ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

SEPTEMBER 2010 VOLUME 11 NUMBER 3

Official Publication of the African Society for Clinical Microbiology

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

GENERAL INFORMATION

Aims and scope

African Journal of Clinical and Experimental Microbiology is the official Journal of the African Society for Clinical Microbiology. It publishes original research, review papers, case reports/series, short communications and letters to the editors, in all aspects of Medical Microbiology including Bacteriology, Virology, Rickettsiology and Chlamydiology, Mycology, Mycobacteriology and Actinomycetes, Parasitology, Clinical Microbiology, and Clinical Veterinary Microbiology

Subscription information

African Journal of Clinical and Experimental Microbiology is an OPEN ACCESS JOURNAL CC BY VERSION 4.0 INTERNATIONAL, and publishes two or three times a year. Free downloads can be made from the website of the world"s largest online library of peer reviewed, Africa published scholarly journals, African Journals OnLine (AJOL): https://www.ajol.info/index.php/ajcem. Subscription is however still open to individuals, libraries, University Departments, Research Institutes and other Multi-reader institutions who may want to have hard copies of the Journal. For each volume (4 issues), subscription rate is £400 (United Kingdom), US $800 (USA/Canada), US $600 (African Countries), US $800 (Other Countries), N28,000 (Nigeria). Additional charges will be made for postage and packaging. A copyright for these is with African Journal of Clinical and Experimental Microbiology.

Subscription enquiries and all other matters relating to the Journal including manuscripts, adverts booking and sponsorship should be addressed to:

Prof Boaz Adegboro (MD)
Editor, African Journal of Clinical and Experimental Microbiology,
Department of Medical Microbiology, Faculty of Health Sciences,
University of Ilorin, Nigeria.
Phone: 031 – 222076-9
Email: ajcem2002@yahoo.com

It is a condition of publication that manuscripts submitted to this Journal have not been published and will not be simultaneously submitted to be published elsewhere except as conference abstracts, for which authors must disclose at the point of manuscript submission. Authors should be aware that electronic journals issues/articles can be accessed free (Open Access) online at the AJOL website: https://www.ajol.info/index.php/ajcem

Responsibility for accuracy of manuscripts lies entirely with the authors. All submissions must conform to the International Committee of Medical Journal Editors (ICMJE) uniform recommendations for manuscripts submitted to biomedical journals (http://www.icmje.org/recommendations/) and follow the guidelines of Committee on Publication Ethics https://publicationethics.org/guidance/Guidelines

Manuscripts should be typewritten with double line spacing and wide margins, following the conventional form: Title, Author's name and full correspondence address, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgment(s), References, Tables, Figures and Legends to Figures. Short Communications and Letters to The Editor are also entertained, and need not follow the above format.

If the research involves the use of human subjects, including collection of human blood or other human specimens, an institutional ethical clearance document should be submitted with the manuscripts. Alternatively, a statement should be made in the "Materials and Methods" section that informed consent of the experimental subjects and the approval of the appropriate ethical committee had been obtained.

All necessary illustrations should accompany the manuscripts, but should not be in the text. The illustrations should be numbered consecutively in the order in which they are referred to in the text. The top of illustration should also be indicated if this is not clear. All x-ray films must be clear and should be in photographic prints. Legends to figures should give sufficient information to make the illustration comprehensive without reference to the text.

References should be listed in their order of appearance in the text; and be indicated in the text by Arabic numbers in brackets e.g. (1), (2, 3, 4), etc (Modified Vancouver style). Accuracy of the references is the responsibility of the authors. The authors‟ names and initials should be followed by the title of the paper, abbreviated name of the journal, which should conform to those used in Index Medicus, year of publication, volume, and the first and last page numbers. Note the following examples.

For Journals:

1. Nsanze, H. Recommendation for management of gonorrhoea and genital ulcers in Africa. Afr J Sex Transm Dis. 1984; 1:5-7

2. Odugbemi, T. O., and Arko, R. J. Differentiation of *Kingella denitrificans* and *Neisseria gonorrhoeae* by growth on a semi solid medium and sensitivity to amylase J Clin Microbiol. 1983; 17: 389-391

For books:

3. Arya, O. P., Osoba, A. O., and Bennett, P. Tropical Venereology, Churchill Livingstone, Edinburgh, 1980 OR when referring to a chapter in a book and where the names of authors are also given, the reference should be as follows:

4. Easmon, C. S. F. Host-Parasite relationship in experimental staphylococcal infections. In: Macdonald, A., and Smith, G. (eds). The Staphylococci. University Press, Aberdeen 1981: 63-72

General:

a. To ensure rapid and accurate publication, it is essential that manuscripts conform to all instructions. Manuscripts, which are not in accordance with these specifications, may be returned.

b. An electronic copy of manuscript typed in Microsoft Word should be sent via email to ajcem2002@yahoo.com

c. An estimation of page charges will be mailed to the author(s) after the paper has been accepted for publication.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)
AJCEM/200972/**21017** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 138-145

MOLECULAR IDENTIFICATION AND PREVALENCE OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX AMONGST PEOPLE LIVING WITH HIV IN OSUN STATE, NIGERIA.

[1]O.A. Terry Alli, [1]Ogbolu, D.O., [1]Salawu, M.A., [2]Oyedeji, J.G., [3]Oladokun, L., and [3]Obaseki, F

[1]Departments of Biomedical Sciences, and [2]Department of Medical Microbiology and Parasitology,College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria; [3]State Hospital, Asubiaro, Osogbo, Nigeria; and [4]Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan.

Runnning title: Molecular identification of *Mycobacterium tuberculosis* complex in people living with HIV

Correespondence: Dr O.A.Terry Alli (alliot@hotmail.com)

Abstract
Human immunodeficiency virus (HIV) infection has created a special niche for *Mycobacterium tuberculosis* complex in humans as a result of the defect/reduction in cell mediated immunity. *M. tuberculosis* still responsible for most cases of death due to infectious diseases after HIV. In this study, prevalence of *M. tuberculosis* was determined in people living with HIV in Osun state of Nigeria with identification of culture positive isolates by polymerase chain reaction. A total of 160 samples were collected from people living with HIV with mean age of 36.8 years old of age (Median -34; age range 16 – 68; 95% confidence interval – 2.49) after seeking ethical approval from the Ministry of Health, Osun State. The result of the microscopy by ZN stain showed that 40 (25%) of the 160 samples were positive for acid fast bacilli while culture on Lowenstein-Jensen (LJ) medium revealed that 30 (18.75%) of the samples processed, grew organism that conformed to the characteristics of *M. tuberculosis* complex. Polymerase chain reaction for IS6110 was used to confirm the identity of the colonies on LJ slope as *M. tuberculosis* complex while the PCR for 260 bp of Rv1255c was used in identifying *M. tuberculosis*. *M. tuberculosis* represented 92% of the *M. tuberculosis* complex. The prevalence of *M. tuberculosis* among people living with HIV was found to be 15% after PCR identification. The effects of socio-demographic factors on the prevalence of TB were analysed. Occupation was found to be associated with the proportional distribution of TB in people living with HIV ($X^2 = 14.85$; $p < 0.05$). The study concluded that PCR should be integrated into the schema for identification of tuberculosis in reference laboratories in developing countries.
Key words: Molecular identification, Polymerase chain reaction, *Mycobacterium tuberculosis* complex; *Mycobacterium tuberculosis*, Prevalence, HIV, Nigeria

Introduction

Tuberculosis (TB) is responsible for most cases of illness and death worldwide, especially in Asia and Africa. Worldwide, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were reported in human immunodeficiency virus (HIV) positive people (1). The problem of TB has been compounded by the emergence of multi-drug resistance *Mycobacterium tuberculosis* and HIV.

The aetiological agent of TB is *M. tuberculosis* complex. *M. tuberculosis* complex is a Gram positive rod which can be best described by the ability to hold on to stain such as carbol fuschin even after decolourization with acid alcohol as acid fast bacilli. *M. tuberculosis* complex so called because of the similarity in pathology of the infection comprises *M. tuberculosis*, *M. bovis* including Bacille Calmette Guerin (BCG) strain, *M. canetti*, *M. africanum*, and *M. microti*. Causative agent of TB is a facultative intracellular organism. The pathogenesis starts with the invasion of alveoli macrophages after inhalation of the contaminated droplets by individuals. The organism able to resist macrophage killing by inhibiting phagosomal-lysosome fusion (2-4) and the detail mechanisms have been described by various researchers (2-7). The initial involvement with macrophages involved the use of receptors (complement receptors, mannose receptor, and type A scavenger receptors) present on the macrophage (8-10). Ability to adapt to the intracellular niche has played important role in the organism. One of the important mechanisms is for the organism to shift majority of its metabolism to fatty acid metabolism (11) and also ability to enter into state of dormancy have played important roles in the parasitism of the host. State of dormancy has been characterized by the increase in expression of α-crystallin protein (12). It has been reported that α-crystallin protein expression increases with anoxic condition (13) and it also increases during intracellular gene expression (14) with other key metabolites. Several workers in the field of mycobacteriology have used different molecular biological techniques such as transposon

mutagenesis (15), microarray (16), RAP-PCR (17) to mention a few, in unraveling the intracellular survival strategy of *M. tuberculosis*.

Epidemiologically, African countries have not been faring well since late 1980s and this has coincided with the HIV pandemic. Co-infection of people living with HIV with *M. tuberculosis* has been shown to increase the mortality rate in sub-Saharan African countries like South Africa, Botswana, and Zambia (18). Nigeria was ranked fifth as high burden country with tuberculosis according to World Health Organization (WHO) report of 2008 (1). According to this report, there were 311 cases of TB per 100,000 population per year. Six hundred and ninety four laboratories performing microscopy for AFB with none of them routinely culturing for mycobacteria and 6 states out of 30 states in Nigeria were mentioned as those involved in TB/HIV collaborative activities. Osun state was not one of the states mentioned in WHO report, suggesting that there is dearth of information on the TB/HIV epidemiology in Osun state. The information on the epidemiology of TB in people living with HIV has been sketchy or not well documented in this part of Nigeria (Osun state) because there is no laboratory facility to do both microscopy and culturing of samples for isolation and identification of mycobacteria routinely. Furthermore, rapid identification of *M. tuberculosis* complex from culture can provide better alternative to identification than the biochemical tests normally employed at reference laboratories. Therefore, the study was aimed at determining the prevalence of *M. tuberculosis* among people living with HIV in Osun State, Nigeria using cultural and molecular methods for identification of *M. tuberculosis*.

Materials and Methods

Sample Population and Selection.

The target population for this study was people that had been confirmed to be infected with HIV by serological diagnosis to be reactive or positive for Human immunodeficiency virus I/II residing in Osun state of Nigeria. Ethical approval was obtained from the Ministry of Health, Osun state, Nigeria before the commencement of the study. Standard questionnaire was provided in order to collect biodata and informed consent was obtained from the patient prior to collection of sputum

sample(s). Samples were collected from the people living with HIV between March and July, 2008.

Sample Collection.

The samples were collected from seven locations in Osun state: Ilesa, Osogbo, Telemu, Ogbagba, Iwo, Ikire, and Ejigbo in Osun State, Nigeria. Samples were collected randomly from people living with HIV/AIDS in those cities/towns aforementioned. Three sputum specimens were collected in wide – mouthed translucent containers (cups). Sputum produced from a deep cough into one of the container on the first day. The subjects took the second sputum cup home and produced early morning sputum from a deep cough. On arrival with the early morning specimen, another sputum specimen (second spot specimen) was collected from each subject. The samples were taken to the laboratory for microbiological analysis.

Mycobacteriology

Processing of Samples

All the sputum samples and cultures were processed in the TB laboratory, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria inside a safety cabinet as described below.

Microscopy.

A loopful of sputum was spread on a clean glass slide uniformly in oval shape by smearing repeatedly in coil-like patterns, approximately 2-3 cm in size. After smearing, the slide was air dried completely. Then, flame-fixed by passing through the flame carefully so that it would not wash off during staining. The smear was stained for presence of acid fast bacilli using Ziehl-Nelseen staining as described previously (19) and examined under light microscope using the oil objective.

Cultural Isolation of Mycobacteria.

Sputum specimens were processed for culture by first decontaminating/digestion. Briefly, 10 ml of 4% NaOH was added to about 5 ml or equivalent proportion, the cap of the container was tightened and shook to digest. It was allowed to stand for 15 min at room temperature with occasional shaking and centrifuged at 2000 x g for 10 min, after which the supernatant was poured off into a stericol disinfectant jar. The deposit was re-suspended in sterile saline and centrifuged at 2000 x g for 15 min. The resultant deposit was inoculated onto Lowenstein-Jensen (LJ) medium. The culture was incubated at 35-37^{o}C until growth was observed for maximum period of 8 weeks. All cultures were compared with the control organism (H37Rv). Every week after the inoculation, LJ slopes were removed from incubator and observed for 4 characteristics: aspect/consistency of the culture media; growth rate of the colonies; morphology of the colonies; and colour of the colonies (presence of pigmentation).

Bioinformatics and Primers design

In order to help in identification of *M. tuberculosis* and *M. tuberculosis* complex, the whole genomes sequences of *M. tuberculosis* (H37Rv) (20) and *M. bovis* (21) were downloaded from the www.sanger.ac.uk. ARTEMIS software designed to view microbial genome was used to view the genomes sequences of both organisms. Artemis comparison tool (22) was used to identify a region that was unique to *M. tuberculosis* that was not present in *M. bovis*. Primers were designed for amplification of the unique region using DNAman. The primers were designed for specificity by submitting the primers sequences to

BLAST programme at National Centre for Biotechnology and information website (www.ncbi.nih.gov/blast).

DNA Extraction

DNA was extracted from the representative colonies suspected to grow organism irrespective of whether the culture resembled *M. tuberculosis* complex culture as described before (23). Briefly, the LJ slope containing suspected colonies was heated at 80°C for 1 h to kill bacteria. Thereafter 3 ml of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 5% mono-sodium glutamate, pH 7.4) was added and the colonies were carefully scraped using a disposable loop and homogenised using a vortexer for 2-3 min to disrupt colonies. Four hundred microlitre of 50 mg/ml lysozyme stock and 10 µl of 10 mg/ml RNAase were added to the suspension and incubated at 37°C for 2 h. Subsequently, 600 µl of 10X proteinase K buffer (100mM Tris-HCl, 50 mM EDTA, 5% SDS, pH 7.8) was added with 150 µl of 10 mg/ml proteinase K and incubated at 45°C for 16 h. The DNA was purified using phenol/chloroform/isoamyl alcohol and precipitated from upper aqeous phase using equal volume of isopropanol in the presence of 0.1 volume of 3 M sodium acetate, pH 5.5 at -20°C for 30 min. The resulting DNA pellet after centrifugation at 3000×g for 30 min was suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C.

