumonia		Proteus spp		P aeruginosa		S. aureus	
	No Tested	% S	No Tested	% S	No Tested	%S	No Tested	%S	No Tested	% S
Cefuroxime	25	32%	17	35%	24	63%	-	-	33	55%
Gentamicin	19	26%	14	43%	31	65%	27	59%	41	68%
Imipenem	9	100%	5	100%	9	78%	27	96%	-	-
Ciprofloxacin	18	67%	18	67%	25	68%	29	66%	24	67%
Amox/Clavulanate	6	83%	5	20%	13	54%	-	-	18	61%
Amoxicillin	-	-	-	-	-	-	-	-	13	53%
Cefotaxime	13	46%	14	64%	17	65%	-	-	-	-
Amikacin	8	88%	6	67%	3	100%	12	83%	12	83%
Ofloxacin	-	-	-	-	-	-	38	61%	32	72%
Ceftazidime	-	-	-	-	-	-	22	60%		
Erythromycin	-	-	-	-	-	-	-	-	24	79%
Clindamycin	-	-	-	-	-	-	-	-	11	55%
Chloramphenicol									12	100%

DISCUSSION

Organisms from 12 different genera were found to be responsible for causing wound infections in the hospital .This study revealed that *S aureus* was the most frequently isolated bacterial pathogen isolated within the period, followed by *P aeruginosa*,*Proteus spp* ,*E coli and K pneumonia*. This finding is consistent with reports of similar studies conducted in various parts of the country such as Ibadan (10), Benin-City (11), Ekpoma (12,13) , Maiduguri (14) and elsewhere outside the country (15,16). A similar study in Enugu, Nigeria however, found *K pneumoniae*as the predominant bacterial isolate (17). Most surgical and traumatic wound infection occurring in hospitalare endogenously acquired, and might explain the predominance of normal flora amongst the pathogens (18). Surgery and gynaecology wards and the accident and emergency unit recorded the highest numbers of infection. These are areas in the hospital that have high numbers of patients with breaches on their skin either from surgery or from road traffic accidents, and are such predisposed to infections (18,19). The out-patient surgery also recorded the highest rate of infection among all the out-patient clinics. This may be as a result of post-discharge infections or infected wounds presenting to surgery from the community.

With the exception of E. coli which showed substantial sensitivity to amoxicillin/clavulanate (83%), and S. aureus to erythromycin 79%) and chloramphenicol (100%), all the five commonest isolates were more than 30% resistant to all the commonly used first line drugs for their treatment, particularly to the third generation cephalosporins and gentamicin. This level of resistance makes the choice for empiric treatment very challenging, with attendant increase in morbidity, cost of care following prolonged hospital stay and use of more expensive drugs and in some cases increased mortality[1].The susceptibility of Staphylococcus aureus to chloramphenicol is particularly interesting, as this is one of the drugs that were commonly used in the past, but rarely used today due to its toxicity in the bone marrow and newborn. In the face of daunting multiple resistance the drug may become useful. Consideration for chloramphenicol has been recommended for eye infection due to Staphylococcus aureus and infection due to susceptible methicillin resistant Staphylococcus aureus (21,21). The substantial level of sensitivity to the carbapenems and amikacin may also provide some kind of fall backposition, but caution must be exercised to avoid losing this window. The findings of this study reinforce the need for antibiotic stewardship and efficient and effective infection control measures.

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MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDICES OF *PSEUDOMONAS* AND *KLEBSIELLA* SPECIES ISOLATES IN LAGOS UNIVERSITY TEACHING HOSPITAL

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ABSRACT
Background/Objectives: *Pseudomonas* and *Klebsiella* infections are important nosocomial infections because of the attendant significant morbidity, mortality and socio-economic impact. These infections are difficult to treat due to the innate and acquired resistance mediated by the organisms' genome and other transferable genetic elements. We determined the multiple antibiotic resistance indices of Pseudomonas spp and Klebsiella spp isolated from clinical specimens in Lagos University Teaching Hospital.
Methods: 110 clinical isolates were evaluated using Microbact™ 24E (Oxoid, UK) and *Pseudomonas* and *Klebsiella* species isolates were 34 and 21 respectively. The antimicrobial susceptibility patterns of the *Pseudomonas* and *Klebsiella* isolates were determined by Kirby-Bauer's disc diffusion method and results interpreted by CLSI interpretative values. Multiple Antibiotic Resistance index (MAR) were calculated.
Results: MAR index of the *Pseudomonas* and *Klebsiella* samples was 0.4, as 31 (91.2%) and 14 (66.7%) of *Pseudomonas spp* and *Klebsiella spp* respectively were multi drug resistant. Isolates of *Pseudomonas spp* demonstrated the highest level of resistance to Ceftazidime (79.4%), Cefixime (76.5%), Cedipime (50%) and Piperacillin (44.1%); while that of *Klebsiella spp* were carbenicillin (76.4%), pipericillin (71.4%), cefixime (52.4%) and cefradoxil (42.9%) respectively. There was a low level of resistance to quinolones and aminoglycosides.
Conclusion: The MAR index shows increase in the rates of resistance among these organisms thus making antimicrobial susceptibility surveillance and testing more crucial in selecting empiric regimen or definitive treatment.

KEY WORDS: Lagos, multiple antibiotic resistance (MAR), antimicrobial susceptibility, *Pseudomonas* and *Klebsiella* spp.

INTRODUCTION:

The World Health Organization (WHO) estimated that infections accounted for 45% of deaths in Africa and South-East Asia and were responsible for 48% of premature deaths worldwide, bacterial infection accounted for a significant proportion of these infections in Africa [1]. The valuable life spans of most antibiotics used in treating these infections have been diminished by the development of resistant strains. Microorganisms use a combination of mechanisms in developing resistance however a dominant mechanism may be identifiable [2,3]. The relationship between antibiotic use and resistance is complex; a major driving factor for antibiotic resistance is antibiotic use/abuse both within medicine and veterinary medicine. In Nigeria 53% of respondents in a survey took incomplete regimen of antibiotics, a significant proportion of which were self prescribed for unspecified ailments [4,5].

Antibiotic resistance increasingly compromises the outcome of many infections that were, until recently, treatable and remain the most common diseases in Africa [6]. The global problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, more expensive agents [6]. Reports from multiple studies from different parts of Nigeria though have observed temporal trends in the prevalence of resistance among enteric organisms have shown increasing prevalence in the last fifteen years [7].

High prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens [8]. Multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [9].

Emergence of antibiotic resistance in *P. aeruginosa* resulting in severe adverse outcomes had been on the rise. In the USA annual prevalence of fluoroquinolones and imipenem resistant *P. aeruginosa* increased from 15% in 1991 to 41% in 2000 1%, and from 1989 to 2006 from 13% to 20% respectively [10]. In Lagos, similar results have been previously documented [11]. These same conclusions had been drawn in Zaria though *P. aeruginosa* there were more susceptible to

fluoroquinolones [9]. In Zaria and Enugu about 60 % , 42%, 33% of clinical isolates of *Pseudomonas spp, Escherichia spp,* and *Klebsiella spp* respectively were noted to be multidrug resistant [12]. In Enugu and Abakaliki 62% of *Klebsiella* isolates were ESBL producers.

Multiple antibiotic resistance (MAR) indexing has been shown to be a cost effective and valid method of bacteria source tracking. Multiple antibiotic resistance index is calculated as the ratio of number of resistant antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed [13, 14]. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used.
This study was carried out to evaluate the MAR indexes values of clinical isolates of *Pseudomonas spp* and *Klebseilla spp* from Lagos University Teaching Hospital (LUTH).

METHOD
Pre-identified clinical isolates of gram negative bacilli species (110 in number) were obtained from Lagos University Teaching Hospital (LUTH) Microbiology laboratory between June and September 2010 and preserved on nutrient agar for further identification. Isolates sub-cultured from nutrient agar slants onto MacConkey agar medium were identified by a combination of colonial morphological characteristics, gram stain, and motility. Further biochemical characterisation was done using Microbact™ 24E (Oxoid, UK). Antimicrobial susceptibility pattern of the isolates were done after they were re-identified. Antimicrobial susceptibility testing was performed using the disc diffusion method (Bauer et al 1966). Antibiotic discs tested were Cefixime, Cedipime, Cefradoxil, Ceftazidime, Cefuroxime, Gentamycin, Amikacin, Piperacillin, Carbenicillin, Ciprofloxacin, Ofloxacin, Imipenem (Oxoid, UK)

Values obtained were interpreted according to the Clinical and Laboratory standards Institute (CLSI, 2010) into resistant, intermediate and sensitive categories. Apart from general caution to strictly follow procedures as described in manufacturer's manual, *Pseudomonas aeruginosa* ATCC 2785 and *Escherichia coli* ATCC 25922 were used as positive and negative controls respectively.
MAR index was determined by following the procedure described by Krumperman 1983 [14]. A MAR index for an isolate is calculated as: Number of antibiotics to which isolate is resistant/ Total number of antibiotics against which isolate was tested.

RESULTS
Total no of clinical isolates obtained from LUTH laboratory was 110. Morphological and biochemical identification revealed that 34 (30.9%) of these were *Pseudomonas* species while 21(19.1%) and 22 (20%) were *Klebsiella* and *Escherichia* species. The rest 33(30%) were made up by a diverse group of the family Enterobacteriacae namely *Serratia, Acinetobacter, Enterobacter, Acetobacter and Citrobacter* species.