Polymerase Chain Reaction (PCR)

After the DNA extraction, about 50 ng of the DNA sample was added to the PCR master mix prepared in the presence of *Taq* polymerase and IS6110F (cctgcgagcgtaggcgtcgg) and IS6110B (ctcgtccagcgccgcttcgg) primers to amplify 123 bp insertion sequence element present in all *M. tuberculosis* complex. The reaction mixture was made up of 1.5 mM $MgCl_2$, 0.2 mM dNTP, 50 mM KCl, 5% glycerol, 1 uM of IS6110F, 1 uM of IS6110B and 25 units of *Taq* polymerase (New England Biolab, USA) in a 20 ul reaction volume. The following cycling parameters were used for amplification of 123 bp IS6110 fragment in positive *M. tuberculosis* complex cultures: 94°C for 5 min; 94°C for 1 min, 68°C for 1 min, 72°C for 2 min – 25 cycles; 72°C for 7 min -1 cycle. The amplicons were run along with DNA size markers on 0.7% agarose gel with positive control (DNA from H37Rv) and negative control (no DNA). The specific *M. tuberculosis* DNA was amplified using the primers stb_F1 (accgagttagcccgcaga) and stb_B1 (cgtcgtggtgatgctca) using the procedure above with different cycling parameters from above to amplify 260 bp: 94°C for 2 min 1 cycle, 94°C for 5 min; 94°C for 1 min, 55°C for 1 min, 72°C for 30 sec – 25 cycles; 72°C for 7 min -1 cycle.

Statistical Analysis

Data were analysed using statistical package within the Microsoft Excel and Epi-info software from Centre for Disease control and prevention, USA. Chi square was used to determine the effect of sex, age, location, and occupation on the data obtained. The p value less than 0.05 was considered to be significant.

RESULTS

In this study, 160 sputum samples were obtained randomly from people living with HIV/AIDS in Osun State of Nigeria of average age of 36.8 years old (Median -34; age range 16 – 68; 95% confidence interval – 2.49). The result of the microscopy by ZN stain showed that 40 (25%) of the 160 samples were positive for acid fast bacilli (AFB) while 42 (26.3%) were positive for culture (see Fig.1). Four of the 120 samples negative by microscopy grew organism suspected to be *M. tuberculosis* complex.

There was no significant difference in the level of detection of mycobacteria between microscopy and culture on LJ ($X^2 = 0.02$; $p > 0.05$). Most of the growths were noticed 4 weeks after incubation. Using culture as the gold standard, sensitivity and specificity for microscopy were found to be 73% and 100%, respectively.

Primers for amplification of the insertion sequence

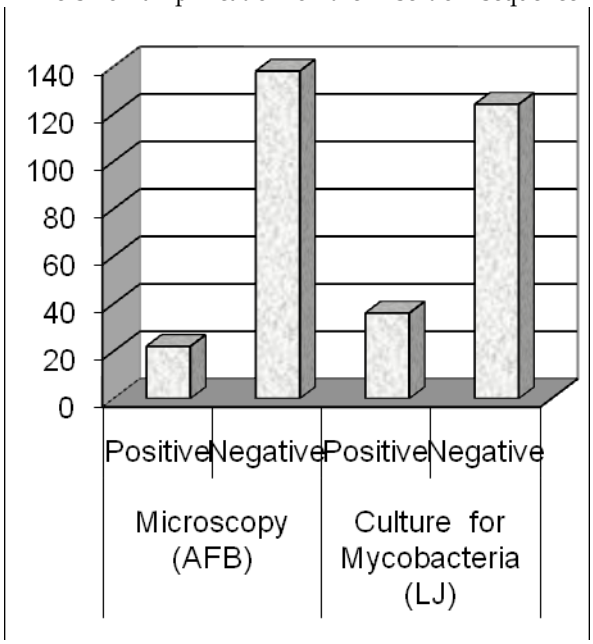


Fig.1. Comparison of number of cases positive by microscopy and culture in diagnosis of tuberculosis. Microscopy was carried out on all the specimens by staining smear of sample using ZN staining technique while culture was done on LJ slope as described in the materials and methods.

element of *M. tuberculosis* complex had been used before by various researchers (24-26) and therefore no need for analysis of the primers for suitability. The primers designed specifically to amplify *M. tuberculosis* were designed based on the comparison of the genomes sequences of *M. tuberculosis* and *M. bovis*.

Various regions of the genome were identified to be unique to *M. tuberculosis*: Rv1255c—R1257c, Rv1369c, Rv1506c, Rv1507c – Rv1516c, Rv1976c, Rv2073c, Rv2106c, to mention few of such regions. One (Rv1255c) of such regions was selected and primers were designed using the primer design programme within DNAman. The primers were submitted for BLAST at ncbi website to make sure that the primers would not amplify any region of the DNA from other organisms including human DNA. Using the IS6110F and IS6110B, all DNA extracted from culture positive samples irrespective of the colonial appearance were subjected to PCR to identify *M. tuberculosis* complex. The result of the PCR for *M. tuberculosis* complex showed that 30 (18.75%) of the 160 samples cultured were positive for PCR for *M. tuberculosis* complex representing 75% of the suspected culture positives. Twenty four (80%) of the 30 *M. tuberculosis* complex isolates were positive for PCR that was designed to identify *M. tuberculosis* alone. Representative of the PCR for *M. tuberculosis* complex and *M. tuberculosis* are shown on Figures 2 and 3, respectively.

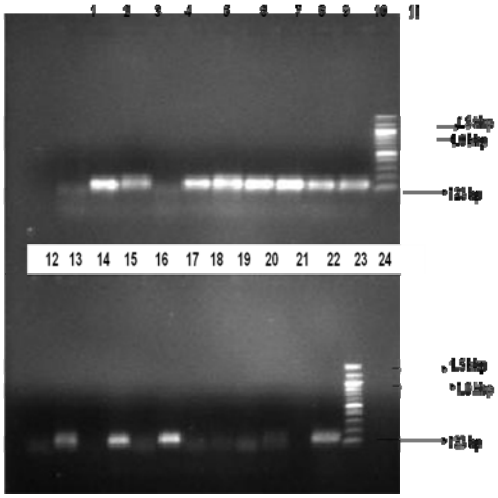


Fig.2. PCR for *Mycobacterium tuberculosis* complex. Agarose gel electrophoresis of representative samples. Lanes 1-10 and 12-21: PCR of various cultures from LJ slopes; lane 22: negative PCR control; lane 23: PCR positive control (H37Rv DNA); and lanes 11 and 24: 100 bp DNA ladder molecular weight size marker. Positive PCR product is indicated by arrow showing 123 bp.

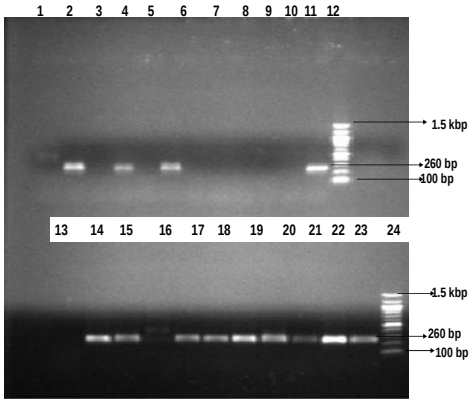


Fig. 3. PCR for *Mycobacterium tuberculosis* Agarose gel electrophoresis of representative samples. Lanes 1-8 and 12-21: PCR of various cultures from LJ slopes; lane 9: negative PCR control; lane 10: PCR positive control (H37Rv DNA); and lanes 12 and 24: 100 bp DNA ladder molecular weight size marker. Positive PCR product is indicated by arrow showing 260 bp.

Table 1 shows the proportional distribution of *M. tuberculosis* complex among people living with HIV according to sex. There
was no significant difference between the prevalence of *M. tuberculosis* complex in male (21.1%) and female (18%) ($X^2 = 0.17$; $p > 0.05$) in people living with HIV.

Table 1. Proportional Distribution of *M. tuberculosis* complex among people living with HIV

Sex	Positive for *M. tuberculosis* complex	Negative for *M. tuberculosis* complex	Total
Male	8 (26.67)	30 (78.95)	38
Female	22 (18.00)	100 (82.00)	122
Total	30 (18.75)	130 (81.25)	160

Number in parenthesis - percentage
Chi square = 0.17 , P=0.89

Furthermore, there was no significant difference between prevalence of *M. tuberculosis* in male (21.1%) and female (13.1%) ($X^2 = 0.88$; $p > 0.05$).). The prevalence of *M. tuberculosis* complex among people living with HIV was found to be 18.75%. There was increase in proportional distribution of *M. tuberculosis* complex with increasing age (Fig. 4). The only age group that did not follow this trend was 21-30 age group which recorded 25% in proportional distribution. With this trend, 61-70 age group recorded the highest proportional distribution (33.3%) while 11-20 recorded the lowest proportional

distribution (0%). There was no association between age and proportional distribution of tuberculosis in people living with HIV (X^2 = 7.16; p > 0.05). Removing the age group 21 to 30 from statistical analysis showed that there was linear association between age and tuberculosis in people living with HIV (X^2 = 4.278; P < 0.05). Fig. 5 shows the occupational distribution of *M. tuberculosis* complex among people living with HIV.

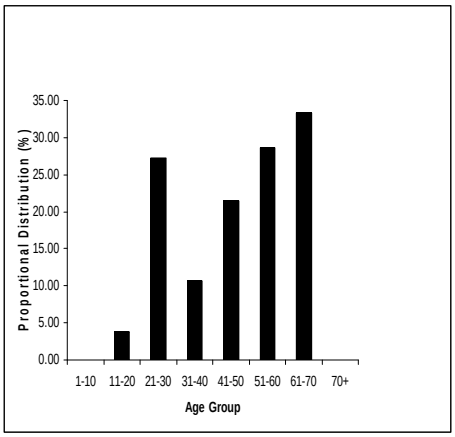


Fig. 4. Age distribution of *M. tuberculosis* complex among people living with HIV.

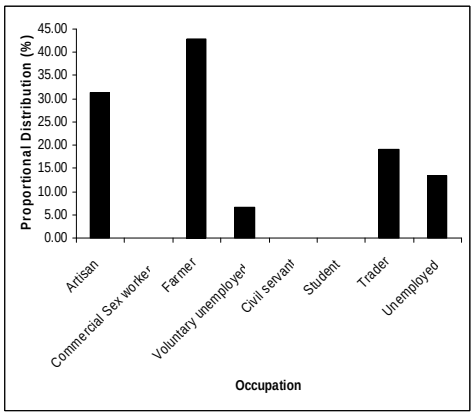


Fig. 5: Occupational distribution of *M.tuberculosis* complex among people living with HIV.

Highest proportional distribution of tuberculosis was recorded among farmers (42.9%), followed by artisans (31.3%), and lowest among students (0%) and commercial sex workers (0%). There was association of occupation with proportional distribution of tuberculosis in people living with HIV (X^2 = 14.85; p < 0.05). Figure 6 shows the distribution of *M. tuberculosis* among people living with HIV in Osun state with respect to the residential location. Ikirun recorded the highest proportional distribution (37.5%), followed by Ilesa (28.6%) with lowest proportional distribution (0%) recorded for Telemu. Association was not found between residential location and prevalence of tuberculosis (X^2 = 9.40; p > 0.05).

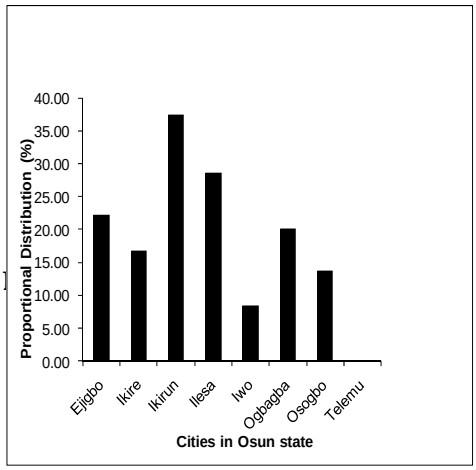


Fig. 6. Distribution of *M. tuberculosis* complex among people living with HIV according to their residential location.

M. tuberculosis is the leading cause of death after HIV from infectious diseases (1, 18). The combination of HIV and *M. tuberculosis* infection increases the mortality rate due to infectious disease. In order to curb the menace due to *M. tuberculosis* there is need for speedy, accurate laboratory diagnosis which will eventually lead to adequate treatment. This study was designed to highlight the scale of the problem facing policy makers in controlling this important infectious agent through adequate laboratory diagnosis. The prevalence of *M. tuberculosis* in people living with HIV in Osun state of Nigeria was found to be 25% using microscopy, and 15% and 18.75% for *M. tuberculosis and M. tuberculosis* complex, respectively, suggesting there is overestimation of TB cases using microscopy. This data is not in agreement with WHO report (2008) on the overall prevalence of TB among people living with HIV that was put at 9.5%. A study carried out on patients attending out-patients clinic in Abeokuta, Nigeria estimated the prevalence of *M. tuberculosis* to be 14% (27) which is in agreement to 15% obtained in this study. Similarly, 16.7% prevalence of TB in people living with HIV/AIDS was reported in Keffi, Nigeria (28).

The overestimation of TB cases by microscopy in this study is not surprising because this method of laboratory diagnosis is not specific as there are other bacteria such as *Nocardia spp* and *Actinomycetes spp*

and fungal elements that are acid fast. The most worrisome is that there is no method that is routinely used to differentiate *M. tuberculosis* complex from *M. avium intracellulare* complex in Nigeria because *M. avium* infection does not respond to usual anti-TB drugs. The delay in treating the infection with appropriate drugs can make a lot of difference between life and death. *M. avium* has been reported in people living with HIV (29). Culture which is a gold standard in laboratory diagnosis estimated the prevalence to be 26.3%, this is without formal identification of the suspected colonies on LJ slope. Formal identification is very important for epidemiological purposes and also it can also give a clue to the way the infection should be treated if anti-TB drug susceptibility testing is not carried out on the isolates. Drug susceptibility testing procedure for mycobacterial infection can only be best handled in reference laboratory. With the advent of several molecular biological techniques in the last 2 decades, this part can be handled with a laboratory that is well funded in developing countries.

In this study, PCR was carried out to identify the species of mycobacteria isolated in people living with HIV. The PCR was based on the IS6110 that was identified more than a decade ago in *M. tuberculosis* by Eisenach et al (24). This insertion sequence (IS6110) has been found in all *M. tuberculosis* complex in high copy number in majority of *M tuberculosis* strains and 1 to 5 copies in *M. bovis* strains (30). The presence of IS6110 in high copy number makes it a good target for PCR because it enhances the sensitivity of detection of *M. tuberculosis* complex. Using IS6110 as target for PCR, 30 (75%) of the 42 isolates were identified as *M. tuberculosis* complex while 24 (63%) of the isolates were identified as *M. tuberculosis*, suggesting there are other species of mycobacteria infecting people living with HIV apart from *M. tuberculosis* within *M. tuberculosis* complex that has been erroneously reported. The prevalence of *M. tuberculosis* in people living with HIV after identification by PCR was estimated to be 15%. A study carried out by Idigbe et al. using biochemical methods of identification (niacin production, nitrate reduction, catalase production, etc) found that not all mycobacteria isolated from patients with tuberculosis had *M. tuberculosis*, with *M. bovis* accounted for small proportion of the mycobacterial isolates (31). A detailed molecular epidemiological analysis of the isolates from Ibadan also showed that *M. africanum* and *M. bovis* accounted for 13% of the mycobacterial isolates (32). Various studies have reported the prevalence of tuberculosis among people living with HIV: Nwobu et al. (33) reported 10.8% and 9.2% in Irrua and Benin, respectively using Ziehl Neelsen staining. Idigbe et al. (34) reported 5.2% in Lagos while Onipede et al (35) reported 12.9% in Ile-Ife (a town about 50 km away from this study site).

The limitation of our study was the inability of the methodology in identifying other species of mycobacteria apart from *M. tuberculosis*, suggesting the prevalence of other species of mycobacteria apart from *M. tuberculosis* cannot be determined with certainty. However, this study still gives an important clue to the most predominating mycobacteria infecting people living with HIV with accuracy and also demonstrates the usefulness of molecular biological technique in mycobacteria identification. It is possible to incorporate this methodology as part of the schema for laboratory diagnosis of tuberculosis.
The influence of different socio-demographic factors on the prevalence of *M. tuberculosis* complex on people living with HIV was examined. We found no association between gender and the prevalence of tuberculosis on the population studied ($X^2 = 0.02$; $p > 0.05$) with prevalence in males and females put at 21% and 18%, respectively. This finding is in conformity with other studies (27, 28, 33, 36) that looked at the effect of gender on the prevalence of TB on people living with HIV. Pennap et al. (28) reported no significant difference in prevalence of TB in males (19.4%) and females (14.4%) co-infected with HIV in Keffi, in Nigeria, suggesting gender plays no role in prevalence of TB in people living with HIV. Although we found no significant association of age ($P > 0.05$) with the prevalence of TB in people living with HIV, there was general increase in the prevalence of tuberculosis as the age progresses with the age group between 61 and 70 recording the highest proportional distribution. The plausible explanation for the highest proportional distribution recorded for 61-70 age group could be attributed to the weakening of the immune system (37). Even in the absence of HIV, this is the age group in general population where reactivation of latent tuberculosis takes place.

Moreover, we found association between tuberculosis and occupation; highest proportional distribution (42.9%) was recorded among farmers living with HIV, followed by artisans. This study is not in agreement with the study carried out by Nwobu et al (33) where they found no association between occupation and tuberculosis in people living with HIV. The plausible explanation for this high prevalence among farmers can be attributed to physical nature of their job which could further weaken their immune system. The same reason can also be given for artisans. Mechanised farming is not adopted by large population of farmers in Nigeria, especially the area where this study was conducted. Animal husbandry especially cattle rearing and exposure to wild animals infected with *M. bovis* could probably expose farmers to

tuberculosis. Our questionnaire did not address the kind of farming operation. Farming operations have been linked with infectious diseases like meliodosis (38) but this has not been proven for tuberculosis due to *M. tuberculosis*.

In conclusion, we have demonstrated the usefulness of PCR in laboratory diagnosis of tuberculosis. We recommend the integration of molecular techniques such as PCR in the final identification of *M. tuberculosis* complex / *M. tuberculosis* as this can provide timely intervention in the diagnosis of tuberculosis and treatment. Identification of suspected *M. tuberculosis* from culture is a prelude to studying the molecular epidemiology of this organism.

Acknowledgements

The molecular biology works were all carried out in the Molecular Biology Laboratory, Department of Biomedical Sciences, Ladoke Akintola University of Technology, Mercyland Campus, Osogbo. We would like to thank the Vice Chancellor (Professor B.B.A. Adeleke) and Management of Ladoke Akintola University of Technology for the establishment of the Molecular Biology Laboratory. We also acknowledge Mr Oyenike and Mr T. Raheem for their technical assistance.

References

1. WHO. Global Tuberculosis Control 2008: Surveillance, Planning, Financing. Geneva, Switzerland; 2008.
2. Brown CA, Draper P, Hart PD. Mycobacteria and lysosomes: a paradox. Nature 1969;221(5181):658-60.
3. Armstrong JA, Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. J Exp Med 1971;134(3 Pt 1):713-40.
4. Armstrong JA, Hart PD. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. J Exp Med 1975;142(1):1-16.
5. de Chastellier C, Thilo L. Modulation of phagosome processing as a key strategy for Mycobacterium avium survival within macrophages. Res Immunol 1998;149(7-8):699-702.
6. Russell DG, Dant J, Sturgill-Koszycki S. Mycobacterium avium- and Mycobacterium tuberculosis-containing vacuoles are dynamic, fusion-competent vesicles that are accessible to glycosphingolipids from the host cell plasmalemma. J Immunol 1996;156(12):4764-73.
7. Sturgill-Koszycki S, Schaible UE, Russell DG. Mycobacterium-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. Embo J 1996;15(24):6960-8.
8. Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA. Phagocytosis of Mycobacterium tuberculosis is mediated by human monocyte complement receptors and complement component C3. J Immunol 1990;144(7):2771-80.
9. Schlesinger LS. Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. J Immunol 1993;150(7):2920-30.
10. Hirsch CS, Ellner JJ, Russell DG, Rich EA. Complement receptor-mediated uptake and tumor necrosis factor-alpha-mediated growth inhibition of Mycobacterium tuberculosis by human alveolar macrophages. J Immunol 1994;152(2):743-53.
11. Alli OAT, Hingley-Wilson S, Ogbolu DO, Spreadbury CL. The Mycobacterium tuberculosis homologue of the Mycobacterium avium mig gene is not specifically expressed in the macrophage. African Journal of Biomedical Research 2008;11:173-181.
12. Yuan Y, Crane DD, Barry CE, 3rd. Stationary phase-associated protein expression in Mycobacterium tuberculosis: function of the mycobacterial alpha-crystallin homolog. J Bacteriol 1996;178(15):4484-92.
13. Cunningham AF, Spreadbury CL. Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. J Bacteriol 1998;180(4):801-8.
14. Yuan Y, Crane DD, Simpson RM, Zhu YQ, Hickey MJ, Sherman DR, et al. The 16-kDa alpha-crystallin (Acr) protein of Mycobacterium tuberculosis is required for growth in macrophages. Proc Natl Acad Sci U S A 1998;95(16):9578-83.
15. McAdam RA, Weisbrod TR, Martin J, Scuderi JD, Brown AM, Cirillo JD, et al. In vivo growth characteristics of leucine and methionine auxotrophic mutants of Mycobacterium bovis BCG generated by transposon mutagenesis. Infect Immun 1995;63(3):1004-12.
16. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, et al. Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment. J Exp Med 2003;198(5):693-704.
17. Alli OAT, Butcher PD, Mangan JA, Akinloye O, Spreadbury CL. The use of RAP-PCR to study intracellular gene expression of Mycobacterium tuberculosis. African Journal of Clinical and Experimental Microbiology 2009;10(3):185-199.
18. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions

with the HIV epidemic. Arch Intern Med 2003;163(9):1009-21.
19. Alli OAT. Development of a RAP-PCR strategy to study mycobacterium tuberculosis gene expression during intracellular infection of macrophages [PhD]. Birmingham: University of Birmingham; 1998.
20. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998;393(6685):537-44.
21. Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, et al. The complete genome sequence of Mycobacterium bovis. Proc Natl Acad Sci U S A 2003;100(13):7877-82.
22. Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. ACT: the Artemis Comparison Tool. Bioinformatics 2005;21(16):3422-3.
23. van Helden P, Victor T, Warren R, van Helden E. Isolation of DNA from Mycobacterium tuberculosis. In: Parish T, Stoker N, editors. Mycobacterium tuberculosis Protocols. Totowa, New Jersey: Humana Press; 2001. p. 19-30.
24. Eisenach KD, Sifford MD, Cave MD, Bates JH, Crawford JT. Detection of Mycobacterium tuberculosis in sputum samples using a polymerase chain reaction. Am Rev Respir Dis 1991;144(5):1160-3.
25. Smith KC, Starke JR, Eisenach K, Ong LT, Denby M. Detection of Mycobacterium tuberculosis in clinical specimens from children using a polymerase chain reaction. Pediatrics 1996;97(2):155-60.
26. Gouveia AC, Eisenach KD, Vinhas SA, Ribeiro FK, Peres RL, Dietze R, et al. Use of in-house PCR for identification of Mycobacterium tuberculosis in BACTEC broth cultures of respiratory specimens. Mem Inst Oswaldo Cruz 2008;103(4):386-91.
27. Ojo D, Mafiana C, Adeniran-Sonola A. Prevalence of Mycobacterium tuberculosis and human immunodeficiency virus infections in Abeokuta, Ogun State, Nigeria. Nigerian Journal of Parasitology 2007;28(1):39-43.
28. Pennap G, Giyan J, Eleboda A. Prevalence of pulmonary tuberculosis (PTB) among people living with HIV/AIDS (PLWHA) in Keffi and its environs. Indian Journal of Microbiology 2009;49:233-236.
29. Ellner JJ, Goldberger MJ, Parenti DM. Mycobacterium avium infection and AIDS: a therapeutic dilemma in rapid evolution. J Infect Dis 1991;163(6):1326-35.
30. Cave MD, Eisenach KD, McDermott PF, Bates JH, Crawford JT. IS6110: conservation of sequence in Mycvobacterium tuberculosis complex and its utilization in DNA fingerprinting. Molecular and Cellular Probes 1991;5:73-80.
31. Idigbe EO, Anyiwo CE, Onwujekwe DI. Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. Journal of Tropical Medicine and Hygiene 1986;89:143-148.
32. Cadmus S, Palmer S, Okker M, Dale J, Gover K, Smith N, et al. Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. J Clin Microbiol 2006;44(1):29-34.
33. Nwobu G, Okodua M, Tatfeng Y. Comparative Study of HIV Associated Pulmonary Tuberculosis in Chest Clinics from Two Regions of Edo State, Nigeria. Online Journal of Health and Allied Sciences 2004;3(4).
34. Idigbe EO, Nasidi A, Anyiwo CE, Onubogu C, Alabi S, Okoye R, et al. Prevalence of Human Immunodeficiency Virus (HIV) Antibodies in Tuberculosis in Lagos, Nigeria. Journal of Tropical Medicine and Hygiene 1994;97(2):91-97.
35. Onipede AO, Idigbe O, A.K. A-N, Omojola O, Oyelese AO, Aboderin AO, et al. Sero-prevalence of HIV antibodies in tuberculosis patients in Ile-Ife, Nigeria. East African Medical Journal 1999;76(3):127-132.
36. Odaibo G, Gboun M, Ekanem E, Gwarzo S, Saliu I, Egbewunmi S, et al. HIV infection among patients with pulmonary tuberculosis in Nigeria. African journal of medicine and medical sciences. 2006;35(4):475-478.
37. Hopewell P, Jasmer R. Overview of Clinical Tuberculosis. In: Cole ST, Eisenach KD, McMurray DN, Jacobs WR, Jr., editors. Tuberculosis and the Tubercle Bacillus. First ed. Washington DC: ASM; 2005. p. 17-31.
38. Dance D. Ecology of Burkholderia pseudomallei and the interactions between environmental Burkholderia spp. and human-animal hosts. Acta Tropical 2000;74:159-168.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/200976/**21018** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 146-149

STUDIES ON BACTERIAL INFECTIONS OF DIABETIC FOOT ULCER

J.vimalin Hena, Lali Growther. Department of Microbiology, Hindustan College of Arts &Science, Coimbatore. India

ABSTRACT

Microbial study for aerobic organisms from 100 cases of diabetic foot ulcers was carried out to determine the etiological agents and their antibiogram. Polymicrobial infection was observed in all the cases. The most frequently isolated aerobic organisms were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antimicrobial sensitivity pattern of the isolates were done in which imepenem was found to be effective. Imipenem belongs to the ß lactam group of new generation antibiotics.

Key words: Diabetes, Imipenam, Foot ulcer.

INTRODUCTION

Diabetes is a metabolic disorder of the endocrine system which plagues approximately 17 million people nationwide. Each year over 700,000 new cases are diagnosed; 12,000 to 14,000 of which are children, teenagers and young adults, while this life threatening disease can be controlled. Diabetes is often accompanied by serious complications, and still today there is no cure (1). By 2010 it is predicted that it will affect 239 million people world wide.

Fifteen per cent of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer (2). Poorly controlled diabetes is prone to skin infections because elevated blood sugar reduces the effectiveness of bacteria fighting cells. Carbuncles, boils, and other skin infections may be hazardous if not properly treated. Even a small cut may progress to a deep, open sore, called an ulcer (3). In most cases ulceration is a consequence of the loss of protective sensation that is ,the loss of awareness of trauma that can cause the breakdown of the skin.

The organisms that occur on foot infections are generally Staphylococcus aureus and *Streptococcus pyogens* arising from the patients' own skin and *Enterococci* from bowel. Among the Gram positive aerobes *Staphyloccoci* are more prevalent. Many of these microorganisms are developing resistance to commonly used antibiotics largely due to their indiscriminate use. The present study was undertaken to determine the microbiology of the diabetic foot ulcers and the antimicrobial sensitivity pattern of the isolates.

MATERIALS AND METHODS: A total number of 100 diabetic patients with foot ulceration were studied during the period of December 2005to Feburary2006. The cases were from Government hospital Coimbatore. Swabs were collected from ulcers that were macroscopically examined and classified based on Wagner's method of evaluation (3, 4). Swabbing was done on slouphy or inflammmed tissue as bacteria tend to present in greater number in these areas. From each patient two swabs were collected. The sterile cotton were moistened with sterile saline before collecting the specimens. One of the swabs was used for the isolation of bacteria. The other swab was used for wet mount microscopy. For the isolation of bacteria the media used were blood agar, and Mac Conckey agar, which were incubated at 37C for 24 hours. The organisms isolated were subjected to antibiotic susceptibility testing on Muller-Hinton agar using Kirbey-Bauer disc diffusion method (5).

RESULTS Of the total 100 diabetic foot patients studied 69 were males and 31 were females, the male: female ratio being 2:1. Their ages ranged from 35 years to 85 years with an average of 58 years. The maximum number of patients having diabetic foot infections belonged to the age group of 56-65 years, the cases was with diabetes ellitus for more than a decade.