Overall antibiotics prescribed by physicians working in all units in LUTH in order of descending frequency included Ampicillin/Cloxacillin, Amoxicillin/Clavulanic acid, Gentamycin, Ciprofloxacin, Cefuroxime, Ceftazidime, Ceftriaxone, Pefloxacin, Ofloxacin, Imipenem.
The total proportion of isolates found sensitive to all antibiotics used in antimicrobial susceptibility testing in *Pseudomonas* and *Klebsiella spp* were 58.4% and 52.2% respectively.

Table 1 and figure 1 show the resistance pattern of *Pseudomonas* to each antibiotic used in the antimicrobial susceptibility testing. Ceftazidime 27 (79.4%) demonstrated the highest levels of resistance followed by Cefixime 26 (76.5%) while other cephalosporins demonstrated moderate activity against *Pseudomonas*. Isolates were most sensitive to Imipenem 32 (94.15), Genticin 31 (91.2%), and Ciprofloxacin 29 (85.3%). 76.5 % of isolates were sensitive to Ofloxacin and Amikacin. Nearly all isolates 31(91.2%) had Multiple Antibiotic Resistance (MAR) index that was higher 0.2 only 3 (8.8%) had a MAR of 0.2 or less. MAR index of the *Pseudomonas* sample is 0.4.

Table 2 shows the resistance pattern of *Klebsiella* to each antibiotic used in the antimicrobial susceptibility testing. The highest levels of resistance were seen in Piperacillin and Ceftazidime 15 (71.4%); other cephalosporin's also demonstrated high levels of resistance. Isolates were most sensitive to Imipenem 20 (95.2%) followed by Gentamycin 19 (90.5%), Ciprofloxacin 19 (90.5%) and Cedipime 18 (85.7%) respectively. Ofloxacin and Amikacin were moderately active against *Klebsiella* isolates. Only a third of the isolates were resistant to two or less antibiotics. The proportion of isolates with Multiple Antibiotic Resistance (MAR) index greater than 0.2 was 66.7% (14) while those with MAR index less than 0.2 was 33.3% (7) showing that a greater proportion of the isolates are likely to be from a high risk source. The MAR index of the *Klebsiella* sample was 0.4.

Figures 1 &2 demonstrate the comparative sensitivity pattern of Pseudomonas and Klebsiella isolates to selected antibiotics in use in the hospital.

TABLE 1: ANTIMICROBIAL SUSCEPTIBILITY TESTING BY DISC DIFFUSION METHOD OF *PSEUDOMONAS ISOLATES*

Antibacterial Agent		Susceptibility Rates n	%S	%I	%R
Cefixime	CFM	34	17.7	5.9	76.5
Cedipime	FEP	34	47.1	2.9	50
Cefradoxil	CFR	34	50	2.9	47.1
Ceftazidime	CA_2	34	17.7	2.9	79.4
Cefuroxime	CXM	34	85.3	2.9	11.8
Imipenem	IPM	34	94.1	0	5.9
Piperacillin	PRL	34	55.9	0	44.1
Carbenicillin	CAR	34	58.8	5.9	35.3
Ciprofloxacin	CIP	34	85.3	0	14.7
Ofloxacin	OFX	34	76.5	5.9	17.7
Gentamycin	GN	34	91.2	2.9	5.9
Amikacin	AK	34	76.5	8.8	14.7

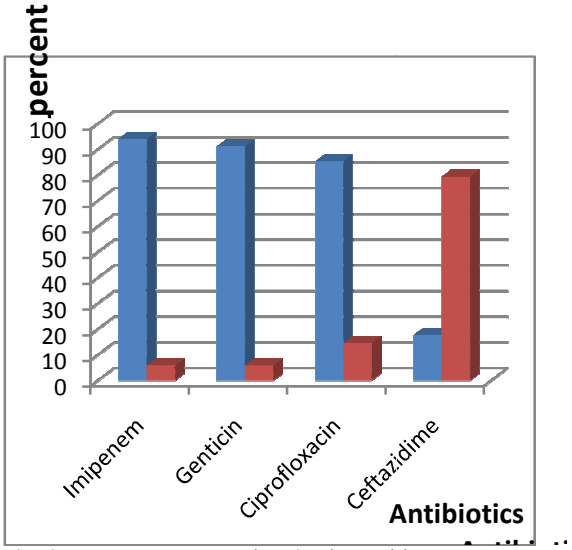
n = no of isolates, S = sensitive, I – intermediate, R = resistant (according to CLSI

TABLE 2: ANTIMICROBIAL SUSCEPTIBILITY TESTING BY DISC DIFFUSION METHOD FOR *KLEBSIELLA ISOLATES*

Antibacterial Agent		Susceptibility Rates n	%S	%I	%R
Cefixime	CFM	21	38.1	4.8	52.4
Cedipime	FEP	21	85.7	4.8	9.5
Cefradoxil	CFR	21	38.1	19	42.9
Ceftazidime	CA_2	21	61.9	0	38.1
Cefuroxime	CXM	21	47.6	28.6	23.8
Imipenem	IPM	21	95.2	0	4.8
Piperacillin	PRL	21	28.6	0	71.4
Carbenicillin	CAR	21	9.5	48	76.4
Ciprofloxacin	CIP	21	90.5	0	9.5
Ofloxacin	OFX	21	61.9	0	38.1
Gentamycin	GN	21	90.5	0	9.5
Amikacin	AK	21	57.1	14.3	28.6

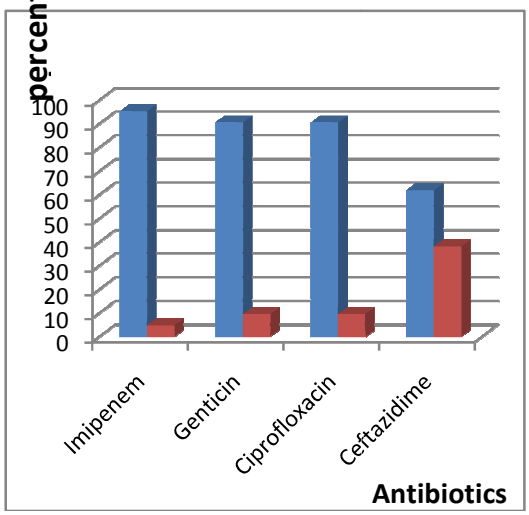
n = no of isolates, S = sensitive, I – intermediate, R = resistant (according to CLSI

FIGURE 1: COMPARISON OF SENSITIVITY PATTERN OF *PSEUDOMONAS* ISOLATES TO IMIPENEM, GENTICIN, CIPROFLOXACIN AND CEFTAZIDIME.



Blue bars represent proportion that is sensitive;
Red bars represent proportion that is resistant.

FIGURE 2: COMPARISON OF SENSITIVITY PATTERN OF *KLEBSIELLA* ISOLATES TO IMIPENEM, GENTICIN, CIPROFLOXACIN AND CEFTAZIDIME.



Blue bars represent proportion that is sensitive;
Red bars represent proportion that is resistant.

DISCUSSION

High prevalence of multidrug resistance indicates a serious need for antibiotics surveillance program. Multiple antibiotic resistance (MAR) analysis has been used to differentiate bacteria from different sources using antibiotics that are commonly used for human therapy. Compared to other methods of bacteria source tracking such as genotypic characterization, the MAR indexing method is cost-effective, rapid and easy to perform. It is also simple and does not require specialized training and expensive equipment [13]. The monitoring of both antibiotic consumption and multiple antibiotic resistances (MAR) especially in nosocomial infections is critically necessary to setting up of effective containment programs and audit of such programs [13, 15].

Pseudomonas and *Klebsiella* are major pathogens in healthcare associated infections (HAI)[12]. This not only because of the attendant significant morbidity associated with infections but also because increasing rates of resistance in them is making it more difficult for them to be treated with cheaper first line antibiotics. Emerging and increasing resistance to newer and otherwise efficacious antibiotics may compound the whole problem[1]. The 30.9% and over 19% prevalence obtained for *pseudomonas* and *klebsiella* respectively out of a sample of 110 clinical isolates approximate to prevalence previously obtained in centre of study [11, 16]

Isolates of both genera demonstrated high levels of susceptibility to the first three drugs (imipenem 32 (94.15%), genticin 19 (91.2%) and ciprofloxacin 29 (85.3%) for *pseudomonas* and *imipenem* 20 (95.2%), Genticin 19 (90.5%), Ciprofloxacin 19 (90.5%) for *klebsiella spp* while varying levels of resistance from moderate to high levels resistance were demonstrated to the cephalosporins. This finding is consistent with that of other studies which reported low resistance profiles for the quinolones and aminoglycosides respectively[8, 12, 16]. However the difference in resistance rates in these works may be (in spite of general similar low rates profile for these drugs) explained by the increasing trend that had been noted globally [1]. This may have implications on the effectiveness of the local and probably national hospital infection and antibiotic resistance control programs. The moderate to high levels of resistance in the cephalosporins underscore the emergence of *B-lactamases,* specifically cephalosporinases among resistant strains of these organisms as previously reported [2,6,10.14.15]. This compromises the clinical efficacy of a very important class of drugs commonly used in the management of many infections in this environment. The clearly visible high rate of resistance of isolates to penicillin (Piperacillin, Carbenicillin) corroborates documented increasing penicillinase-producing *B-lactamases* strains among these organisms [10].

Analysis of the MAR index of isolates showed that 91.2% and 66.7% of *Pseudomonas* and *Klebsiella*

isolates were resistant to three or more antibiotics. These figures were higher than was seen in the series done by Olayinka et al. This may be due to either the difference in the definition used for multidrug resistance in both works or may be an indication of a significant jump in the emergence of multi-drug resistance strains. Olayinka et al in their series estimated their proportion on the basis of resistance to four drugs or more. Three was used in this study as it approximates more with the general definition of multi-drug resistance [2].