TABLE 1: AEROBIC BACTERIAL ISOLATES

Culture isolate	Number of isolates	percer
S.aureus	47	42.3
C.koseri	3	2.70
E.coli	17	15.3
K.pneumoniae	10	9.0
Ps.aeruginosa	27	24.3
P.vulgaris	7	6.3

Of the 100 patients, 48 patients had some other complications, such as peripheral vascular disease, neuropathy, nephropathy, retinopathy, cataract, ischaemic heart disease or hypertension along with diabetes mellitus. Peripheral neuropathy has a central role and is present over 80% of diabetic patients with foot lesions

From the 100 patients studied, aerobic bacteria in the pure form were isolated in all the cases in which 47 were *S. aureus*, 3 were *C. koseri,* 17 were E. coli, 10 were *K. pneumoniae*, 27 *Ps. aeruginosa*, 7 were *P. vulgaris.*

TABLE 2: ANTIBIOTIC SENSITIVITY PATTERN OF THE AEROBIC GRAM POSITIVE ISOLATES

Antibiotics	*S.aureus* (n=47)number of sensitive strains(percentag
Amikacin(30 mcg)	19(40.4)
Cloxacillin(30 mcg)	11(23.4)
Cefepine(30 mcg)	11(23.4)
Chloramphenicol(30 mcg)	33(70.2))
Ciprofloxacin(25 mcg)	12(25.5)
Cotrimoxazole(25 mcg)	-
Gentamycin(10 mcg)	18(38.2)
Pencillin(100 mcg)	2(4.2)
Tetracyclin(30 mcg)	5(10.6)
Gatifloxacin(5 mcg)	30(63.8)
Vancomycin(30 mcg)	14(29.7)

TABLE 3a: ANTIBIOTIC SENSITIVITY PATTERN OF THE AEROBIC GRAM NEGATIVE ISOLATES

Antibiotics	*S. aureus* (%) n = 37 Number of Sensitive Strains
Amikacin (30mcg)	19 (70.3)
Cloxacillin (30mcg)	11 (40.7)
Cefepine (30mg)	11 (40.7)
Chloramphenicol 30mcg	23 (85.1)
Ciprofloxacin (25mcg)	12 (44.4)
Cotrimoxazole(25mcg)	-
Gentamycin(10mcg)	18 (66.6)
Penicillin(100mcg)	2 (7.4)
Tetracyclin(30mcg)	5 (18.5)
Gatifloxacin(5mcg)	20 (74)
Vancomycin(30mcg)	14 (51.8)

TABLE 3b: ANTIBIOTIC SENSITIVITY PATTERN OF AEROBIC GRAM NEGATIVE BACTERIA

Antibiotics	*E.coli* n = 17	*C.koseri* n = 3	*K.pneumoniae* n = 10	*P.vulgaris* n = 7	*Pseudomonos* n = 27
Ciprofloxacin (5mcg)	9 (52.9)	3 (100)	5 (50)	5 (71.4)	6 (22.2)
Ceftazidine (30mcg)	11 (64.7)	2 (50)	3 (30)	5 (71.4)	11 (40.7)
Piperacillin(100mcg)	12 (70.5)	3 (100)	5 (50)	4 (57.1)	22 (81.48)
Ceftoxine (30mcg)	10 (58.8)	3 (100)	2 (20)	2 (28.5)	3 (11.1)
Amikacin (30mcg)	10 (58.8)	3 (100)	8 (80)	4 (57.1)	13 (48.14)
Imipenam(10mcg)	16 (94.1)	3 (100)	10 (100)	7 (100)	27 (100)
Chloramphenicol (30mcg)	6 (35.2)	2 (50)	7 (70)	-	8 (29.6)
Gentamycin(10mcg)	10 (58.8)	-	6 (60)	5 (71.4)	4 (14.8)
Gatifloxacin (5mcg)	12 (70.5)	3 (100)	8 (80)	5 (71.4)	17 (62.96)
Cotrimoxazole (25mcg)	2 (11.7)	-	-	-	-
Tetracyclin(30mcg)	2 (11.7)	-	-	-	2 (74.07)

Over 63.8% strains of *S.aureus* were sensitive to gatifloxacin. Only 25.5% of strains were sensitive to ciprofloxacin (Table 2). While *E. coli* was highly sensitive to the antibiotics tested, Pseudomonas was highly resistant to them (Table 3).

DISCUSSION

The presence of S. *aureus, Proteus species* and other aerobic gram negative bacilli in septic complications of infected diabetic feet have been reported in various studies.

The infections are usually polymicrobial in nature, caused by aerobic Gram- positive *S. aureus*, and by Gram-positive bacilli like *E. coli, Klebsiella species* and *Proteus* (5). In the present study S. aureus (43.2%) was predominantly isolated. As regards the aerobic Gram negative bacilli, Pseudomonas aeruginosa (24.3%) E. coli (15.3%) C. koseri (2.7%) P. vulgaris(6.3%) and K. pneumoniae(9%) were the common organisms isolated. When testing the susceptibility to fluroquinolones, individual drugs must be included, as susceptibility to one drug cannot be taken as evidence of susceptibility to other fluroquinolones .

Among the 17 isolates of *E. coli* quinolone and cephalosporin group of antibiotics showed a higher sensitivity than to commonly used chloramphenicol. The 3 isolates of *C.koseri* were found to be sensitive to all major groups of antibiotics except to tetracycline so further analysis of its prevalene of resistance is required.10 isolates of *K.pneumoniae* showed marked difference in their sensitivity pattern to the quinolone group of antibiotics there was a pattern of resistence to ciprofloxacin and sensitivity to gatifloxacin .The 7 isolates of *Proteus* were resistant to major group of antibiotics but a high sensitivity pattern was shown towards to gatifloxacin. In *Pseudomonas* the total isolates were resistant to the most commonly used antibiotics like ciprofloxacin, ceftoxime, amikacin, chloramphenicol and tetracycline,but all were sensitive to the beta lactum antibiotic imipenem.

In this study all the Gram negative isolates were susceptible to carbapenem beta lactum antibiotic imipenem, which is resistant to inactivation by most bacterial beta lactamases and so it has the widest spectrum of antibacterial activity. Imipenem should therefore be used as a monothreraphy against polymicrobial infections in difficult gram negative infections. It produces a response rate comparable to that of third generation cephalosporins.

ACKNOWLEDGEMENT

We thank Dr. Parimala and Mr. Ramanathan for their guidance and moral support.

REFERENCES

1. Lipsky, B.A., Pecoraro, R.E. Larson S.A., Hanley, M.E. Ahroni, J.H (1990) Outpatient management of uncomplicted lower –extremity infection in diabetic patients Arch. Intern. Med. 150: (790-797).

2. Ramani, A., Ramani, R., Shivananda, P.G and Kundaje, G.N(1991). Bateriology of diabetic foot ulcers. Indian. J pathol . Micronbiol. 34: (81-87)

3. Boulton., AJ(2000). The diabetic foot: a global view. Diabetes Metab Res Rev:16

4. Pathare, N.A., Bal,A., Talvalkar, G.V.and Antani D.V(1998). Diabetic foot infections a study of microorganisms associated with the different Wagner grades. Indian J Pathol Microbiol 41(4):437-441.

5. Prabhakar P Rao and Hira J(1981): Bacterilogical study of diabetic foot ulcers. Trop.geogr. Med. 33: 249-252.

6. Anandhi.c., Alaguraj and Natrajan(2004). Bacteriology of Diabetic foot: lesions. Indian Journal of microbiology.volume:22,Issue:3(175-178).

7..Sally.M.and and Marshell(2004).Diabetic Medicine. Journal of Diabetes U.K.volume 21:Issue 7pp(37-87)

REVIEW ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/200981/**21019** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 150-158

GLOBAL TREND OF METHICILLIN-RESISTANT STAPHLOCOCCUS AUREUS AND EMERGING CHALLENGES FOR CONTROL

O. AZEEZ-AKANDE , DEPARTMENT OF MEDICAL MICROBIOLOGY, AND PARASITOLOGY, FACULTY OF MEDICINE, BAYERO UNIVERSITY, P.M.B 3011, KANO – NIGERIA
E-MAIL: akadaze@yahoo.com

ABSTRACT
Background: Following its first recognition in early 1960s, the increasing incidence of nosocomial and community-acquired methicillin resistant *Staphylococcus aureus* (MRSA) infections has become a global problem. The emergence of multiple-drug resistant MRSA strains and dissemination of epidemic antibiotic clones including presence of wide spectrum of virulence and predisposing risk factors complicate diagnosis, chemotherapy and control causing significant morbidity and mortality. Detection of MRSA strains in domestic animals and protozoan has widened the epidemiologic characters of the organism and may influence infection control policies.

Objectives: To review the emergence and epidemiologic spread of resistant strains of MRSA, molecular/genetic basis of resistance in the organism and challenges facing control strategies worldwide. It also aims to suggest intervention strategies so as to checkmate the spread of MRSA infections.
Methods: By reviewing local and international literatures on MRSA infections coupled with practical experience in the field of this endeavour.
Result/Conclusion: MRSA has shown increasing endemic and epidemic spread in the last four decades causing serious medical and socio-economic difficulties. Routine and regular surveillance (uncommon in poor-resourced developing areas of especially sub-Saharan Africa), good hospital practices and personal hygiene, public enlightenment, development of effective therapeutic agents and rational administration of antibiotics based on reliable test results will limit the spread of MRSA infections.
Key words: MRSA, incidence, morbidity, mortality, surveillance, control.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important bacterial pathogen causing nosocomial and community-onset infections which has shown increasing endemic and epidemic spread in the last four decades (1,2) ,while its control has become a serious concern worldwide (3,4). These MRSA associated infections impose a serous burden in terms of medical and socio-economic costs and cause significant morbidity and mortality (5-7).

S. aureus (including MRSA strains) are cluster-forming, facultative aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning Mannitol Salt Agar (MSA) yellow (8). The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, often called enzymes (clumping factor) used for their identification. MRSA strains exhibit resistance to oxacillin or methicillin (1 or 5µg/disk: zone of inhibition < 14mm in diameter used as marker for all β-lactams) and other antimicrobial agents (8,9).

MRSA has been severally shown to cause variety of diseases ranging from mild, superficial dermatological diseases to severe and potentially fatal systemic debilitations (10-11). In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an important and increasing cause of post-surgical wound infections (12,13). some invasive infections such a nosocomial bacteremia and septicemia (sepsis) (14), acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections (STIs),[13,15]. The increasing prevalence of MRSA multiple-drug resistant strains which limits the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide [2].

This paper aims to review the emergence and global epidemiologic spread of MRSA including multiple-resistant strains, molecular/genetic basis of resistance in this organism and the challenges facing the control strategies worldwide. It also aims to suggest ways of overcoming these challenges in order to limit the spread of MRSA and associated health problems.

Emergence and global epidemiological trend of MRSA

Strains of MRSA were first detected in the United Kingdom (UK) in the early 1960s[16] soon after methicillin antibiotic was introduced for clinical use. During the next decade, the existence of MRSA

strains was reported in the United States (US) with prevalence rate of less than 1%.[17] Since then, the endemic and epidemic outbreaks of the organism have been reported worldwide[18-20] but overwhelmingly from developed economies of the world. Presently, hospital of all sizes, other care centers, and increasing number of different population groups at various communities globally are facing the problem of MRSA infections (1,7,21).

Reports emanating from different parts of the world revealed increasing rates in the incidence of MRSA and population at risk. The epidemiological data from north America including Center for Disease Control and Prevention (CDC) in the US showed that the prevalence of MRSA strains in both large and small hospitals located at different regions increased progressively over the years (21-25). Other studies conducted at various health care centers in the country revealed that out of all hospital bacterial isolates, the prevalence of MRSA, cumulatively increased from 6% in 1998 to 50% in 2002.[4] A similar study conducted earlier elsewhere in the country showed a 30% increase (from 20% to 50%) in MRSA prevalence within a two year period (i.e. from 1988 to 1990) (26) .

The published data from some countries of Europe and Asia presented identical scenario with significant increase in the outbreaks of MRSA infections. For instance, Mangeney and co-workers [27] documented MRSA prevalence of 33%-62% (in relation to *S. aureus* isolates) in their hospital wards in France. The report of De Sousa and colleagues [28] from Portugal though indicated downward trend in the outbreaks of MRSA infections but showed consistently high prevalence of MRSA during the last decade e.g. 65% in 1992; 49% and 47% in 1993 and 1994 respectively.

In contrast, lower prevalence rates of MRSA have been reported in certain parts of Europe. For example, Harbath and co-researchers[29] reported 3% MRSA prevalence in their hospital in Geneva, Switzerland though further evidence showed that the MRSA were not nosocomial strains and could have originated from the community. In a similar study carried out in the UK,[30] the prevalence of MRSA was found to be comparatively low (<10%) but never the less significant in the population group studied. Identical scenario has been observed in Scandinavia and the Netherlands where incidence of MRSA appears relatively low compared to other European countries (31).

In earlier studies carried out in Asia, a remarkable upsurge in MRSA prevalence has been documented in many areas, including Taiwan where an increase in MRSA prevalence from 4.3% in a period covering 1981-1986 was reported (32). In Japan (33) and Republic of Koria (34), MRSA prevalence of up to 54% and 70% have been documented respectively. Both endemic and epidemic outbreaks have continued to rise in that region (35). On the other hand , reports from Africa on MRSA are scanty. Nonetheless, in study conducted in a Khartoum hospital, Sudan, a prevalence of 11% was documented (36). More so, the results of surveillance studies carried out by Kesah and other researchers (37) in some parts of Africa (including Lagos-Nigeria; Cameroon, Kenya and Algeria) and Malta between 1996 and 1997 revealed rates of 21 – 30% MRSA prevalence among the participating countries of the sub-Saharan region (e.g. Nigeria, Cameroon and Kenya) while that of North Africa (e.g. Algeria) and Malta presented lower rates of below 10%. However, results of similar studies carried out at different locations in Nigeria viz: Ilorin, (38) Calabar, (39) Jos, (40) (41) revealed higher MRSA prevalence rates of 34.7%, 36.4%, 43% and 49.1% respectively. More studies are clearly needed particularly in all regions of Africa including Nigeria and other developing poor-resourced areas to determine the current epidemiologic outlook of this increasingly important nosocomial and community-acquired pathogen.

Emergence of MRSA multiple antibiotic clones and public health implications According to Olive and Bean(42), clonally related organisms are mostly members of the same species that share certain characteristics such as virulence factors, biochemical and genetic traits. These traits may aid the pathogenicity, resistance to drugs, or the critical mechanism for survival by the organism. They may also facilitate the general identification of such group of organisms Information from different geographical locations showed that clones, particularly the antibiotic types and those withinherent repertoires of virulence factors are spreading in various care centers, hospitals, among members of different sporting teams and in the communities (7, l1) that hitherto were not considered to be at risk of MRSA infections. Furthermore, MRSA strains have also been detected in domestic animals and birds such as horses, cattle, chickens and dogs as well as associated individuals (43,44). More so, a group of researchers in England has suggested that a protozoan, *Acantha - amoeba polyphaga* which is found virtually everywhere in the environment may aid the spread of MRSA. This is significant because other scientific evidence suggests that pathogens that emerge from amoebas may exhibit broader resistance to antibiotics and may be more virulent with serious epidemiologic and clinical implications (45). Data published by CDC (25) suggested that community - acquired MRSA (Ca - MRSA) is becoming more prevalent. Consequently, the array of virulence factors possessed by Ca-MRSA and broader resistance

to antimicrobial agents being exhibited by the organism as compared to hospital - associated MRSA (Ha -MRSA) is of concern. Subsequently, Ca-MRSA had been implicated in various clinical conditions affecting some persons without any notable risk factors and in many cases the management of such conditions has resulted in poor prognosis (7). In addition, it has been proposed[3, 42, 46] that one clone with homogenous or heterogeneous resistance profile may be present in the same location at any given time thus compounding diagnostic problems (especially in poor - resourced areas) and treatment failures arising from empirical chemotherapy. The situation is even more compounded in patients with critical underlying ailments such as cancer, HIV/AIDS and those undergoing immunosuppressive therapy. Such conditions obviously constitute serious risk factors for MRSA infection and may results in severe complications and fatal consequences, especially if multiple - antibiotic resistant strains are involved.

Molecular and genetic factors predisposing to antimicrobial resistance in MRSA

It is now recognized that coding for methicillin resistance in MRSA is facilitated by the mec A gene, which is located on the staphylococcal cassette chromosome (SCC), a large mobile genetic element which differs in size and genetic composition among different strains of MRSA (4, 47). Consequently, at least five types of this organism have been classified accordingly [48-50] (Table I shows types I - IV as thus classified).

The mechanism of resistance in this manner involves changes or defects brought about by mutation on mec A gene which result in modification to penicillin - binding protein 2a (PBP - 2a) product. The outcome of the event is that it renders the organism resistant to β-lactams and other antibiotics with the same target site. [4] In addition, other antibiotic resistance genes may be present in the cassette thus conferring on the organism, multiple - resistance to other antibiotics.[49] Apart from the inability of an antibiotic to bind to the target site due to structural defect of such site, other mechanisms that may generally play significant roles in the development of resistance in bacteria such as MRSA include efflux phenomenon (also a product of structural modification of cellular component e.g. cell wall/membrane protein) resulting in continuous pumping of antimicrobial drugs out of the bacterial cell. Others are alteration in the outer-membrane proteins which limit the access of drugs to the cell; resistance can also arise from high level production of β lactamase (51-53).

On the other hand, PBP - mediated resistance in MRSA is suggested to take various forms and may arise from (a) overproduction of a PBP, (b) acquisition of a foreign PBP with low affinity, (c) recombination of susceptible PBP with more resistant varieties or (d) may be due to specific point mutations within PBPs that consequently lower their affinity for β lactams (10,53,54). Consequently, the occurrence of any of these events may lead to changes in antibiotic phenotypic characters of affected *S. aureus* strains. The classification of MRSA strains based on SCCmec present has notably revealed certain epidemiological features of these bacterial strains including possible sources of acquisition and dissemination (nosocomial versus community), nature of drug - resistance (single versus multiple), as well as genome characteristics viz: size of genome and type of ribosome found in individual strain type (Table 1). It is note worthy that type IV MRSA strains originating from the community (Ca-MRSA) were found to possess higher prevalence of certain virulence factors as compared with non methicillin - resistant *S. aureus* (NMRSA) and health care associated MRSA (Ha - MRSA). These factors include enterotoxin, Panton - Valentine leucocidin (PVL) e.g. as observed in USA 300 and 400 strains, toxic shock syndrome toxin 1 (TSST-1), and other superantigens with serious clinical implications.[21,55,56] Consequently, the Ca- MRSA is believed to have inherent potential for greater disease than NMRSA, and broader antibiotic resistance than typical Ha – MRSA (57). The increasing prevalence of Ca-MRSA coupled with associated battery of virulence factors and wide spectrum of resistance to chemotherapeutic agents pose serious challenges for diagnosis, management and general control of MRSA infections.

TABLE 1: CLASSIFICATION OF M RSA BASED ON SCCMEC-TYPE PRESENT (50,51)

Type of SCCmec	Source	Resistance	Genome Size (kb)	Ribotype
I	Hospital	Methicillin	34.3	Conserved
I	Hospital	Multi-drug	53.0	Conserved
III	Hospital	Multi-drug	66.9	Conserved
IV	Community	Methicillin	21- 24	Variable

Risk factors for the acquisition of MRSA

Traditionally, MRSA is associated with health care institutions (11) following its first detection in the UK in the early 1960s (16). However, though Ca - MRSA became prominent during the last decade nevertheless its extent in most communities is uncertain (36,57). In addition, the frequency of Ca - MRSA infections among otherwise healthy persons without typical Ha - MRSA risk factors is increasing with concomitant health problems (58). Reports of Ca - MRSA prevalence indicated high rates among certain population groups such as those living in close contact or proximity to one another such as prisons, barracks and care centres (11,21, 24). The presence of several risk factors (Table 2) , which increasingly predispose the general populace to MRSA infection is raising concern worldwide(2,7, 55).

The above developments therefore constitute a challenge to the researchers in health sector, epidemiologists, policy makers and health care providers at all levels in the developing world to fashion out a coordinated effort at ascertaining the current epidemiologic profile in various communities and groups for necessary intervention programmes. On the other hand Ha - MRSA strains, are usually introduced into the health care institutions by either an infected or colonized health care worker (62).

Several risk factors have been suggested for the acquisition of nosocomial MRSA. They include previous or excessive antimicrobial therapy (4), previous hospitalization (1), autoimmune diseases including immunosuppressive therapy as well as surgery and prolonged hospital stay *(>8.4* days) (4). Others are enteral feeding, mechanical ventilation, implantation of prosthetic devices (63) and nasal carriage of MRSA (64).

The increased use of indwelling devices coupled with increasing number of immunocompromised patients such as HIV/AIDS and cancer patients particularly in the developing countries of sub-Saharan Africa and parts of Asia compound the problem of control of MRSA. The existing control strategies have progressively been inundated by the massive increase in antibiotic use in the hospitals worldwide and in the communities (through self medication) especially in developing countries resulting in selective pressure and emergence of highly resistant MRSA strains. In addition, the recognition of several risk factors for MRSA infection and wide spectrum of resistant pattern to antimicrobial drugs by the organism clearly make effective control highly tasking and have been considered a global crisis (5,65).

TABLE 2: RISK FACTORS FOR MRSA (57-60)

Susceptibility patterns of MRSA and antibioticreatment of associated infections.

- **Direct contact with an infected or colonized individual**
- **Crowded and unhygienic living conditions.**
- **Recent long-term antibiotic use (within six months) or history of frequent antibiotic use.**
- **Frequent antibiotic use or abuse (including suboptimal dose) for chronic problems such as otitis media, atopic dermatitis (or eczema) and pharyngitis.**
- **Recent or frequent outpatient visits (including outpatient surgical procedures).**
- **Injection or intravenous drug use and homosexuals.**
- **Shared clothing and/or equipment and other items.**
- **Underlying chronic illness (especially dermatologic diseases) and HIV/AIDS.**
- **Contact with family member or household working in health care facility e.g. Nurses, Doctors, Laboratory scientists e.t.c.**
- **Caretaker for person with unknown history of MRSA infections.**
- **Participation in close contact sports such as wrestling, football e.t.c.**
- **Contact with toiletries or bed linens of an infected individual.**
- **Individuals living in close proximity and with frequent close contact such as prisons, dormitories, army barracks and child care settings.**
- **Regular exposure to clinical specimens without adequate preventive measures (e.g. in hospital wards and laboratories).**

Various reports (4,5,16,30) have described high-level resistance of MRSA to antibiotics apart from methicillin as a common phenomenon. The reports further stated that in the developed economies of North America for instance, up to 90%, 95% and 83% of MRSA infections were resistant to fluoroquinolones, erythromycin and c1indamycin respectively. The organism has also expressed high level resistance to other antimicrobial agents like gentamycin (75-93%), ketolides (82-98%) and trimethoprim/sulfamethoxazole (16-65%) (Table 3). However, rates of resistance to some antibiotics, such as inducible c1indamycin was lower among methicillin-susceptible *S. aureus* than MRSA in some locations (2,6). Certain drugs also were less effective against MRSA infections due to some factors and characteristic nature of such drugs. For example, vancomycin used to treat MRSA systemic infections was found to be less effective in the treatment of ventilator-associated pneumonia (VAP) due to its poor lung penetration unlike such

drugs as linezolid with effective penetration of the body including tissue, muscle, fat and bone (57). On the other hand, antibiotic susceptibility pattern of MRSA is not uniform or clear cut in the developing countries and thus varies from one geographical location to another depending on various factors (36,37-42).

In view of this scenario the following factors are imperative for consideration in the selection of antibiotics for the treatment of MRSA infection. They include: (a) susceptibility of the organism to antibiotics (b) the type of infection (c) drug characteristics such as activity (i.e. bactericidal versus bacteriostatic) (d) mechanism of action (e) blood level/tissue penetration (f) toxicity (g) cost and availability (h) early initiation of appropriate antimicrobial chemotherapy is also essential for favourable prognosis and outcome (4,11).

Antidote for increasing MRSA acquisition dissemination and spread

In the developed countries of North America e.g. US and Canada, and parts of Europe, considerable attention has been given to infection control programme which has led to significant reduction in the incidence of many infectious diseases.(1,2) However, the situation in many developing regions of the world appears gloomy due to inadequate or poor implementation of policy on infection control due to lack of political will, inadequate resources including shortage or ill-equipped manpower, poor motivation of health care workers and researchers. Other militating factors include extreme poverty and ignorance on the part of the general populace. However, in order to bring the present situation under control, some holistic measures are imperative for strict implementation at local and national hospital levels, communities and corporate establishments involving health care workers, researchers, epidemiologists and drug anufacturers. These include:- (a) regular surveillance of endemic and epidemic outbreaks of MRSA especially in high risk centres, (b) screening before employment and periodic test of health care workers and identification of carrier status plus adequate treatment to eliminate potential source of infection,

TABLE 3: RESISTANCE RATES OF MRSA STRAINS TO ANTIBIOTICS (4,15,32)

Antimicrobial agent	Rate of resistance (%)
* Erythromycin	90 – 95
* Gentamycin	75 – 93
* Fluoroquinolones	30 – 90*
* Clindamycin	75 – 83
* Ketolides	82 – 98
* Tetracycline	18 – 82
* Trimethoprim/sulfamethoxazole	16 – 65
* Quinupristin/dalfopristin	4 – 31
* Fusidic acid	5 – 10
* Vancomycin	0 – 5
* Oxazolidinones (e.g. Linezolid)	0 – 1
* Tigecycline	0
* Daptomycin	0

*-variable in developing countries and may be lower in some cases

(c) screening of high risk patients and other individuals, (d) routine laboratory diagnosis (which is lacking in most developing areas) and prompt identification of MRSA and determination of antibiotic susceptibility profile while (e) empirical treatment of MRSA infection should be based on prior determination of local resistance patterns. Other measures involve (f) the enforcement of good hospital practices, (g) provision and adequate implementation of educational programs on hospital, community and personal hygiene, and (h) development of effective chemotherapy to replace those drugs to which the organism has developed resistance. More so, rational administration of antibiotics based on rapid and reliable laboratory test results will go along way in reducing the cases of treatment failures, and selective pressure leading to the proliferation of MRSA resistant strains.

CONCLUSION

The increasing prevalence of MRSA infections in the hospitals, other care centres and lately in the community has become a worldwide phenomenon. The wide spread dissemination of multiple - drug resistant strains and antibiotic clones of the bacterium facilitated by inherent or acquired molecular/genetic element is worrying as it complicates diagnosis and chemotherapy. More so, the presence of wide array of virulence and potential risk and spreading factors compounds morbidity and control measures. There is need for adequate policy framework on infection control that will reflect the current realities on the epidemiologic characters of MRSA as well as strict implementation of such control program to checkmate the spread of MRSA infections.

REFERENCES

1 Kuehnert, M.J., Hill, H.A., Kupronis, B.A Methicillin-resistant *Staphylococcus aureus* hospitalization, United States, *Emerg. Infect. Dis.* 2005; 11 (6): 868 - 872.

2 Frazee, B.W., Lynn, J., Charlebois, E.D., Lambert, L, Lowery, D., Perdreau-Remington, F. High prevalence of methicillin - resistant *Staphyloccus aureus* in emergency department skin and soft tissue infection. *Ann. Emerg. Med.* 2005; 45(3): 311 - 320.

3 Pere - Roth, E., Claverie - Martin, F., Villar, J., Mendez - Alvarez, S. Multiplex PCR for simultaneous identification *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J. *Clin. Microbiol.* 2001; 39(11): 4037 - 4041.

4 Stevens, D.L Optimizing outcomes in methicillin_resistant *Staphylococcus aureus* infections: focus on nosocomial pneumonia and SSTI, highlights from a satellite symposium at the 11th annual international congress on infectious disease (ICID), Cancun, Mexico, 2004; PP 1 - 8. ·

5 Maranan, M.C., Moreira, B., Boyle - Vavra, S., Daum, R.S Antimicrobial resistance in Staphylococci: epidemiology, molecular mechanisms and clinical relevance *Inf. Dis. Clin. N. Am.* 1997; 11:813 - 849.

6. Carbon, C. Costs of treating infections caused by methicillin resistant staphylococci and vancomycin-resistant enterococci. J. *Antimicrob. Chemother.* 1999; 44:31 - 36.

7. Bratu, S., Eramo, A., Kopec, R., Coughlin, E., Ghitan, M., Yost R., Chanpnick, E.K., Landman, D; Wuale, J. Community - associated methicillin - resistant *Staphylococcus aureus* in hospital nursery and maternity units. *Emerg. Infect. Dis* 2005; 11(6): 808 - 813.

8. Bannerman, T. L. Staphylococcus, Micrococcus and other catalase-positive cocci that grow aerobically In: *Manual of Clinical Microbiology* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. pfaller and R. H. Yolken eds. 8th edition, 2003 ASM Press Washington DC Pp 384 - 404.

9. Duguid, J. P. Staphylococcus: Cluster-forming Gram-positive Cocci In: *Mackie and McCartneyPractical Medical Microbiology* J. G. Collee, J. P. Duguid, A. G. Fraser, B. P. Marmion eds. 13 edition, 1989 Churchill Livingstone London, Pp 303 - 316.

10. Chambers, H.F. Methicillin - resistant in staphylococci: Molecular and biochemical basis and clinical implications. *Clin. Microbiol. Rev.* 1997; 10: 781 - 791.

11. Moran, GJ., Amii, R.N., Abrahamian, F.M., Talan, D.A Methicillin - resistant *Staphylococcus aureus* in community - acquired skin infections. *Emerg. Infect. Dis* 2005; 11 (11): 928 - 930.

12. Gottileb, G.S., Fowler, V.G. Jr., Kong, L.K. *et al. Staphylococcus aureus* bacteremia in the surgical patients: a prospective analysis of 73 postoperrative patients who developed *Staphylococcus aureus* bacteremia at a tertiary care facility. *J. Am. Coll. Surg.* 2000; 190:50 - 57.

13. Graffunder. E.M., Venezia, R.A Risk factors associated with nosocomial methicillin - resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J. Antimicrob. Chemother.* 2002; 49:999 - 1005.

14. Mylotte, J.M., Tayara, A. *Staphylococcus aureus* bacteremia: predictors of 30 - day mortality in a large cohort. *Clin. Infect. Dis.* 2000; 31: 1170 - 1174.

15. Rello, J., Diaz, E. Pneumonia in the intensive care unit. *Crt. Care Med.* 2003; 31: 2544 - 2551.

16. Jevons, M.P. "Celbanin" - resistant staphylococci. *Sr. Med J.* 1961; 1: 124 - 125.

17. Peacock, J.E. Jr., Marsik, FJ., Wenzel, R.P Methicillin - resistant *Staphylococcus aureus:* introduction and spread within a hospital. *Ann. Intern. Med* 1980; 93: 526 - 532.

18. Chambers, H.F. The changing epidemiology of *Staphylococcus aureus? Emerg. Infect. Dis.* 2001; 7: 178 - 182.

19. Ayliffe, A.G. The progressive intercontinental spread of methicillin - resistant *Staphylococcus aureus. Clin. Infect. Dis.* 1997; 24:574 - 579.

20. McDougal, L.K., Steward, C.D., Killgore, G.E. *et al.* Pulse field gel electrophoresis typing of oxacillin - resistant *Staphylococcus aureus* isolates from the United States establishing a national database. J. *Clin. Microbiol.* 2003; 41:5113 - 5120.

21. Mulvey, M.R., MacDouglall, L., Cholin, B. *et al.* Community associated methicillin - resistant *Staphylococcus aureus,* Canada. *Emerg. Infect. Dis.* 2005; 11(6):844 - 850.

22. Horan, T., Culver, D., Jarvis, W. Pathogens causing nosocomial infections: preliminary data from the national nosocomial infections surveillance system. *Antimicrob. Newsl.* 1988; 5: 56 67.

23. Hughes, J.M. Setting priorities: nationwide nosocomial infection, prevention and control programs in the U.S.A *Eur.* J. *Clin. Microbiol.* 1987;6:348 - n351.

24. Centers for Disease Control. Public health dispatch outbreaks of community - associated methicillin - resistant *Staphylococcus aureus* skin

infections - Los Angels County California. Morbid. *Mortal. Weekly Rep.* 2003; 1: 52 - 88.