All these findings have implications for the choice of antibiotics for empiric management of infections, continuous surveillance of antibiotic susceptibility patters and effective hospital infection control. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are curtailed and continuous education of infection control practices maintained.

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URINARY TRACT INFECTIONS IN A TERTIARY HOSPITAL IN ABUJA, NIGERIA

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ABSTRACT
Background: Urinary tract infections (UTIs) are among the most common bacterial infections. In uncomplicated cases the infection is easily treated with a course of antibiotic, but there is increased resistance to many of these antibiotics.
Objective: To determine the profile of UTI among patients using National Hospital Abuja and the antibiotic susceptibility profile of isolated uropathogens.
Methods: This prospective study was carried out at the department of Medical Microbiology of National Hospital, Abuja over a period of three years (January 2010 – December 2012). A total of 6763 urine samples were analyzed for age, gender, distribution, yield and antibiotics sensitivity.
Results: Of 6763 urine samples, 885 (13.1%) yielded uropathogens, with the highest percentage yield in the below one year and above 57 years age groups. The mean age was 33.9 years and modal group was 25 -32 years. The most common isolates were Escherichia coli 323 (37%) and Klebsiella spp 202 (25%). Although more infections occurred in outpatients than inpatients, the rate was more with inpatients (36% vs 11%). 97% of Klebsiella spp, 89% of E coli and 83% of P.aeruginosa were sensitive to imipenem, while their respective sensitivities to amikacin were 65%, 98% and 96% Most isolates showed high levels of resistance to many other antibiotics tested.
Conclusion: High levels of resistance exist among uropathogens in our study area. This calls for regular surveillance and improved antibiotic stewardship.
Keywords : Urinary tract infection, National Hospital Abuja, antibiotic

INTRODUCTION

Urinary tract infection or UTI is said to exist when a significant number of microorganisms, usually greater than 10^5 cells per millilitre of urine, are detected in properly collected mid-stream " clean catch"urine(1,2). The gold standard for diagnosis is the detection and identification of the causative pathogen in the urine(3).
UTI is one of the most common infections to plague man worldwide(4,5). Over 150 million people worldwide experience an episode yearly, costing the world economy over 6 billion US dollars in treatment and workloss(6). In the USA alone this result in over 8 million visits to the physician office and 1.5 million emergency room visits with about 300,000 admissions(7,8).

The common etiologic agents of UTI include enterobacteriacae like E. coli and Klebsiella spp, as well as Gram positive organisms like Staphylococci and enterococci(9-11) and Candida albicans in patients with underlying physiological debilitation(12). Studies carried out in Enugu, Yola, Zaria and Ife(13-16) show that these are the same etiological agents isolated in Nigeria.

UTI is commonly treated with oral antibiotics like amoxicillin-clavulante, nitrofurantoin, cephalosporins, fluoroquinolones and trimethropin-sulfamethoxazole(17). However, available data indicate that Nigeria has isolates with high levels of resistance(18-21), thus underscoring the need for regular institutional, regional and national surveillance in order to optimize care. The study was designed to profile UTIs as seen at the National Hospital Abuja in order to provide guidelines for optimization of antibiotics treatment of UTIs.

METHOD

The biodata for this study namely, age, gender, ward and clinic, were extracted from the request forms of the patients sent to the laboratory for UTI investigations from January 1, 2010 to December 31, 2012. Samples with incomplete information were excluded from the study. All samples were processed in the Medical Microbiology laboratory of National Hospital, Abuja using standard procedures(22,23). Briefly, samples were examined macroscopically and microscopically, and thereafter inoculated onto CLED and blood agar media, and incubated in air at 35-36^{o}C for 16-24 hours. Significant isolates were identified biochemically and antibiotic sensitivity performed by disc method using modified Kirby Bauer method. Data were analyzed using Microsoft excel 2007 software.

RESULTS

A total of 14700 urine samples were sent to the Medical microbiology department for urine microscopy, culture and sensitivity within the three year study period. 6763 (46%) met the inclusion criteria. 4125 (61%) were from females while 2638 (39%) were from males. There were 5380 (80%) adult samples and 1383 (20%) from children. 6215 (92%) samples were from outpatients while 548 (8%) samples were from in-patients (table 1).

TABLE 1: OUTPATIENT AND INPATIENT DISTRIBUTION OF PATIENTS

Source	Total samples	Positive samples
Outpatients	6215 (92%)	689 (11%)
Inpatients	548 (8%)	196 (36%)
Total	6763 (100%)	885 (13%)

885 (13%) specimens yielded significant growth. The yield from female samples was 14% compared to 12% from males, while it was 13.6% among adults and 11% among children. 36% and 11% of in-patient and outpatient samples respectively yielded significant growth (table 2).

TABLE 2: AGE AND GENDER DISTRIBUTION OF PATIENTS

Age Distribution	Male		Female		Total	
	Total samples	Positive samples (%)	Total samples	Positive samples (%)	Total samples	Positive samples (%)
0 – 1	40	18(45)	32	15(50)	72	33(46)
2 – 8	391	26(7)	399	41(11)	808	67(8)
9 – 16	216	20(9)	337	32(10)	553	52(9)
17 – 24	66	10(15)	402	50(12)	468	60(13)
25 – 32	930	60(7)	1997	200(10)	2927	260(9)
33 – 40	213	26(12)	438	70(16)	651	96(15)
41- 48	266	25(9)	237	50(22)	503	75(15)
49 – 56	118	21(18)	119	30(25)	237	51(22)
57 – 64	104	22(21)	86	38(44)	190	60(32)
>64	222	78(35)	182	53(29)	404	131(32)
Total	2566	306 (12)	4197	579 (14)	6763	885 (13)

The age group with the highest yield was the neonate (46%) and the above 64 year old with 32.4%, while the age group of 25-32 years had the lowest.

The most frequently isolated pathogen was E. coli 37%, followed by Klebsiella spp 25%, P. aeruginosa 8.4%, Proteus spp 7.5%, S. aureus 6.8%, Enteroccocus spp 6.2% and Candida spp at 5.3% (table 3). 50% of all isolates from ICU were Candida spp.

TABLE 3: FREQUENCY OF ISOLATES

Isolates	Number (%)
E. coli	323 (37%)
Klebsiella spp	202(25%)
P. aeruginosa	75 (8.4%)
Proteus spp	67 (7.5%)
S. aureus	60 (6.8%)
Enterococcus faecalis	55 (6.2%)
Candida spp	47 (5.3%)
Others	56 (6.3%)
Total	885 (100%)

97% of Klebsiella spp, 89% of E coli and 83% of P. aeruginosa were sensitive to imipenem, while their respective sensitivities to amikacin were 65%, 98% and 96% (table 4). Sensitivity of P aeruginosa to ceftazidime, ciprofloxacin and gentamicin were 33%, 44% and 24% respectively. 81% of S. aureus isolates were sensitive to nitofurantoin, 80% to amikacin, 73% to amoxicillin/clavulanate and 63% to ciprofloxacin. 83% of all tested isolates of E. faecalis were sensitive to amoxicillin/clavulanate, while 67% and 66% were so for cefuroxime and nitrofurantoin respectively.

TABLE 4: ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES

Antibiotics	Isolates					
	E. coli	Klebsiella spp	P. aeruginosa	Proteus spp	E. faecalis	S. aureus
	T (%S)	T (%S)	T (%S)	T (%S)	T (%S)	T (%S)
Ampicillin	31(1)	-	-	3(0)	3(33)	10(40)
Amoxicillin/ Clavulante	185(27)	71(25)	-	48(19)	24(83)	26(73)
Ceftriaxone	176(67)	54(48)	-	3(0)	6(67)	15(60)
Ceftazidime	-	66(55)	48(33)	45(60)	-	-
Ciprofloxacin	103(53)	28(43)	41(44)	12(25)	6(17)	19(63)
Imipenem	45(89)	37(97)	35(83)	-	4(100)	-
Nitrofurantoin	265(79)	98(40)	-	60(45)	29(66)	43(81)
Gentamicin	143(43)	46(39	42(24	21(43)	5(40)	25(48)
Amikacin	84(98)	62(65)	25(96)	54(67)	14(21)	20(80)

T =Total tested, %S= percentage sensitive

DISCUSSION

The prevalence rate of UTI from this study was 13%, which is lower than figures from previous studies in Yola (67.2%), Enugu (77.9%), South Africa (51%) and India (27%)(14,15,24,25). It is however, higher than the figure for pregnant women in Ghana 9.5%26. The variation in rates may be partly explained by the differences in study populations and in the criteria used by centres in selecting urine samples for culture. Some centers exclude samples from patients clinically diagnosed with UTI or previous antibiotic use(27). The taking of antibiotics prior to presentation at the hospital maybe a key factor in bacterial yield(28).These factors were not considered in this study.

Most of the requests for UTI investigation came from the outpatient department, which sees most of the cases coming in directly from the community, and by extension serves as the clearing house for the specialists' clinics/units. An uncomplicated UTI is unlikely to be admitted into the wards. This finding is consistent with findings in studies from Botswana and the United States(29,30) . However, the yield from inpatient samples was more than three times that of outpatients. This may be due to primary diseases of the patients, some of which compromise the immunity of the patients and the use of invasive devices such as urinary catheters in the hospital setting(8,9,11).

The finding that UTI was more frequent in women than men is in agreement with previous studies(14,24,25,31). However, the yields from samples were not markedly different in the two groups (14% vs 12%). The higher frequency in females has been attributed to the shorter female urethra and the proximity of this to the gastrointestinal outlet, hence making it easier for enteric flora to colonize this area(32,33). Other contributory factors may include the use of contraceptives, childbirth and menopause(34,35).