25. Centers for Disease Control. M RSA August 2008. http:jwww.cdcjgov.ncidodjhipjARESISTjmrsajfag.htm. retrieved September 22, 2008.

26. Mangeney, N., Bakkaouch, A, Pons, J.L Methicillin - resistant *Staphylococcus aureus* isolates from trauma patients. J. *Clin. Microbiol.* 1998; 36 (2): 414 - 420.

27. Mangeney, N., Bakkauch, A, Pons, J.L., Dupeyon, C., Niel, P., Leluan, G. methicillin - resistant *Staphylococcus aureus* subtyping: interest of combined antibiotyping and esterase electrophoretic typing. J. *Appl. Bacteriol.* 1995; 79: 347 - 351.

28. De sousa, M.A Sanches, 1.S., Ferro, M.L. *et al.* Intercontinental spread of a multidrug - resistant methicillin - resistant *Staphylococcus aureus* clones. J. *Clin. Microbiol.* 1998; 36: 2590 - 2596.

29. Harbath, S., Francois, P., Schrenzel, J. *et aI.,* Community - associated methicillin -resistant *Staphylococcus aureus* Switzerland. *Emerg. Infect. Dis.* 2005 2005; 11(6).

30. Grundmann, H., Tami, A, Hori, S., Halwani, M., Slack, R. Nottingham *Staphylococcus aureus* population study: prevalence of MRSA among elderly people in the community. *BMJ. 2002;* 324: 1365 - 1366.

31. Murchnas, S., Kaufmann, 1.E., Deplano, A., Ryck, R., Sruelens, M., Zinn, C.E. Harmonization of pulsed - field gel electrophoresis protocols for epidemiological typing of strains of methicillin - resistant *Staphylococcus aureus.* a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J. Clin. Microbiol. 2003:* 41(4): 1574 - 1585.

32. Hsueh, P., Chen, M.L, Sun, C.C., W.H., Pan, HJ., Yang, L.S., Chang, S.C. Antimicrobial drug resistance in pathogens causing nosocomial infections at a university hospital in Taiwan 1981 - *1999. Emerg. Infect. Dis,* 2002; 8(1): 63 - 68.

33. Lotus, D.K., Imamura, T., Tukamine, F. Current status of antimicrobial susceptibility in MRSA isolates typed by coagulase and phage typing in Okinawa, *Acta. Med. Okayama.* 1995; 49: 81- 89.

34. Woojoo, K. Seunchill, P. Bacterial resistance to antimicrobial agents: an overview from Korea. *Yon Med. J.* 1999; 39(6): 488 494.

35. Euichong, K., Hyunjin, J., Myoung Don, O. *et al.* Epidemiological typing of methicillin-resistant *Staphylococcus aureus* out break isolates by pulsed-field gel electrophoresis and antibiogram *Trop. Dis. Bull.* 2008 (10): 1023-1028

36. Musa, H.A., Shears, P., Khagali, A. First report of methicillin resistant *Staphylococcus aureus* from hospitalized patients in Sudan. *J. Hosp. Infect.* 1999; 42(1): 74 -'- 75.

37. Kesah, c., Rdjeb, B., Odugbemi, T. *et al.* Prevalance of methicillin - resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clin Microbiol. Infect.* 2003; 9(2) 153 - 156.

38. Taiwo, S.S., Onile B.A., Akanbi, A.A. Methicillin - resistant *Staphylococcus aureus* (MRSA) isolates in Nigeria. *Afr. J. Clin. Exptl. Microbiol.* 2004; 5(2): 189 - 197.

39. Azeez, O.A., Utsalo, SJ., Epoke, J. Distribution and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates in a University Teaching Hospital in Nigeria. *Sahel Med.* J. 2008, 11(4): 142-147.

40. Ikeh, E.I. Methicillin - Resistant *Staphylococcus aureus* (MRSA) at Jos teaching hospital. *Afr,* J. *C/in. Expl. Microbiol.* 2003; 4(1): 52 - 55.

41. Olayinka, B.O., Olayinka, A.T., Onaolapo, J.A., Olurinola, P.F.
Pattern of resistance to vancomycin and other antimicrobial agents in staphylococcal isolates in a university teaching hospital *Afr,* J. *C/in Exptl. Microbiol.* 2005; 6(1): 21 - 27.

42. Olive, D.M Bean, P. Principles and applications of methods for DNA - based typing of microbial organisms: Minireview. 1. *Clin. Microbiol.* 1999; 37(6): 1661 - 1669.

43. Weese J.S., Archambault, M., Willey, B.M *et al.* Methicillin resistant *Staphylococcus aureus* in horses and horse personnel, 2000 - 2002. *Emerg. Infect. Dis,* 2005; 11(3): 430 - 435.

44. Cuny, C, Kuemmerie, J., Stannek, C, Willey, B., Strommenger, B., Witte, W. Emmergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison with MRSA from humans *Euro Surveil* 2006: 11(1): 44 - 47

45. Birchard, K. Amoeba aids spread of MRSA bacterium.
http://www.medicalpost.comjmpcontentjarticle.jsp. Retrieved August 16, 2008.

46. Oren, H., Leejene, T., Pnachyr, Y., *et al,* Dissemination of two methiocillin - resistant *Staphylococcus aureus* clones exhibiting negative staphylase reactions in intensive care units. J. *Clin. Microbiol.* 2007; 37(3): 504 - 509.

47. Euichong, K. Hyunjin, J., MyongDon, 0., Hoanjong, 1., Hyamgsoon, 0., Kangwon, CO. Epidemiological typing of methicillin - resistant

Staphylococcus aureus outbreak isolates by pulsed - field gel electrophoresis and antibiogram. *Yon. Med.* J. 1998; 39(6): 587 - 594.

48. Daum, R.S., Ito, T., Hiramatsu, K. *et al.* A novel methicillin resistance cassette in community acquired methicillin - resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds J. *Infect Dis* 2002; 186: 1344 - 1347.

49. Ito, T., Katayama, Y.I., Asada, K. *et al.* Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin - resistant *Staphylococcus aureus Antimicrob. Agents Chemother.* 2001: 45: 1323 - 1336.

50. Ito, T., X.S., Takeuchi, F., *et al.* Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase cere. *Antimicrob. Agents Chemother.* 2004; 48: 2637 - 2651.

51. Rasheed, J.K., Tenover, F.e. Detection and characterization of antimicrobial resistance genes in bacteria in: *Manual of Clinical Microbiology.* Eds. P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken. 8th edtn. 2003 ASM press, Washington DC PP. 1197 - 1212.

52. Rice, L.B., Sahm, D., Bonomo, R.A. Mechanisms of resistance to antimicrobial agents in: *Manual of Clinical Microbiology eds.* P.R. Murray, EJ. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken 8th Edtn. 2003, ASM press, Washington De. PP 1674 - 1101.

53. Hackbarth, CJ. Kacagoz, T. Kacagoz, S., Chambers, H.F. Point mutations in *Staphylococcus aureus* PBP 2 gene affect penicillin binding kinetics and are associated with resistance. *Antimicrob. Agents. Chemother.* 1995; 30: 103 - 106.

54. Vandenesch, F. Naimi, T. Enright, M.C *et al.* Community acquired methicillin - resistant *Staphylococcus aureus* carrying panton - valentine leucocidin genes: worldwide emergence. *Emerg. Infect. Dis.* 2008; 9: 978 - 984.

55. Fey, P.O., Said - Salim, B., Rupp. M.E. *et al.* Comparative molecular analysis of community - or hospital acquired methicillin resistant *Staphylococcus aureus. Antimicrob. Agents Chemother.* 2003; 47: 196 - 203.

56. Ochoa, TJ., Mohr, J., Wanger, A., Murphy, J.R., Haresi, G.P. Community - associated methicillin - resistant *Staphylococcus aureus* in pediatric patients. *Emerg. Infect. Dis* 2005; 11(6): 966 – 968.

57. Said - Salim, B., Mathema; B., Kreiswirth, B.M. Community acquired methicillin - resistant *Staphylococcus aureus.* an emerging pathogen. *Infect Contr. Hosp. Epidemiol.* 2003; 24(6): 451 - 455.

58. Centers for Disease Control. MRSA. *http.!/www.cdc/gov.ncidod/hip/ARESIST/mrsa/fag.htm* retrieved August 10, 2008.

59. Salgado, C.D., Farr. B.M., Calfee, D.P. Community - associated methicillin - resistant *Staphylococcus aureus.* a meta - analysis of prevalence and risk factors. *Clin. Infect. Dis.* 2003; 36: 131 139.

60. Basco, W.T. Jr. Community acquired MRSA: evolving pathogens. *http./ /www.edscape.com/viewarticlej507869*retrieved February 28, 2008.

61. Fang, H., Hedin, G. Rapid screening and identification of methicillin - resistant *Staphylococcus aureus* from clinical samples by selective - broth and real - time PCR assay. J. *Clin. Microbiol.* 2003; 41(7): 2894 - 2899.

62. Haddad, S.H., Arabi, Y.M. Memish, Z.A, AI-Shimemeri, AA. Nosocomial

infective endocarditis in critically ill patients: a report of three cases and review of the literature. *Intern.* 1. *Infect. Dis.* 2004; 8: 210 - 216.

63. Becker, K., Friedrich, AW., Lubritz, G., Weilert, M., Peters, G., Von Eiff, C. Pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J. *C/in. Microbiol.* 2003; 41(4): 1434-1439.

64. Matinez, J.L., F. Mutation Frequencies in antibiotic resistance.
Antimicrob. Agents Chemother. 2000; 44: 1771 - 1777.

65. Lovering, A. M., Zhang, J., Bannister, G. C. *et al.,* Penetration of Iinezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement J. *Antimicrob. Chemother.* 2002; 50: 73 - 77.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/200985/**21020** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 159-163

PREVALENCE AND MANAGEMENT OF *FALCIPARIUM* MALARIA AMONG INFANTS AND CHILDREN IN OTA, OGUN STATE, SOUTHWESTERN NIGERIA

[1]* GI Olasehinde, [1]AA Ajayi, [1]SO Taiwo, [1]BT Adekeye, and [2]OA Adeyeba. 1Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria; and 2Deptartment of Medical Microbiology and Parasitology, LAUTECH Teaching Hospital, Osogbo, Nigeria
*** Correspond: golasehinde@yahoo.com**

Abstract
Studies were carried out to determine the prevalence of malaria parasite infection among infants and children (0-12yrs) in Ota, Southwestern Nigeria between April and December 2008. The two hospitals used were Ota General Hospital and Covenant University Health Centre, Canaanland, Ota. Thick and thin films were made and stained using standard parasitological procedures. Structured Questionnaires were distributed to ascertain the age, sex, drugs or insecticides used and state of health of the subjects before recruiting them into the study.
Overall, 215 (80.5%) of the 267 children investigated were found to have malaria infection. Age group (0-5 years) had the highest frequency rate of 84.7% with mean parasite density of 900 and the difference between the age groups was statistically significant ($p<0.05$). Children of illiterates from suburb villages had the highest mean parasite density of 850 with 78.1% prevalence rate. 20% of the children were given local herbs and 22% used orthodox medicine as prophylaxis. Only 18% used insecticide treated mosquito nets while 24% of the parents spray insecticides to prevent mosquito bites.
There is therefore need for more awareness on effective use of drugs and Insecticide Treated bed nets in malaria hyperendemic regions.

Key words: % Prevalence, malaria parasite, *Plasmodium falciparum*, infants, Children

INTRODUCTION

Malaria is the most prevalent tropical disease in the world today. Each year, it causes disease in approximately 650 million people and kills between one and three million, most of them, young children in Sub-Saharan Africa (1). Nigeria is known for high prevalence of malaria and it is a leading cause of morbidity and mortality in the country. Available records show that at least 50 per cent of the population of Nigeria suffers from at least one episode of malaria each year and this accounts for over 45 per cent of all out patient visits (2). Malaria infection during the first five years of life is a major public health problem in tropical and subtropical regions throughout the world (3 and 4).

The disease accounts for 25 per cent of infant mortality and 30 per cent of childhood mortality in Nigeria thereby imposing great burden on the country in terms of pains and trauma suffered by its victims as well as loss in outputs and cost of treatments (5). It has also been documented that children visiting their country of origin import malaria from Nigeria and other hyperendemic countries to the low and non-endemic countries (6).The disease is caused by protozoan parasites of the genus *Plasmodium*. The most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax* (3). The parasite is transmitted by female anopheles mosquitoes (7).

Consequences of severe malaria include coma and death if untreated, young children are especially vulnerable (8). In endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten (9). For reasons that are poorly understood, but which may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage (8 and 9). Malaria has been found to cause cognitive impairments, especially in children. Malaria causes widespread anemia during a period of rapid brain development and also direct brain damage and this neurologic damage results from cerebral malaria to which children are more vulnerable (10). Over the longer term, developmental

impairments have been documented in children who have suffered episodes of severe malaria (3). Considering the above complications associated with malaria infection in children, this study therefore was carried out to assess the prevalence of malaria parasite infection among children between zero and twelve years of age in relation to attitudes and social pattern of living as factors affecting their exposure to malaria parasite infection.

MATERIALS AND METHODS

Two hundred and sixty seven infants and children between the ages of 0 and 12 years that were referred to the laboratory for malaria diagnosis test at the General Hospital, Ota and Covenant University health centre, Canaanland Ota, Ogun State, South West Nigeria between April and December, 2008 were recruited for this study.

Study Site

Ota, Ogun State, Nigeria

Safety procedures were adopted in the collection of finger-prick blood samples by swabbing the area to be sampled with 70% alcohol and allowed to dry before collection. Experienced medical laboratory scientists did sample collection in the hospitals. Thick and thin blood films were made on clean slides and labeled accordingly as recommended by the World Health Organization (WHO) (11).

The thin films were fixed with methanol and all films were stained with 3% Giemsa stain at pH 7.0 for 30 min as recommended by WHO (12). Blood films were examined microscopically using 100X (Oil immersion) objectives as described by Cheesbrough (11). The thick films were used to determine the parasite densities while thin films were used to identify the parasite species and infective stages. Parasite density per microlitre of blood was estimated from the thick film, taking the number of leucocytes per microliter of blood as 8,000 and was expressed as:

$$\text{Parasite density} = \frac{\text{Parasite count} \times 8{,}000}{\text{No of WBC counted}}$$

Questionnaires were designed and administered to parents of infants and older children determine the age, sex, drug usage for prophylaxis, attitude to use of Insecticide treated mosquito nets. The statistical analysis for significance was done using chi-square test.

Scientific and Ethical permit/clearance was obtained from the Nigerian Institute of Medical Research - Institutional Review Board (NIMR-IRB) and Covenant University Ethics Committee before this research work was carried out. The Ogun State Ministry of health (Hospitals Management Board) was also informed before this research was carried out. Consent for the children was provided by the parents/guardians while some of the participants provided the assents by nodding.

RESULTS

The prevalence of malarial parasite in infants and children according to age is shown in Table 1. Of the 267 samples examined, 215had malaria parasite in their blood ($p < 0.05$). The distribution of malaria parasite according to the methods used for prevention of mosquito bite by the patients (Table. 2) revealed that those who did not use any method to prevent the bite of mosquitoes have the highest prevalence rate of (90.9%) and mean parasite density of 900.

Table 1: Prevalence of Malaria Parasite in children according to age

Age group (yrs)	No. Examined	No Infected (%)	Mean Parasite Density (MPD)
0-5	177	150(84.7)	900
6-12	90	65(72.2)	600
Total	267	215(80.5)	750

Table 2: Distribution of Malaria Parasite according to mosquito prevention Method

Preventive Methods used	Total No. of infants and children (%)	No Infected out of patients using method (%)	Mean Parasite Density (MPD)
Bed nets (ITN)	48 (18)	23(47.9)	580
Insecticides	64 (24)	45(70.3)	750
Mosquito repellent cream	20 (7.5)	16(80.3)	910
Window/door nets	113 (42.3)	111(89.5)	870
None	22 (8.2)	20(90.9)	900
Total	267(100)	215(80.5)	750

The prevalence rate of malaria and mean parasite density (580) was significantly low (47.9%) among the children who used Insecticide treated bed nets. Prevalence of malaria parasite in children based on drug used as prophylaxis is shown in Table 3. There is a significantly low prevalence (55.2%) among those who used antimalarial drugs as prophylaxis when compared with patients who did not use any drug as prophylaxis (92.5%). Those who took local herbs (indigenous drug) had prevalence rate of 64.8%.

Table 3: Prevalence of Malaria Parasite in relation to prophylactic Antimalarial drugs used by study population

Drug used	Total No. of those using drug (%)	No. Infected (%)	Mean Parasite Density
Quinolines	21(7.8)	18(85.5)	890
Sulfonamides	17(6.4)	9(52.9)	800
Artemisinin Combination Therapies	16(6.0)	5(40.1)	760
Local herbs	53(19.9)	35 (64.8)	800
No drugs	160(59.9)	148(92.5)	990
Total	267(100)	215(80.52)	750

Table 4: Prevalence of Malaria Parasite According to Place of Residence

Place of residence	Number Examined	Number Infected (%)	Mean Parasite Density
Boarding House	52	47(90.4)	870
Campus Quarters	81	60(74.1)	750
Housing Estate	23	12(52.2)	760
Town/city setting	80	71(88.8)	800
Village/Remote setting	32	25(78.1)	850
Total	267	215(80.5)	750

The distribution of malaria parasite according to residential environment of the parents and children where the highest prevalence of 90.4% and parasite density of 870 was found among a set of students from a boarding school in the suburb of Ota (Table 4).

DISCUSSION AND CONCLUSION

Malaria remains one of the world's greatest childhood killers and is a substantial obstacle to social and economic development in the tropics. *Plasmodium falciparum* infection is the major cause of morbidity and mortality especially among the vulnerable groups to which children, especially aged less than 5 years belong (8).

In this study the prevalence of *falciparum* malaria in children between 0 and 12 years is 80.5% with mean parasite density of 750. This is in agreement with the findings of other workers in malaria endemic countries (13 and 14). The high prevalence could be due to the fact that this study was conducted just before the onset of rains till the end of raining season. This period has been marked as a period of high transmission (15).

Prevalence rate of 84.7% was recorded among children between zero and five years. It was observed that newborns and infants between age 5 days and three months who presented with fever were found to be positive for *falciparum* malaria. Similar observations have been reported in endemic and hyperendemic areas (14 and 16), where it was found that the parasite rate increases with age from 0-10% in the first three months of life to 80 to 90% by one year of age and the rate persists at a high level during early childhood. The presence of malaria parasites in

the blood of newborns may be as a result of congenital malaria as reported by Sotimehin *et al*. (14). Congenital malaria, defined as the presence of malaria parasites in the erythrocytes of newborns aged <7 days, was considered rare in endemic areas until recent studies started reporting high prevalence rates (14). The high prevalence rate in the study area could result to childhood anaemia and other severe conditions such as cerebral malaria as reported by other workers (3 and 9).

Following the demonstration that vector control measures can substantially reduce malaria transmission, malaria morbidity and all-cause child mortality, it has been suggested that insecticide-treated materials (ITMs) may also curb the spread of antimalarial drug resistance (17). There is a significantly low prevalence rate of malaria (47.9%) among the children that used Insecticide Treated Nets, although only 18% of the sample population used Insecticide Treated Nets (ITNs). Majority of households do not prevent mosquito bites and disease transmission in any way and a high prevalence rate of 90.9% and mean parasite density of 900 was recorded among this group of children. There is therefore the need to intensify awareness and education and to make the ITNs available at affordable prices in these malaria hyper endemic areas as the use of insecticide-treated nets decreased the number of malaria cases in children. Boarding house students should be encouraged to use bed nets so as to reduce transfer of infection among the students as the highest prevalence rate of 90.4% was found among students who live in the same hostel in a boarding house system.

It is also essential to avoid stagnant pools and poor environmental conditions, which encourage the breeding of mosquitoes. This is demonstrable as the prevalence rate among the children whose parents live in clean and hygienic environment like campuses and well laid out housing estates was low i.e. prevalence rates of 52.2 and 74.1 in housing estates and University campus respectively.

It has been observed in this study that antimalarial drugs are used as prophylaxis without doctors' prescription. Even artemisinin Combination Therapies (ACTs) have been used. Self-medication is a practice that is very common among people living in malaria endemic areas (18). This study shows that the number of children who had taken antimalarial drugs as prophylaxis was considerably high. This could be as a result of awareness created through education on home management and control of malaria among the pregnant women and mothers. Parents should therefore be educated on the safe effective use of antimalarial prophylactic drugs as too much exposure to antimalarial drugs could lead to the development of drug resistance. It was also observed from this study that there was low prevalence rate (64.8%) among the children that were given local herbs. However the community and the producers of these indigenous herbs need to be educated on strict adherence to environmental hygiene. There is also urgent need to encourage and empower researchers to identify the active ingredients in the effective local herbs for mass production. These may eventually lead to drug discovery in the bid to eliminate malaria.

Acknowledgements

We wish to acknowledge the management of Covenant University for funding this research. We also acknowledge the members of staff, Medical diagnostic laboratory of Covenant University health Centre and General hospitals, Ota and Abeokuta for their assistance in the collection of samples.

REFERENCES

1. Hay S, Guerra C, Tatem A, Noor A and Snow R (2004). "The global distribution and population at risk of malaria: past, present and future." Lancet 4(6):327-236.
2. Ojurongbe O, Ogungbamigbe TO, Fagbenro-Beyioku AF, Fendel R, Kremsner PG, Kun JF 2007. Rapid detection of Pfcrt and Pfmdr1 mutations in *Plasmodium falciparum* isolates by FRET and in vivo response to chloroquine among children from Osogbo, Nigeria. *Malar J 6*: 41.
3. Trampuz A, Jereb M, Muzlovic I, Prabhu R (2003). "Clinical review: Severe malaria." *Crit Care* 7 (4): 315-23.
4. Greenwood BM, Bojang K, Whitty CJ, Targett GA (2005). "Malaria". *Lancet* 365: 1487-1498.
5. Ogungbamigbe T, Ogunro P, Elemile P, Egbewale B, Olowu O, Abiodun O 2005. Presciption patterns of antimalarial drugs among medical practitioners in Osogbo Metropolis, South-West Nigeria. *Trop Med Health 33*: 201-208.
6. Katrin S., Fabrice L., Gérard K., Nicola L., David B., Meghna D., Simone G., Stefania D., Yasutaka M., Ragnhild J., Eskild P., John K.,

Robert S., and Patricia S. (2009). Imported Malaria in Children in Industrialized Countries, 1992–2002. *Emerg. Infect. Dis. 15* (2):185

7. Adefioye, O.A., Adeyeba, O.A., Hassan W.O. and Oyeniran, O.A. (2007). Prevalence of Malaria Parasite Infection among Pregnant Women in Osogbo, Southwest, Nigeria. *American-Eurasian Journal of Scientific Research* 2 (1): 43-45.
8. Idro, R, Otieno G, White S, Kahindi A, Fegan G, Ogutu B, Mithwani S, Maitland K, Neville BG, Newton CR. (2007). "Decorticate, decerebrate and opisthotonic posturing and seizures in Kenyan children with cerebral malaria". *Malaria Journal* 4 (57). Online biomed Central.
9. Mockenhaupt F, Ehrhardt S, Burkhardt J, Bosomtwe S, Laryea S,
   Anemana S, Otchwemah R, Cramer J, Dietz E, Gellert S, Bienzle U
   (2004). "Manifestation and outcome of severe malaria in children in
   northern Ghana." *Am J Trop Med Hyg* 71 (2): 167-72.
10. Boivin, M.J.(2002). "Effects of early cerebral malaria on cognitive ability in Senegalese children," *Journal of Developmental and Behavioral Pediatrics* 23 (5): 353–64.
11. Cheesbrough M (2000). District laboratory practice manual in Tropical Countries pt 2. Cambridge University press.
12. WHO. (2000). Malaria diagnosis, New Perspectives. Geneva, World Health Organisation.WHO/CDS/RBM/2000.14, WHO/MAL/2000.1091.
13. Aribodor U.D.N., Njoku O.O., Eneanya C.I. and Onyali I.O. (2003). Studies on prevalence of malaria and management Practices of the Azia community, Ihiala LGA, Anambra State, South East Nigeria. Nigerian Journal of Parasitology 24:33-38.
14. Sotimehin, S.A., Runsewe-Abiodun T.A., Oladapo, O.T. Njokanma O.F. and Olanrewaju D.M. (2008). Possible Risk Factors for Congenital Malaria at a Tertiary Care Hospital in Sagamu, Ogun State, Southwest Nigeria. Journal of Tropical Pediatrics, http://tropej.oxfordjournals.org/misc/terms.shtml
15. Ukoli FMA (1990) Introduction to Parasitology in Tropical Africa. Chichester, Wiley & Sons, 1990, pp 399–429.
16. Kakkilaya B.S (2006). www.malariasite.com.
17. Diadier A.D., Colin S., Issa N, Amadou T.K., Rosalynn O., Hirva P., Cally R., Edith I, Brian, M.G. and Simon N.C. (2007). Curtains are not associated with greater circulation of drug-resistant malaria parasites, or with higher risk of treatment failure among children with uncomplicated malaria in Burkina Faso. *Am. J. Trop. Med. Hyg.*, 76(2): 237-244.
18. Akanbi O.M, Odaibo A.B., Afolabi K.A. and Ademowo, O.G. (2005). Effect of Self-Medication with Antimalarial Drugs on Malaria Infection in Pregnant Women in Southwestern Nigeria. Med Princ Pract 2005; 14:6–9.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/200988/**21021** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 164-169

PLASMODIUM FALCIPARIUM PARASITEMIA IN PREGNANCY IN RELATION TO MATERNAL ANAEMIA

[1]AKINBORO R. A., [2]OJURONGBE O., [*3]AKINDELE A.A., [2] ADEFIOYE O.A., [2]BOLAJI O. S.,[4]OLANIRAN O, [2]ADEYEBA O.A.

1. Department of Medical Microbiology and Parasitology, LAUTECH Teaching Hospital, Osogbo, Nigeria. 2. Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria. 3.Department of Community Medicine, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria. 4. Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria.

ABSTRACT

This study was aimed at examining existing relationship between peripheral parasitaemia of *Plasmodium falciparum* and anemia among pregnant women in a secondary hospital and a tertiary hospital in Osogbo, South-Western, Nigeria. Two hundred and twenty five (225) patients were enrolled into this study, one hundred and fifty (150) from Asubiaro General Hospital, Osogbo and seventy five (75) from LAUTECH Teaching Hospital, Osogbo. A total of 30 (13.3%) women carrying the first pregnancy (primigravida), and 195 (86.6%) multiparous women (2-5) were enrolled. Mean age of recruited women was 31.511± SD 1.03, mean gestational age was 2.4267 ± SD 0.72 and mean packed cell volume was also 26.889 ± SD 0.43. Overall prevalence of malaria parasitemia was 63.6% while mean malaria parasite density was 461.33 among women infected with malaria parasite. Prevalence of malaria in pregnancy was highest amongst women with first pregnancy and in the age bracket 26 – 30 years (26.7%) and least among women greater than 40 years. Parasitemia decreased as parity increased, as women acquire immunity to malaria progressively with multiple pregnancies. Mild to moderate anaemia was also found to be prevalent among primigravida (11.6%) and this was associated with malaria parasitemia among these women .No correlated relationship was established between malaria parasitemia and age, gravidity, trimester of pregnancy, and Packed cell volume. Malaria chemoprophylaxis and other methods of malaria control should be sustained and advocacy for inclusion of malaria treatment in safe motherhood should be continued because of its beneficial potentials.

Key words: Malaria, Pregnancy, anaemia.

[*]Correspondence Author: Akindele A.A., Department of Community Medicine, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.(e-mail: akindeleaa@yahoo.com)

INTRODUCTION

Malaria has been described as a disease of poverty and underdevelopment (1). It remains a complex and overwhelming health problem, with 300 to 500 million cases and 2 to 3 million deaths per year (1). About 90 percent of all deaths attributable to malaria

occur in sub-Saharan Africa. Although 40 percent of the world's population is at risk for malaria, in pregnant women the disease

has been most widely evaluated in sub-Saharan Africa.

Plasmodium falciparum infection during pregnancy increases the chance of maternal anemia, abortion, stillbirth, prematurity, intrauterine growth retardation, and low birth weight (defined as a weight of 2500 g), the greatest risk factor for neonatal mortality (2). Malaria during pregnancy is therefore a serious problem in sub-Saharan Africa, affecting an estimated 24 million pregnant women annually (2). Although *P. falciparum* infection in pregnancy could be asymptomatic, it often contributes to adverse perinatal outcomes with a high risk for infant death, maternal morbidity, including fever and severe anemia, abortion, and placental malaria particularly in areas of lower malaria endemicity (3). On the other hand anaemia in pregnancy is thought to be one of the commonest problems affecting pregnant women in developing countries.

In 1993, the World Bank ranked anaemia as the 8th leading cause of disease in girls and women in the developing world (4). Data collected from all over the world indicate that a total of 2170 million

people (men, women and children) are anaemic by WHO criteria (4). The most affected groups, in approximately descending order are pregnant women, the elderly, school children and adult men. In developing countries, prevalence rates in pregnant women are commonly estimated to be in the range of 40-60%. Among non-pregnant women this is 20%-40% and in school aged children and adult men the estimate is around 20% (2).

In sub-Saharan Africa, it is estimated that between 200,000 and 500,000 pregnant women develop severe anemia as a result of malaria (3). *P. falciparum* malaria in pregnancy is the primary cause of up to 10,000 maternal anemia-related deaths in sub-Saharan Africa annually (4). However, there have been conflicting reports from parts of sub-Saharan Africa on the relationship between placental malaria and maternal anemia. An earlier report from the Ubangi district of Zaire noted that malarious placentas had no consistent relationship to maternal anemia (5).

In other studies, maternal anemia and placental malaria were associated in all gravidity and age groups, with maternal anemia higher among women with placental malaria than those without placental malaria (3). This study was therefore aimed at examining existing relationship between peripheral parasitaemia and anemia among pregnant women in a secondary and one tertiary institution in Osogbo, South-Western, Nigeria.

MATERIALS AND METHODS

STUDY LOCATION

The study was carried out in Olorunda Local Government with headquarters in Osogbo, the capital of Osun State, Nigeria. Osogbo is in the tropical rain forest belt of Southwestern part of Nigeria, it is about 500kilometers from Abuja the capital city of Nigeria. It lies approximately on latitude 40^0N of equator and longitutude 7.34^0E of Greenwich meridian. The sites selected for this study are Ladoke Akintola University of Technology Teaching Hospital, and General Hospital, Asubiaro, in Osogbo.