The incidence of UTI was highest among infants, and thereafter dropped sharply from age two and maintained a rise until age 25-32 when it dropped slightly before maintaining the rise again. Previous studies have shown the incidence of UTI in infants to range from 0.7 – 7% with girls having lower rates than boys(4,36). The very high incidence recorded in this study likely represent contaminations and poor handling of the samples. Further study will be required to determine the true incidence of UTI in our environment. The age group with the highest sample is paradoxically among the group with least incidence. This is a very sexually active age group, and the age that most commonly abuse antibiotics(37), thus explaining the relatively low yield of samples. The high incidence in both sexes with advancing age has been attributed to the presence of a number of risk factors such as prostatic enlargement in males, diabetes mellitus, reduced ambulation, osteoporosis, interventional instrumentations like catheterization and weak bladder sphincter(12).

Gram negative Enterobacteriacae led by E coli and Klebsiella pneumoniae dominated the uropathogens seen in this study, similar to results of other studies elsewhere(14,15,38). S. aureus and enterococcus faecalis were the two Gram positive isolates and made up only 13% of all isolates. Previous studies have found Staphylococcus as an increasing cause of UTI, and attributed this to increased instrumentation like bladder catheterization(11,12,39). The rate of candiduria found in this study is of concern, considering that previous studies in Israel and Italy found rates ranging from 0.14-0.77%(40,41). Although the 5.3% rate in this study is lower than that found in a European study (9.4%)(42), we did not have data to differentiate between true infection and contamination.

The majority of isolates showed resistance to drugs commonly used to treat UTIs. Imipenem was broadly the most sensitive drug, followed by amikacin, and ceftriaxone, and these are not drugs often deployed as first line in the treatment of uncomplicated UTI. Although different studies in different parts of the world and in different parts of the same country found different resistance rates to different drugs over time(14-17,43-50) it is important that emphasis be paid to local resistance patterns as these have the greatest impact on care. These variations in susceptibility may be due to the prescription habits in different localities as inappropriate exposure to antibiotics drives development of resistance. From the results of this study it is certain that choosing drugs for empiric treatment will be challenging as no single common drug can conveniently be recommended for

that. This reinforces the need for mandatory urine culture for all suspected UTIs to properly guide therapy.

In conclusion, E. coli remains the most frequent isolate from our environment causing UTI. However this pathogen, as well as other enterobacteriaceae especially K. pneumoniae, isshowing increased resistance to beta-lactam, aminoglycoside and fluoroquinolones. Regular antibiotic surveillance and practice of antibiotic stewardship will help stem the tide of resistance and improve care.

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KNOWLEDGE, ATTITUDE AND PRACTICE OF BLOOD CULTURE: A CROSS SECTIONAL STUDY AMONG MEDICAL DOCTORS IN A NIGERIAN TERTIARY HOSPITAL.

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ABSTRACT

Background: Blood culture is one of the most important investigations done in clinical microbiology laboratories. Not only has it been long recognized as the "gold standard" for diagnosis of Blood Stream Infections (BSIs), very important decisions regarding septicaemic patients' management are based on it. Being a user-dependent diagnostic test, quality of results often depends on the performer.

Aim: To study the knowledge, attitude and practice of blood culture among doctors in a Nigerian tertiary hospital.

Materials and Methods: A pre-tested self-administered semi- structured questionnaire developed by the research team was used to access the biodata, knowledge, attitude and practice of blood culture among doctors in our institution.

Results: Forty-eight (54.5%) out of the 88 doctors studied had good knowledge regarding blood culture, 34 (38.6%) moderate knowledge and 6 (6.8%) poor knowledge. Majority of the senior registrars (75.0%), registrars (64.3%) and house officers (65.9%) studied had good knowledge while majority of the consultants (75.0%) had moderate knowledge. Doctors from paediatrics (62.5%) and internal medicine (60.0%) departments had higher proportions with good knowledge compared to those from surgery (57.9%) and obstetrics and gynaecology (45.0%) (p = 0.240). Majority of the doctors with <10 years experience as doctors (57.0%) had good knowledge compared to 33.3% recorded among those ≥10 years. Attitude and practice was generally positive.

Conclusion: Through this study areas of unsatisfactory knowledge, attitude and practice of blood culture were identified. This will help in designing an educational intervention programme for the purpose of addressing identified problems areas in blood culture.

KEYWORDS: Blood culture, Knowledge, Attitude, Practice, Doctors.

INTRODUCTION

Blood culture is one of the most important investigations done in clinical microbiology laboratories. It has long been recognized as the "gold standard" for diagnosis of Blood Stream Infections (BSIs) which accounts for 10% of all nosocomial infection with mortality approaching 15% [1]. Not only will blood culture help in the isolation of offending pathogens, it also allows susceptibility tests to be carried out on isolates. Thus very important decisions regarding the choice of antibiotics for managing patients with BSI are based on blood culture results. It is therefore very crucial that the test must be done with best practices.

Evidence has shown that this very important test is often sub-optimally done. According to American Society of Microbiology, the rate of contaminants of blood cultures should not exceed 3% [2]. However, the baseline contamination rates of many institutions in the developed countries are often higher than this rate [3] [4] [5]. The situation in developing countries like Nigeria may even be worse. The consequences of increased contamination rate of blood culture are grave. Hospital bill are usually increased while clinicians are confused, especially when there is discordance between results and clinical features [2] [6].

Being a highly user-dependent diagnostic test, the quality of blood culture results does not only depend on the nature of the underlying infectious process but more importantly on the performer [7]. For example when aseptic procedures during specimen collection are strictly adhered to, there were significant reductions in contamination rates [8] [9]. Also correct timing of sampling in relation to fever and antibiotics administration; and sampling of adequate volume of blood are other user-dependent factors that affect yield [7] [8] [9]. Whereas the level of knowledge, training and years of experience of medical personnel impacts so much on resource utilization and diagnostic test use [7] [10], there is a dearth of information on the knowledge, attitude and practice of blood culture among doctors. This study was therefore aimed at studying the knowledge, attitude and practice of blood culture among medical doctors in our institution.

METHODOLOGY

This descriptive cross-sectional study was carried out among doctors working at the University of

Uyo Teaching Hospital, a tertiary institution located at the south-south region of Nigeria.

A pre-tested self-administered semi- structured questionnaire developed by the research team was used to access the biodata, knowledge, attitude and practice of blood culture among doctors in our institution. The study population comprised of different cadres of doctor viz: house officers, registrars, senior registrars and consultants, from different departments including internal medicine, paediatrics, surgery, obstetrics and gynaecology and others.

Verbal consents were sought and obtained from the different heads of departments and the doctors themselves, and each consenting doctor was handed the questionnaire to complete. The mean time for completing the questionnaire was 10 minutes. Confidentiality was assured and strictly maintained. Completed questionnaires were collected by the investigators and data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.

The questionnaire comprised of three sections viz; sociodemographic, knowledge assessment (comprising of seven questions) and attitude and knowledge (comprising of seven questions) sections. The seven questions used to access level knowledge of blood culture covered what a set of blood culture comprised, the number of sets required in standard blood culture, nature of organisms supported by a blood culture set, temperature at which blood cultures are incubated, the necessity of strict asepsis during sampling and the effect of prior antibiotics use and volume of blood sampled on recovery of organisms. Correct answer for each question was scored 2 and incorrect or unsure answers were scored zero. Total scores of 0-4, 5-9 and 10-14 were categorized as poor, moderate and good knowledge. Fisher's exact or Chi square, where appropriate, was used to assess associations between level of knowledge and socio-demographic variables. Significant association was presumed at p value less than 0.05.

RESULTS

A total of 88 doctors took part in the study comprising of 56 (63.6%) males and 32 (36.4%) females. Majority of the doctors studied (58.0%) were in the age bracket 20-29 years, followed by 30-39 years age bracket (27.3%); age bracket ≥50years had only one representation (1.1%) as shown in Table 1. More than half of the doctors studied (54.5%) were house officers, 28 (31.8%) registrars, 8 (9.1%) consultants, and 4 (4.5%) senior registrars (Table 1).

Of the 88 doctors studied, 24 (27.3%) were from paediatrics department, 20 (22.7%) each from internal medicine and obstetrics/gynaecology respectively, 19 (21.6%) from surgery and 5 (5.7%) from others departments (Haematology{x2}, Family Medicine, Clinical Chemistry, and Psychiatry). Majority of the doctors studied (89.7%) had practiced for less than 10 years (Table 1).

Regarding the individual questions assessing knowledge of blood culture, more than half of those that responded (57.6%) knew that a set of blood culture comprises of two blood culture bottles, 80.5% were aware that a set of blood culture should support the growth of both aerobic and anaerobic organisms while 57.5% knew that standard blood culture should comprise of 2-3 sets of blood culture bottles (Table 2).

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF DOCTORS STUDIED.

Characteristics	Number	Percent (%)
Gender		
Male	**56**	**63.6**
Female	**32**	**36.4**
Age groups (years)		
<20	**6**	**6.8**
20-29	**51**	**58.0**
30-39	**24**	**27.3**
40-49	**6**	**6.8**
≥50	**1**	**1.1**
Rank		
House officer	**48**	**54.5**
Registrar	**28**	**31.8**
Senior registrar	**4**	**4.5**
Consultant	**8**	**9.1**
Department		
Paediatrics	**24**	**27.3**
Internal Medicine	**20**	**22.7**
Obstetrics/Gynaecology	**20**	**22.7**
Surgery	**19**	**21.6**
Others	**5**	**5.7**
Years of Experience (years)		
<10	**79**	**89.7**
≥10	**9**	**10.3**

TABLE 2: DISTRIBUTION OF RESPONDENTS BY KNOWLEDGE OF BLOOD CULTURE

Variable	Frequency (%)
A set of Blood culture comprises of two blood culture bottles.	
Agree	47 (57.6)
Unsure	19 (22.4)
Disagree	17 (20.0)
Total	83
A set of Blood culture should support the growth of both aerobic and anaerobic organisms.	
Agree	70 (80.5)
Unsure	7 (8.0)
Disagree	10 (11.5)
Total	87
Standard Blood culture should comprise of 2-3 sets of blood culture bottles.	
Agree	50 (57.5)
Unsure	26 (29.9)
Disagree	11 (12.6)
Total	87
Blood cultures are usually incubated at 37^{0}C.	
Agree	33 (37.9)
Unsure	22 (25.3)
Disagree	32 (36.7)
Total	87
Strict asepsis is necessary during sampling.	
Agree	72 (83.7)
Unsure	12 (14.0)
Disagree	2 (2.3)
Total	86
Antibiotics use before sampling affects organisms yield.	
Agree	85 (96.6)
Unsure	0 (0.0)
Disagree	3 (3.4)
Total	88
Volume of blood sampled affects quality of result.	
Agree	24 (28.6)
Unsure	42 (50.0)
Disagree	18 (21.4)
Total	84

Further analysis of data showed that 48 (54.5%) out of the 88 doctors studied had good knowledge regarding blood culture, 34 (38.6%) moderate knowledge and 6 (6.8%) poor knowledge (Table 3). The proportion of female that had good knowledge (56.3%) was slightly more than the males (53.6%) (p = 0.967) as shown in Table 4. Furthermore good knowledge did not vary in any particular direction with age. Majority of the senior registrars (75.0%), registrars (64.3%) and house officers (65.9%) studied had good knowledge while majority of the consultants (75.0%) had moderate knowledge (Table 4).

TABLE 3: LEVEL OF KNOWLEDGE OF RESPONDENTS

Level of Knowledge	Total score	N (%)
Poor	0-4	6 (6.8)
Moderate	5-9	34 (38.6)
Good	10-14	48 (54.5)

TABLE 4: ASSOCIATION OF DEMOGRAPHIC CHARACTERISTICS WITH LEVEL OF KNOWLEDGE

Characteristics	Level of Knowledge			P value(χ^2 test/ Fisher Exact test)
	Poor (0-4)	Moderate (5-9)	Good (10-14)	
Gender				
Male	4 (7.1)	22 (39.3)	30 (53.6)	0.967
Female	2 (6.3)	12 (37.5)	18 (56.3)	
Age group (yrs)				
<20	0 (0.0)	3 (50.0)	3 (50.0)	
20-29	4 (7.8)	19 (37.3)	28 (54.9)	
30-39	2 (8.3)	7 (29.2)	15 (62.5)	0.477
40-49	0 (0.0)	5 (83.3)	1 (16.7)	
≥50	0 (0.0)	0 (0.0)	1 (100.0)	
Rank				
House officer	4 (10.5)	9 (23.7)	25 (65.9)	
Registrar	1 (3.8)	9 (32.1)	18 (64.3)	0.135
Senior Registrar	1 (25.0)	0 (0.0)	3 (75.0)	
Consultant	0 (0.0)	6 (75.0)	2 (25.0)	
Department				
Surgery	1 (5.3)	7 (36.8)	11 (57.9)	
Internal Medicine	0 (0.0)	8 (40.0)	12 (60.0)	
Paediatrics	1 (4.2)	8 (33.3)	15 (62.5)	0.240
Obs & Gynae	4 (20.0)	7 (35.0)	9 (45.0)	
Others	0 (0.0)	4 (80.0)	1 (20.0)	
Years of Experience				
< 10 years	6 (7.6)	28 (35.4)	45 (57.0)	0.900
≥10 years	0 (0.0)	6 (66.7)	3 (33.3)	

Greater proportions of doctors from paediatrics (62.5%) and internal medicine (60.0%) departments had good knowledge compared to their counterparts from surgery (57.9%) and obstetrics and gynaecology (45.0%) (p = 0.240). Majority of the doctors with <10 years experience as doctors (57.0%) had good knowledge as against 33.3% recorded among those ≥10 years (Table 4).

Regarding attitude and practice of blood culture, while 95.5% of respondents agreed that they sometimes make diagnosis requiring blood culture, only 39.8% of those studied always request for the test when such diagnosis is made (Table 5). Two-third of the respondents (66.7%) still request for blood culture if required when patient is already on antibiotics while 26.4% will not in a similar situation. Majority (82.4%) agreed that drawing blood for routine culture from an intravenous catheter was a wrong practice; only 18.4% practiced single needle technique during sampling against 79.3% that practiced double needle technique (Table 5).

TABLE 5: DISTRIBUTION OF RESPONDENTS BY ATTITUDE AND PRACTICE

Variable	Frequency (%)
I sometimes make diagnosis requiring blood culture	
Agree	**84 (95.5)**
Unsure	**1 (1.1)**
Disagree	**3 (3.4)**
Total	**88**
I always request for blood culture each time I make diagnosis requiring it.	
Agree	**33 (39.8)**
Unsure	**0 (0.0)**
Disagree	**50 (60.2)**
Total	**83**
If patient is already on antibiotics, I still request for blood culture if indicated	
Agree	**58 (66.7)**
Unsure	**6 (6.9)**
Disagree	**23 (26.4)**
Total	**87**
I do deliver the blood sample collected into the culture bottle with the same needle used for venipuncture rather than changing it.	
Agree	**16 (18.4)**
Unsure	**2 (2.3)**
Disagree	**69 (79.3)**
Total	**87**
It is wrong practice to take blood samples from intravenous catheters for routine blood culture	
Agree	**70 (82.4)**
Unsure	**13 (15.3)**
Disagree	**2 (2.3)**
Total	**85**
Methylated spirit swab of proposed venipuncture site is sufficient skin preparation before sampling	
Agree	**53 (62.4)**
Unsure	**9 (10.6)**
Disagree	**23 (27.1)**
Total	**85**
Am satisfied with the results I get from blood cultures	
Agree	**24 (31.2)**
Unsure	**16 (20.8)**
Disagree	**37 (47.1)**
Total	**87**

TABLE 6: REASONS GIVEN FOR NOT ALWAYS REQUESTING FOR BLOOD CULTURE WHEN REQUIRED (N=20).

Reasons	Frequency (%)
Delay in getting results	**6 (30.0%)**
Blood culture bottles not readily available	**5 (25.0%)**
Cost consideration for the patients	**5 (25.0%)**
Results often not convincing	**2 (10.0%)**
Patients already on antibiotics	**1 (5.0%)**
Not a requirement for treating every case	**1 (5.0%)**

TABLE 7: REASONS GIVEN FOR THINKING BLOOD CULTURE RESULTS ARE NOT SATISFACTORY (N = 17).

Reasons	Frequency (%)
Results usually delayed	**6 (35.3)**
Results often negative	**4 (23.5)**
Always growing Staphylococcus	**3 (17.6)**
Don't isolate anaerobes	**2 (11.8)**
Results often not agreeing with clinical signs	**1 (5.9)**
Most patients on antibiotics prior to culture	**1 (5.9)**

Table 6 shows the reasons why doctors studied do not always request for blood culture when they make diagnosis requiring it. "Delay in getting results" was the main reason (6/20; 33.3%), followed by "blood culture bottles not readily available" and "cost consideration for the patients", each accounting for 25.0% of reasons given. Out of the 17 respondents that gave reasons why they thinks blood culture results were not satisfactory, 6 (35.3%) felt "delay in getting result" was their problem, 4 (23.5%) felt result were often negative, while 3 (17.6%) felt blood cultures are always growing staphylococcus (Table 7).

DISCUSSION

There are limited studies on knowledge, attitude and practice of doctors on blood culture among medical doctors. Doctors are the ones that request for blood cultures and in most hospitals, especially tertiary institutions, are responsible for sampling for blood culture and transporting same to the laboratories for incubation and further processing. Therefore by virtue of training and practice they are expected to have good knowledge of blood culture. In this study 54.5% of the doctors studied demonstrated good knowledge of blood culture.

There are however specific areas of knowledge regarding blood culture that lower than expected performance was recorded. Regarding the temperature at which blood cultures are incubated, for instant, only 37.9% of doctors studied agreed that 37 ^{0}C was the temperature for incubation, 25.3% were unsure while 36.7% disagreed. Perhaps the reason for the lower performance on this question is that most doctors, apart from the laboratory physicians, do not have sufficient knowledge of the happenings in the laboratory. As soon as specimens are submitted at the receptions of the laboratories they are done and only wait for the results. It is advocated that all doctors are made to rotate through the laboratories to acquaint themselves with how specimens are further processed beyond reception, as experience garnered during undergraduate laboratory posting appears not to be sufficient.

Another specific area where level of knowledge was below expectation in this study was regarding volume of blood sampled affecting quality of result as only 28.6% agreed that volume of blood sampled affects quality of result; the rest either disagreed or were not sure. Volume of blood per culture has always been known as the single most important variable affecting recovery of microorganisms from patients with sepsis. Several studies have confirmed that the higher the volume cultured, the higher the rate of detection of bloodstream infection, reporting increase in yield from 0.6-4.7% per extra ml of blood cultured [11] [12]. Inadequate volume of blood is a common problem observed during blood culture sampling. Connell et al, in their study reported that only 46.0% of blood from infants and children submitted for culture in their centre had adequate volume of blood [13]. However after an educational intervention, there was a significant increase in the proportion of adequate volume of blood collected to 63.9% [13]. Similar educational intervention might be necessary in the study area to bridge the gap in knowledge thus improving the quality of blood culture result.

This study equally revealed that while greater proportion of senior registrars, registrars and house officers had good knowledge of blood cultures, majority of the consultants had moderate knowledge. The reason for this disparity is not known but may be connected to the fact that residents and house officers are more practically involved in blood cultures than the consultants. Patients from paediatrics and internal medicine wards often present with septicaemia more than those in surgery and obstetrics/gynaecology wards [14], thus doctors from paediatrics and internal medicine departments would more than their counterparts from other specialties request for and be more conversant with blood culture. It therefore follows that level of knowledge of blood culture among doctors from paediatrics and internal medicine, as found in this study, is expected to be higher than other specialties.

The attitude and practice of blood culture from this study was generally positive. It is of note that whereas 95.5% of the doctors studied sometimes make diagnosis requiring blood culture, only 39.8% always request for the test whenever such diagnosis is made. This figure is considerably low. Out of the 50 (60.2%) doctors that did not always request for blood cultures when indicated only 20 (40.0%) indicated why, with the most common reason given being "delay in getting results"(30.0%). Timeliness of results reporting has been a major concern in most clinical laboratories due to increasing pressure from clinicians to report results rapidly. Even though there are only sparse data, timeliness in reporting of laboratory results undoubtedly affects clinician and patient satisfaction as well as length of hospital stay [16]. Improving turnaround time (TAT) is a complex task involving education, equipment acquisition, and planning [15]. Other common reasons given are "unavailability of the blood culture bottle" (25.0%) when needed, a peculiar problem in the study area requiring attention, and "cost consideration for the patients" (25.0%), an important factor affecting utilization of hospital services this part of the world.

Blood culture yields are known to be significantly lower among patients with pre-culture antibiotic use compared with those without antibiotic use [16]. This perhaps may have influenced the opinion of 26.4% of respondents who would not request for blood culture when indicated if the patients are already on antibiotics. The implication is that the few cases of BSI that would have yielded positive

blood culture are missed and may not be properly treated, especially in this part of the world where most of our patients have taken some antibiotics before presenting to the hospital.

CONCLUSION

Through this study areas of unsatisfactory knowledge, attitude and practice of blood culture were identified. This will help in designing an educational intervention programme for the purpose of addressing identified problems areas in blood culture.

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PRESUMPTIVE DIAGNOSIS OF SCHISTOSOMA HAEMATOBIUM AND SCHISTOSOMA MANSONI USING MICROSCOPY AS GOLD STANDARD IN A RIVERRINE COMMUNITY OF SOUTHWESTERN NIGERIA

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ABSTRACT
A cross-sectional study was carried out in Ilie community of Olorunda Local Government Area in Osun state, southwestern Nigeria to comparatively evaluate the presumptive diagnosis of schistosoma infections using microscopy as gold standard. One hundred and thirty seven consented primary school children aged 4 to 15 years were examined for presence of schistosome eggs. The urine samples were analyzed with urinalysis strips for microhaematuria as indicators of presumptive diagnosis for urinary schistosomiasis while fecal samples were analyzed with fecal occult blood test kits for occult blood detection as an indicator of presumptive diagnosis for intestinal schistosomiasis. The indicators of presumptive diagnosis were compared with microscopy examination of urine and stool while sensitivity and specificity of the presumptive diagnostic methods were determined. The results of the prevalence showed that 107(78.1%) had co- infection and overall prevalence of 73.5% and 26.3% recorded for both *S. haematobium* and *S mansoni* infection respectively. It was observed that the use of microhaematuria alone had 52% sensitivity and 91.67% specificity while stool occult blood recorded 73.685 and 66.67% for sensitivity and specificity respectively. This study shows that presumptive diagnosis of urinary schistosomiasis is significantly more sensitive (P<0.05) than intestinal schistosomiasis. Also, various degrees of co- infections were observed across all age groups of study subjects with age group 10-12years exhibiting highest co- infection rate 48(13.4); and tendency towards increased transmission and re-infection. Use of these alternatives is recommended in resource limited settings, to be confirmed by gold standard when feasible.

Keywords: Presumptive diagnosis, *Schistoma haematobium*, *Schistoma mansoni*, Microscopy, Holoendemic Community.

INTRODUCTION

Schistosomiasis is one of the most prevalent parasitic infection after malaria, with nearly 207 million people infected, and 779 million currently at risk in 76 countries of the tropics where the disease is endemic (1).In sub- Sahara Africa, about 192 million are found to be infected with the disease (2).It is the most prevalent of the waterborne parasitic diseases and one of the greatest risks to health in rural areas of the developing world and the intensity of the infection rises with age and peaks usually between 15and 20 years of age (3). Schistosomiasis is a parasitic disease caused by blood flukes (Trematode). Intestinal Schistosomiasis caused by *S mansoni* occurs in 52 nations including Caribbean countries (4) while *S haematobium*,- causative agent of urinary schistosomiasis- is endemic in 54 countries mainly in Africa and Eastern Mediterranea (5).Co-Infection of S.haematobium and S.mansoni has been reported in various parts of Africa and Nigeria (6,7and 8). Most studies on the epidemiology of schistosomiasis are usually based on microscopic parasitological technique which is often cumbersome, time consuming and reagent dependent (9) .The resources to accomplish this is often absent in rural areas of Nigeria. The lack of a widely accepted alternative diagnostic technique in these areas, are responsible for the inadequate containment of the continued transmission of these infections. Thus, this study is directed at establishing presumptive diagnostic methods and comparing same with microscopy as gold standard.

METHODOLOGY

Study Area: The study was carried out in Ilie community of Osun State. Ilie is situated in Olorunda Local Government Area of Osun State, Southwestern Nigeria. Its geographical coordinates are 7° 58' 0" North, 4° 32' 0". The community has a population less than 5000 persons with an annual rate growth of about 3%. The inhabitants are of the ethnic Yoruba speaking group with farming and fishing as their predominant occupation. In the community, there are two predominant primary schools with one secondary school. More than 70% of the population of the community access water from the local river for use.

Sampling Technique

A total of 137 subjects within the age range of 4-15 years were randomly selected among the primary and junior secondary schools in the study area. At least, 10 eligible subjects were selected in each class until maximum sample size was reached. Before the commencement of sample collection and questionnaire administration, Ethical approval were obtained from both Osun State Universal Basic Education Board and Local inspector of Education while informed consents were obtained from the parents/guardians through Parents Teachers Association(PTA) forum. A Pre-survey visit was made to the school in order to familiarize and educate the school authorities about the importance of the study.

Sample Collection and Processing

Two specimen containers were given to each subject and the procedure for introduction of stool and urine specimens into the containers was carefully explained to them .Stool and urine samples were labeled before processing. The urine samples were analyzed within 6 hours of collection to prevent hatching of the schistosome eggs. The urine specimens were observed for presence of colour, visible blood and turbidity. Microhaematuria and proteinuria analysis were carried out by using urinalysis strip (combistic 9) Urine deposit obtained by centrifuging 10ml of urine in a conical tube 1000g was examined microscopically using 10x objective with condenser closed sufficiently to give good contrast. Stool analysis, and faecal occult blood test were carried out as described by Chessbrough (2005) (9). Data obtained from stool and urine analysis were used to evaluate their sensitivity and specificity according to World Health Organisation Format (10) using microscopy as Gold standard.

Sensitivity (%) =Total true positive x100/Total true positive + Total false positive

Specificity (%) = Total true negative x100/Total true negative+Total false positive

RESULTS

From a total of 137 examined, 107(78.1%) were found to have co- infection and the prevalence did not show significant variation with age ($P<0.05$). An overall prevalence of 73.5% urinary schistosomiasis and 26.3% of intestinal schistosomiasis was observed in this study (Table 1).

TABLE1: DITRIBUTION OF SCHISTOSOMA MANSONI AND SCHISTOSOMA HAEMATOBIUM AMONG STUDY SUBJECTS

Age	No Examined infected	Freq (%) *S.mansoni*	Freq (%) *S.haematobium*	Freq (%)
4-6	33	30(25.3)	17(16.2)	25(25.7)
7-9	55	52(29.00	29(16.3)	44(24.6)
10-12	21	15(33)	32(36.3)	35(44.3)
13-15	28	10(30.0)	3(15.0)	9(31.5)
TOTAL	137	107(73.5)	81(26.3)	113(17.6)

Using strip microhaematuria test alone as an indicator of presumptive diagnosis of urinary schistosomiasis in this study, it was observed that there was 38.2% true positive results, 35.3% false negative results, 2.2% false positive results and 24.3% true negative results (Table2).

Table 2: SENSITIVITY AND SPECIFICITY OF MICROHAEMATURIA TECHNIQUE FOR SCHISTOSOMA HAEMATOBIUM USING MICROSCOPY AS GOLD STANARD

	Micro - haematuria(%)	Microscopy(%)
No of true Positive(%)	38.2	50.3
False Negative(%)	35.3	62.3
False Positive(%)	2.2	20.3
True Negative(%)	24.3	13.4
Sensitivity(%)	52.0	
Specificity(%)	91.67	

TABLE 3: SENSITIVITY AND SPECIFICITY OF OCCULT BLOOD TECHNIQUE FOR SCHISTOSOMA MANSONI AND MICROSCOPY GOLD STANARD

	Occult Blood(%)	Microscopy(%)
No of true Positive(%)	14.8 0	26.30
False Negative(%)	5 3.2 0	62.30
False Positive(%)	26.6 0	12.20
True Negative(%)	62.70	76.30
Sensitivity(%)	73.68	
Specificity(%)	66.67	

Table 4: DISTRIBUTON OF CO-INFECTION AMONG AGE –GROUPS

Co-Infecton	Age(years)	Freq (%) Infected
A.lumbricoide+ *S.haematobium*	4-9	39(17.40)
S.haematobium+ *S.mansoni*	10-12	48(34.40)
A.lumbricoides+ *S.mansoni*	12-13	38(11.70)
S.mansoni + *A.duodenale*	13-15	12(4.30)

This indicator thus has 52% sensitivity, 91.67% specificity, 94.55% positive predictive value and 40.74% negative predictive value. Considering sensitivity and specificity of stool occult blood technique with microscopy for the diagnosis *Schistosoma mansoni*, it was observed that this indicator has 14.89% true positive results, 5.32% false negative results, 26.60% false positive results and 53.19% true negative results(Table3). This indicator thus has 73.68% sensitivity and 66.67% specificity with a positive predictive value of 35.90% and a negative predictive value of 90.9%.the co-infection observed include *Ascaris lumbricoides* and *Schistosoma haematobium*, *Schistosoma haematobium* and *Schistosoma mansoni*, *Ascaris lumbricoides* and *Schistosoma mansoni*, Schistosoma mansoni and hook worms and highest co infection observed among the age group10-12 years.

DISCUSSION

This research work revealed the possibility of the use of presumptive diagnosis in rural communities that lack the facilities of microscopy. This study agrees with similar studies in endemic areas (8). High prevalence of Schistosomiasis recorded suggests endemicity and probably leads to higher degree of environmental contamination which as a result of water contacts activities; promotes the infection rate. *S. hamatobium* are more common than *S.mansoni* infection and this finding agrees with studies carried out by Gundiri and Okwuosa (2001) which reported that urinary schistosomiasis is more prevalent than intestinal schistosomiasis (10).

Using micro haematuria as indicators of presumptive diagnosis, for urinary schistosomiasis with microscopy as the gold standard, the indicator is not reliable enough for presumptive diagnosis. High true positive results recorded may probably due to other health conditions and subjects into further investigation hence microscopy which is the gold standard remains the choice of examination of urinary schistosomiasis. Fecal occult blood for intestinal schistosomiasis reveals 73.68% sensitivity and 66.67% specificity, thus, making it a good alternative for investigation of intestinal schistosomiasis in an area where microscopy method is absent. This finding agrees with the results of previous studies around the globe (11, 12 and 13) and in Nigeria (14, 15, 16, 17 and 18). Other parasites discovered in this course of study are *Ascaris lumbricoides* and *hookworm*. This indicates that more research needs to be done on the prevalence of helminthic infections in this community

In conclusion, more investigations need to be done on the use of microhaematuria and proteinuria as indicators of presumptive diagnosis for urinary schistosomiasis in other endemic areas in Nigeria because most places lack adequate facilities for the use of microscopy method. Also, adequate interventional strategies should be put in place at the endemic regions to prevent transmission and re-infection. This should be done with adequate information of the etiological and disease transmission knowledge of schistosomiasis to local inhabitants of endemic areas. Deployment of more human and material resources to endemic communities will help in stemming the spread of both urinary and intestinal schistosomiasis.

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THE RELATIONSHIP BETWEEN PERCEPTION AND PREVALENCE OF FAECAL-ORALLY TRANSMITTED PARASITIC INFECTIONS AMONG SCHOOL CHILDREN IN A RURAL COMMUNITY IN CAMEROON

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ABSTRACT

Background: Faecal-orally transmitted parasites are those which are spread through faecal contamination of food and drinks. Infections with these parasites are responsible for high morbidity and mortality, especially in children in developing countries.

Objective: This study was carried out to determine school children's perception of faecal-orally transmitted parasitic infections and the relationship between that perception and the prevalence of the infections.

Methods: Data were collected through questionnaires and laboratory analysis of stool samples. The study was conducted in two phases. In phase 1 questionnaires were administered to determine children's knowledge on the cause, risk behaviours and prevention of the faecal-orally parasite infections. Stool specimens were analyzed using the formol-ether concentration technique. Health education was utilized in the experimental village, but not the control. Phase 2 was conducted six months later during which questionnaires were distributed and stool samples analyzed from both villages.

Results: A total of 370 children were enrolled in this intervention study, out of which 208 were from Kake II (experimental arm) and 162 from Barombi-kang (control arm). At Kake II there was a significant increase in awareness in relation to the source of infection (9.5% vs. 62.5%, P< 0.001), risk behaviour (12.4% vs. 83.7, P<0.001) and prevention (17.9% vs. 84.8%, P<0.001) between the first and second phase of the study, followed by a significant change in the prevalence of *Ascaris lumbricoides* (24.9% vs. 3.4%, P<0.001), *Entamoeba coli* (12.9% vs. 6.5%, P<0.001), *Trichuris trichiura* (22.4% vs. 12.5%, P=0.004) and *Entamoeba histolytica* (6.0% vs. 1.9%, P=0.035). In Barombi-kang the change in the awareness was not significant (P>0.1) and there was no significant change in the prevalence of any of the faecal-orally transmitted parasites detected. The relationship between the perception and the prevalence of feacal orally transmitted parasitic infections showed a strong negative correlation (r dispersed between -0.97 and -99)

Conclusion: Health education applied in the experimental village was responsible for the changed perception of infection by children and consequently for the reduction of infestation rate. Good perception of the infection was inversely proportional to its prevalence. Therefore, health education through the framework of school proved to be an effective control method for faecal-orally transmitted parasite infections. We recommend this inexpensive method to be adopted as a national policy in developing countries, especially in rural communities.

Key Words: Perception and prevalence, Faecal-orally transmitted parasitic infections, School children, Cameroon

INTRODUCTION

Parasitic infections, caused by intestinal parasites, are among the most prevalent infections in humans in developing countries and are responsible for considerable morbidity and morality in endemic countries. Most of them are transmitted by the faecal-oral route. In general, situations involving close human-human contact and unhygienic conditions promote transmission (1).These infections are globally endemic and have been described as constituting the greatest single worldwide cause of illness and disease (2). They are associated with poor hygiene and lack of access to safe water. Several factors including temperature, humidity, natural disasters, socioeconomic status and customary nutrition of people play a role in the distribution of these parasites (3). Food handlers also play an important role in their transmission (4,5).

The most common of these faecal-orally transmitted parasites are the soil transmitted helminth (STH) which include *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Necator americanus* and

Ancylostoma duodenale), and in developing countries it is common that children are parasitized with more than one species at the same time, with resultant impairments in physical, intellectual, and cognitive development, if heavy infections are left untreated (6,7). These infections rarely cause death. Instead, the burden of disease is related less to mortality than to the chronic and insidious effects on health and nutritional status of the host (7).

Like the majority of the parasitic diseases, these infections are influenced by human behaviour especially their hygienic practices, and also failure to take advantage of available screening services or comply with treatment (8). Control measures against intestinal parasites include chemotherapy and sanitation. Chemotherapy is an effective short term measure while sanitation is effective long term control measure (9). Control in school age children delivered through school system is the main intervention strategy in a community (10). Morbidity and worm load have been found to decrease considerably where health care has been efficiently integrated with education programmes (11).

In Cameroon, intestinal parasite infections are recognised by the ministry of public health to be an important public health problem ranking second to malaria (12). Soil transmitted helminths are highly endemic in the south west region of the country, particularly in rural areas where sanitary conditions are poor (13,14). Control typically focuses on periodic antihelminthic treatments targeted at specific risk groups, for example; school children. Re-infection in endemic areas is continuous (15,16). Chemotherapy remains the main means of managing morbidity from faecal-orally transmitted parasites. However, the prevention of re-infection and the reduction in the incidence of infection require that population have a good perception of the disease as a health problem. This study therefore aims at determining school children's perception of faecal-orally transmitted parasitic infections and evaluates the relationship between that perception and infection prevalence.

MATERIALS AND METHODS

Ten villages in the Kumba district in the South West Region of Cameroon were grouped in pairs. Each pair was made of 2 rural communities sharing the same social, geographical and climatic features. The pair comprising Kake II and Barombi-Kang was randomly selected among five. For the selection of experimental and controls arm, a coin was tossed and the decision taken was based on the observed side. An intervention study was conducted in the primary schools of the selected villages (Kake II and Barombi-kang). They are separated by a distance of 30 Km. The former village served as the experimental arm, whilst the latter served as the control arm. The two villages are typical African rural communities. There is no pipe-borne water, electricity, or drainage system. Each of the villages has a government owned primary school, but no secondary school. The inhabitants are farmers practicing peasant farming and petty trading.

Prior to the start of this study, permission was sought from the school authorities and parents were informed about the purpose, objectives and benefits of the study, as their involvement was a key factor for its success. They were made to understand that it was not a school obligation to take part in the study, neither was it a prerequisite for accessing publicly available health facilities. Written informed parental assent forms were distributed one week prior to the beginning of data collection. An ethical clearance was obtained from the Institutional Review Board (IRB) of the Regional Delegation of Health (Ref.R11/MPH/SWP/RDPH/FP-R/5341/94).

Phases of the study

The study was conducted in two phases.

Phase 1

Data were obtained from questionnaire and laboratory analysis of stool samples. School children were administered questionnaires structured to provide information on personal bio data (name, age, sex) and their awareness toward faecal-orally transmitted parasites. Children questionnaires were prepared and distributed through the head masters of the schools and the teachers with the aim to test their perception of the source of infection, the prevention measures and the treatment seeking behaviour. Questionnaires were returned through the same channel. Stool samples were collected in well labelled 50 ml screw-cap vials and then transported to the Kumba district hospital laboratory. Samples were collected from volunteer pupils aged between 5-15 years. Stool specimens were analyzed using the formol-ether concentration technique as already described elsewhere (17). All slides were read within 24 h of preparation to avoid the degeneration of *Ancylostoma sp*. eggs. Following sample collection, health education was given to the pupils in Kake II school (experimental arm), and it was aimed at promoting and reinforcing health behaviour with particular reference to the need to encourage aspects of personal hygiene relevant to the control of faecal-orally transmitted parasitic infections. Focus group discussions were held in the school using pictorial cards. Basic messages communicated were: what faecal-orally transmitted parasites are, how the infections are acquired, what the signs and symptoms are, what can be done to prevent these parasites, and the importance of visiting the health centre for treatment. In Barombi-Kang (control arm), only the baseline data were collected without any health education.

Phase II

This phase was conducted 6 months after the end of the first intervention. It consisted of administering the same pre-designed and pre-tested questionnaire to assess children's perception and collecting stool samples for laboratory analysis. For ethical reasons, pupils in the control village (Barombi-kang) received health education at the end of the sample collection.

Data Management and Analysis

Data was entered using Epi-Info 6.04 (CDC) and analyzed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc. 2008). The Chi-Square test was used to compare proportions before and after the health education intervention at significant level of 0.05.

RESULTS

Table 1 shows the perception of faecal-oral parasite infection in Kake II (experimental arm) and Barombi-Kang (control arm) before and after health education intervention. Awareness levels in the two villages was low during the first intervention (dispersing between 9.5% and 18.4%). In the second phase, the level of awareness increased significantly in Kake II with respect to the source of infection (9.5% vs. 62.5%, P<0.001), risk behaviour (12.4% vs. 83.7%, P<0.001) and prevention and/or treatment (17.9% vs. 84.6%, P<0.001) of faecal-oral parasite infection. There was no significant increase in the control arm with respect to the source of infection (6.7% vs. 10.1%, P=0.271), risk behaviour (17.2% vs. 20.8%, P=0.397) nor prevention and/or treatment (18.49% vs. 25.6%, P=0.115).

TABLE 1: CHILDREN'S PERCEPTION OF FAECAL-ORAL PARASITES INFECTION IN KAKE II (EXPERIMENTAL ARM) AND BAROMBI-KANG (CONTROL ARM) BEFORE AND AFTER HEALTH EDUCATION (HE) INTERVENTION

Perception	Number (%)* of pupils with good knowledge in Kake II (experimental arm)		Number (%)* of pupils with good knowledge in Barombi-kang (control arm)	
	Before health education	After health education	Phase 1 intervention	Phase 2 intervention
Source of infection	19 (9.5)	131 (62.5)	11 (6.7)	17 (10.1)
Risk behaviour	25 (12.4)	174 (83.7)	28 (17.2)	35 (20.8)
Mode of prevention and/or treatment	36 (17.9)	178 (84.6)	30 (18.4)	41 (25.6)

P< 0.001 in Kake II (experimental arm); P>0.1 in Barombi-Kang (control arm)
***Percentage is based on the number of respondents**

TABLE 2: PREVALENCE OF DIFFERENT SPECIES OF FAECAL-ORALLY TRANSMITTED PARASITES IN KAKE II (EXPERIMENTAL ARM) AND BAROMBI-KANG BEFORE (CONTROL ARM) BEFORE AND AFTER HEALTH EDUCATION INTERVENTION

Parasite species	Number (%) of infected stool samples in Kake II (experimental arm)		Number (%) of infected stool samples in Barombi-Kang (control arm)	
	Before health education	After health education	Phase1 intervention	Phase 2 intervention
*A. lumbricoides**	50 (24.9)	7 (3.4)	28 (17.3)	29 (18.1)
*T. trichiura**	47 (22.4)	26 (12.5)	18 (11.1)	16 (0.1)
E. vermicularis	3 (1.5)	4 (1.9)	10 (6.2)	9 (5.6)
Ancylostoma sp.	8 (4.0)	7 (3.4)	12 (7.4)	18 (11.2)
H. nana	0 (0.0)	1 (1.6)	1(0.6)	2 (1.2)
*E. histolytica**	12 (6.0)	4 (1.9)	16 (9.8)	15 (9.3)
*Entamoeba coli**	29 (12.9)	8 (6.5)	21 (12.9)	20 (12.5)
G. lamblia	3 (1.5)	2 (1.0)	6 (3.7)	7 (4.3)
B. coli	1 (0.5)	1 (0.5)	1 (0.6)	1 (0.6)

*** P< 0.005 in Kake II (experimental arm)**

Table 2 shows the prevalence of different species of faecal-orally transmitted parasites in Kake II (experimental arm) and Barombi-Kang (control arm), before and after the health education intervention. In Kake II, the change in the prevalence of parasites was more significant for *Ascaris lumbricoides* (24.9% vs.

3.4%, P<0.001), *Entamoeba coli* (12.9% vs. 6.5%, P<0.001), *Trichuris trichiura* (22.4% vs. 12.5%, P=0.004) and *Entamoeba histolytica* (6.0% vs. 1.9%, P=0.035). In Barombi-Kang, there was no significant change in the prevalence of any of the faecal-orally transmitted parasites detected.

DISCUSSION AND CONCLUSION

The purpose of this study was to determine the relationship between the perception and the prevalence of faecal-oral parasite infection among school children in a rural community in Cameroon. The ten villages from which our study communities were selected are all found in the the Kumba District. The choice of this District was motivated by previous reports on high prevalence of soil transmitted infection in this area of the country (13,14,15,18). The study was targeted at school children as they are mostly the heavily infected individuals in the community and are at risk of developing severe disease (11). School children are also likely to contribute to transmission and are readily accessible through the framework of school.

According to WHO (9), understanding how people regard a problem such as faecal-orally transmitted parasitic infections makes it easier to communicate with them about it. The results of this study have indicated that before health education sessions were held, only a few school children in Kake II (experimental arm) and Barombi-kang (control arm) had a good perception of faecal-orally transmitted parasite infections. Majority of children linked the infection to eating of sweet foods such as overripe fruits (mostly mangoes) and candies. The misconception of parasitic infections due to ignorance is common in most rural communities in the world, as shown by earlier reports (19,20,21).

A comparison of how children perceived faecal-oral parasites; before and after health education intervention show a significant difference between the first and second phase of the study, in Kake II (experimental arm) but not in Barombi-kang (control arm). This result portraying the impact of health education on disease perception is in accordance with previous findings (19,22), and proves that children who are conversant with the health knowledge of the disease are more knowledgeable of the consequences of many attitudes and practices than those who have not been exposed to such an opportunity.

Comparing the prevalence of different parasites in the experimental village where health education was implemented, there was a significant drop in *Ascaris lumbricoides, Entamoeba coli, Trichuris trichuira* and *Entamoeba histolytica*. No significant change was observed in the control arm. The significant change in prevalence observed in Kake II can not be attributed to social or seasonal variation; the thwovillages share the same social, geographical, and climatic features. Only health education applied in the experimental village could have accounted for the observed prevalence decrease. This is in accordance with a previous study carried out in Cameroon (19) and Indian (23). Following health education, children have probably changed their sanitary behaviour and hygienic practices, adopting those which place them less at risk of becoming infected; they might have visited their local health centre for consultation and treatment. However, It can also be argued, that low infestation rate recorded during the second phase of our study in the experimental village is attributed to self-medication on the part of the children and their parents. It is possible that following health education school children developed a tendency to purchase drugs from street vendors and use them indiscriminately without medical supervision. This argument is reinforced by the fact that in Cameroon, like in many other African countries, drugs such as albendazole, Mebendazole and metronidazole can easily be purchased from street vendors at very low cost. The tendency of people to practice self medication is likely to induce long term resistance of parasites to these drugs with consequences on the transmission of these diseases (24).

The relationship between the perception (knowledge of the source of infection, risk behaviour and mode of prevention and/or treatment) and the prevalence of feacal orally transmitted parasitic infections showed a strong negative correlation (r dispersed between -0.97 and -99).

There was a negative correlation between the perception and the prevalence of faecal-oral parasites in Kake II indicating that the prevalence (percentage of infected subjects) was inversely proportional to the awareness. In other words, the greater the awareness about the cause, risk behaviour and prevention/treatment of faecal-oral parasitic infections, the lower the prevalence of the disease. This can be explained by the fact that children who are conversant with the knowledge of the disease are aware of the consequences of many attitude and practices than those who have not been exposed to such opportunity. It follows that through health education, a child is exposed to health ideals which enhance the promotion of his physical, social and psychological health which ought to be pursued all through life. It is therefore important for health education to be successful, to select appropriate strategies which should address the factors contributing to behaviour that influences health (9). It is of great desire also educating mothers since their literacy will influence change in behavior of children and enhance hygienic practices. In a study on mother

knowledge, attitudes and perception regarding intestinal parasite and diarrhoea in 3 regions of Gaza strip, Palestine, it was observed that mother's education had a positive effect for decreasing parasitosis among children (24).

This study has also shown that in rural areas, lack of adequate knowledge on faecal-orally transmitted parasitic infections improves the transmission of these diseases. Health education increases the knowledge and change the overall perception of the infections, and consequently the prevalence. We conclude that health education through the framework of school is an effective control method for faecal-orally transmitted parasitic infections. We recommend this inexpensive strategy to be adopted as a national policy in developing countries, as a supplement to mass distribution of drugs, especially in rural communities.

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