STUDY SUBJECTS

The study subjects consisted of 250 pregnant women on regular ante-natal visit at Ladoke Akintola University of Technology Teaching Hospital, and General Hospital, Asubiaro, Osogbo between December 2008 and June, 2009. The women were of varying age ranging from 18-45 years.

BLOOD SAMPLE COLLECTION

Safety procedures were adopted in the collection of venous blood samples by swabbing the ante cubital fossae with 70% alcohol and 5mls of blood was drawn into EDTA bottle with sterile hypodermic needle. Thick and thin films were made on clean slides and labelled accordingly as recommended by WHO (3).

MICROCOPIC EXAMINATION

The thin films were fixed with methanol and all films were stained with 3% Giemsa stain of pH 7.0 for 30 min as recommended by WHO (6). Taking the number of leucocytes per micro liter of blood as 8,000, parasite density of blood using the thick film was expressed as: parasite count (x) 8,000 divided by number of WBCs counted. The thick films were used to determine the parasite densities while thin films were used to identify the parasite species and infective stages. Stained slides were examined under the light microscope using x100 objective lens (immersion oil) (3).

HAEMOGLOBIN DETERMINATION

Five ml of blood were collected inside EDTA bottle .Non heparinised capillary tubes were filled with blood sample from the EDTA bottle.The tip of the capillary tubes were cleaned with cotton wool and they were arranged inside the haematocrit centrifuge.They were centrifuged at revolution per min. The Packed cell volume was determined by using haematocrit reader to read the level of the haemoglobin (7).

STATISTICAL ANALYSIS

Data was analyzed using Statistical programmed for service solution (SPSS) 16.0 (SPSS Chicago Inc., IL, U.S.A.), the statistical significance of variables was estimated using chi-square test. Pearson correlation analysis shall be used to establish possible relationship or correlation between *Plasmodium falciparum* parasitemia and gravidity, age, trimester, packed cell volume.

P-values of equal to or less than 0.05 will be taken as measures of significance.

RESULTS

Two hundred and seventy five (275) women were enrolled into this study, one hundred and fifty pregnant women (150) from Asubiaro General Hospital, Osogbo and seventy five pregnant women (75) from LAUTECH Teaching Hospital, Osogbo. A total of fifty (50) ages matched, non-pregnant women were recruited as controls. They had no symptoms of malaria, and were recruited among members of staff, students and traders within the Teaching Hospital complex.

A total of 30 (13.3%) women carrying the first pregnancy (primigravida), and 195 (86.6%) women with multiple parity (2-5) (multigravida) were enrolled. All enrolled women in this study are married. Mean age of recruited pregnant

TABLE I: Prevalence of Malaria according to age

	Pregnant women n=225		**Non pregnant women n=100**	
Age (year)	Frequency (%)	Positive (%)	Frequency (%)	Positive (%)
16-20	11(4.9)	7(3.1)	3(3.0)	0(0)
21-25	44(19.6)	33(14.7)	14(14.0)	1(1.0)
26-30	95(4.22)	60(26.7)	22(22.0)	5(5.0)
31-35	52(23.1)	30(13.3)	20(20.0)	3(3.0)
36-40	21(9.3)	11(4.9)	30(30.0)	20(20.0)
41-45	2(0.9)	2(0.9)	11(11.0)	5(5.0)
Total	**225(100)**	**225(100)**	**100(100)**	**34(34)**

women was 31.511± SD 1.03 (controls; 32.22± 1.32) mean gestational age was 2.4267 ± SD 0.72 and mean packed cell volume was also 26.889 ± 0.43 for patients while controls was 35.5± .27405 .

Overall prevalence of malaria parasitemia was 63.5% for pregnant women while prevalence among controls was (12.0%). Meanwhile, means of malaria parasite density accounted for 461.33 among pregnant women infested with malaria 100 (100) 31(31) parasite in this study, same was 22.40 among non-pregnant control women.

Table I shows age distribution of recruited pregnant women in relation to malaria positivity. Women between ages 26 – 30yrs accounted for 42.2% of the study population. Followed by 31 – 35yrs which accounted for 23.1% .Other age distributions are 16 – 20yrs – 4.9%, 21 – 25yrs – 19.6 %, 36 – 40 - 9.3 % and > 40yrs - 0.9% . Prevalence of malaria in pregnancy was highest amongst women in the age bracket 26 – 30 years (26.7%), same was 2.0% among non pregnant controls. Prevalence was least among women greater than 40 years. Statistically however, there was no significant difference in the trend. ($\chi 2$ = 5.54, df =5, p >0.05).

Table II: Prevalence of malaria according to Gestational age

Gestational Age	**Frequency (%)**	**No. +ve for malaria (%)**	**No –ve for malaria (%)**
1st Trimester	**31 (13.8)**	**20 (8.9)**	**11 (4.9)**
2nd Trimester	**67 (29.8)**	**41 (18.2)**	**26 (11.6)**
3rd Trimester	**127 (56.4)**	**82 (36.4)**	**45 (20)**
Total	**225 (100.0)**	**143 (63.6)**	**82 (36.4)**
X^2 = 0.23, df =2, p>0.05			

Table III: Prevalence of Malaria according to Parity

Parity	**No. Examined**	**No. +ve for Malaria**	**No. -ve for Malaria**
1 + 0	**92**	**58(25.8)**	**34 (15.1)**
2+ 0	**68**	**45 (20)**	**23 (10.2)**
3 + 0	**39**	**25 (11.1)**	**14 (6.2)**
4 + 0	**18**	**11(4.9)**	**7(3.1)**
≥ 5	**8**	**4 (1.7)**	**4 (1.7)**
Total	**225**	**143 (63.6)**	**82 (36.4)**

Table IV: Relationship between PCV and malaria

PCV(%)	No. Examined (%)	Patients		Controls	
		No. +ve for malaria(%)	No. - ve for malaria (%)	No. + ve for malaria (%)	No. -ve for malaria (%)
10-20	2 (0.9)	0 (0)	2 (0.9)	0 (0)	0 (0)
21-30	66 (29.3)	47 (20.9)	19 (8.4)	3 (6.0)	5 (10.0)
31-40	157 (69.8)	96 (42.7)	61 (27.1)	3 (6.0)	39 (78.0)
Total	225 (100.0)	143 (63.6)	82 (36.4)	6 (12.0)	44 (88.0)

Table II is a frequency table which shows the relationship between gestational age (trimesters) and prevalence of malaria positivity. One hundred and twenty seven women (56.4%) were in the third trimester, while 29.8% (67) were in the second trimester. Meanwhile only 13.3% (30) were in their first trimester. Prevalence of malaria positivity increased progressively across trimesters, from first (8.9%), second (18.2%) to third trimester (63.5%).However, there was no existing significant difference between malaria positive and negative cases with respect to trimester. ($\chi 2 = 0.23$, df =2, $p > 0.05$).

Malaria parasitemia was prevalent among the primigravida (women with first pregnancy) 58/225 (25.8%) and parasitemia decrease as parity increases, among the secondigravida (women with second pregnancy) prevalence was found to be 20% (45/225) while Parity 3, 4, and 5 have a prevalence of 11.1% (25/225) ,4.9% (10/225) and 1.7% (4/225) respectively as shown in table III.There was no significant difference between malaria positive and negative cases with respect to parity . ($\chi 2 = 0.89$, df =4, $p > 0.05$).

Mild to moderate anemia (PCV; 21 - 30) as found in the study population accounted for 29.3% (66/225) and was prevalent, while severe anaemia was uncommon 0.9% (2/225) in the study population. A large population of pregnant women had PCV 31 – 40% as shown in the frequency table IV. Of the 2 patients that had severe anaemia (PCV≤ 20), none was positive for malaria parasite, While 44 (19.6%) of 65 patients that had moderate anaemia demonstrated malaria parasite positivity. Pearson Chi-square test showed no correlation between malaria positive and negative cases in relation to packed cell volume. (($\chi 2 = 5.55$, df = 2, $p > 0.05$). (Table IV).

DISCUSSION

Malaria during pregnancy is a serious problem in sub-Saharan Africa, affecting an estimated 24 million pregnant women. Pregnancies in women living in malaria endemic regions, particularly in sub-Saharan Africa are associated with a high frequency and density of *Plasmodium falciparum* parasitaemia, with high rates of maternal morbidity including fever and severe anaemia, with abortion and stillbirth, and with high rates of placental malaria and consequently low birth weight in newborns caused by both prematurity and intrauterine growth retardation (8).

In highly endemic malarious area where semi-immune adults usually have substantially acquired resistance to local strains of plasmodia, the prevalence of clinical malaria is higher and its severity greater in pregnant women than non-pregnant women (2). This was found true in this study, as prevalence of malaria parasitemia was found to be 63.6% which was higher than prevalence of 12.0% among non pregnant women used as control. This prevalence is similar to 72% found by Adefioye *et al* (9) in a study among pregnant women in Osogbo. It is also worth mentioning that Okwa in Lagos (10) found a comparable prevalence of 60%. However, Huddle *et al* (11) in rural Malawi study found extremely high prevalence of 83%. In studies conducted by Brabin in 1991 (12), the primigravidae were more susceptible to malaria infection than the multigravidae, which was also confirmed by this study. Prevalence was highest among the primigravidae (25.8%) and malaria positivity decreases as parity increased. Among the secondigravida, prevalence was found to be 20% while Para 3, 4, and 5 had a prevalence of 11.1%, 4.4% and 1.7% respectively. Onwere *et al*. (13) in

Aba found higher prevalence among primigravidae (39.3%).

Younger women appeared to be susceptible to malaria in this study as prevalence was highest among age group 26 – 30 (26.7%), a lower prevalence of 2.0% was found among the non pregnant controls. This contradicts the findings of Adefioye *et al* (9) that found 36 – 39 year age group to be more susceptible. However 3rd trimester prevalence in this study is a deviation from previous studies as Brabin in 1983 (2) found in Western Kenya that prevalence was highest at 13-16 weeks gestation (1st trimester), and found similar number of recoveries in both groups during the 2nd and 3rd trimesters. The loss of immunity in early pregnancy was equivalent to an 11-fold decrease in the rate of recovery from infection. The recovery seen in late pregnancy suggests that the women mount a satisfactory immune response to malaria infection, reacquiring their pre-pregnancy immune status at about the time of delivery (14). However, reasons that may be adduced to high prevalence of parasitemia include the fact that, large numbers of women in this study were in their 3rd trimester, and registered late for care, which is extremely common in this environment.

It is not clear whether a natural immune depression in pregnancy or factors associated with the placenta encourage parasite multiplication and determines clinical manifestations. Pregnant women with malaria in pregnancy in Tanzania had significantly higher total and free serum cortisol concentrations than controls without malaria whether nulliparous or multiparous. High plasma corticosteroid levels may have an immunosuppressive effect on cell mediated immune responses. Cell-mediated immune responses to malaria antigens are more markedly suppressed in first than in subsequent pregnancies (2).

The multigravidae are presumably less affected because immunological memory from first pregnancy is retained. In first and second pregnancies women are especially vulnerable. Mcgregor (15) identified the factors responsible for susceptibility of primigravidae to malaria as inhibition of type 1 cytokine responses (interferon, interleukins 2 and 12 and TNF). At pregnancy, immunity has been altered; hence, with malaria 70–80% of pregnant women in malarious areas are susceptible to anaemia (2).

Mild to moderate anaemia (mean PCV: 26.1%, Control: 35.5%) was prevalent generally in this study (28.9%) and was highest in the 2nd trimmester. This prevalence seems low when compared with other studies. Reasons for this might be that most of these women are on routine folic acid, iron and other haematinics which might had brought up their PCV. Idowu *et al* (16) observed higher prevalence of anaemia in pregnanacy (76.5%) in Abeokuta because women attending traditional birth home (TBH) were recruited into his study. Most of the women were taking herbal remedies made from tree barks, leaves and roots of undisclosed plants.

The iron supplementation is necessary to prevent anaemic condition during increased physiological burden of pregnancy (2), same was lacking among women in the TBH, this might had contributed to the higher prevalence recorded in that study. The peak of anaemia recorded in this study (2nd trimester) also coincides with the period when haemodilution get to its maximum peak. This may have contributed to the high prevalence recorded in the 2nd trimester, showing that anaemia is further aggravated by haemodilution in addition to other possible factors. However, this finding contradicts the report of WHO in which anaemia is said to be significantly higher in the 3rd trimester of pregnancy than the first two trimesters. More so most of these women are in the 3rd trimester in which tendencies to anaemia is much reduces. This aggregation in 3rd trimester might be due to late antenatal booking.

CONCLUSION

This study recorded high prevalence of malaria parasitemia among pregnant women attending antenatal clinic in Osogbo. The study also recorded mild to moderate anaemia among pregnant women with malaria parasitemia compared to those without malaria parasite and the controls. The high prevalence of malaria parasites can be traced to the fact that Osogbo is in the rain forest belt of southwestern Nigeria, which is a good environment for mosquito breeding especially during the rainy season when this study was conducted.

Malaria parasitemia and its attendant's complications have been demonstrated to be worst in pregnancy in this study. Proper malaria control programme is highly necessitated among this highly susceptible group especially in the tropics. With good ambient temperature, humidity, and mosquito will conveniently transmit malaria. There are stagnant pools and blocked drainages in Osogbo, many of the pregnant women came from shanty region of the city. Pregnant women must therefore be

adequately protected from double edged sword of malaria and anaemia. Advocacy for inclusion of malaria treatment into Safe Motherhood Initiatives must be sustained, because of these overwhelming implications of malaria parasitemia in pregnanacy.

It is recommended that routine intermittent preventive treatment of malaria is therefore recommended in this environment, for women in pregnancy. Several studies have shown that protection against malaria contributes to the prevention of anaemia in pregnancy, thus highlighting the importance of chemoprophylaxis and use of other methods of malaria control like insecticide impregnated bed net. These good practices must however be sustained and recommended to be included in Safe Motherhood Initiatives because of its beneficial potentials in our resource poor setting. Early ante natal booking for effective monitoring and prompt treatment of both malaria and anaemia in pregnanacy will contribute significantly in reducing maternal mortality and morbidity and its attendant perinatal mortality. Regular environmental sanitation to dislodge mosquitoes from their breeding places will also go a long way to reduce prevalence of malaria in shanty towns and villages commonly seen in the tropics which we belong.

REFRENCES

1.WHO Expert Committee on Malaria. Twentieth report. Geneva, World Health Organization, 2000 (WHO Technical Report Series, No. 892).

2. Brabin BJ. An analysis of malaria in pregnancy in Africa . Bull World Health Organ.;61(6):1005-16.

3. World Health Organization. The Prevalence of Anaemia in Women: A tabulation of available information. 2nd Ed. Geneva: WHO; 1992.

4. Steketee RW, Nahlen BL, Parise ME, Menendez C . The burden of malaria in pregnancy in malaria-endemic areas. Am J Trop Med Hyg 2001; 64: 28–35.

5. World Malaria Report. 2008. www.cdc/malaria epidemioloy .com

6. Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science 1976; 193: 673-5.

7. Beales PF. (1997)**.** Anaemia in malaria control. A practical control. Amer J Trop Med Parasitol 1997; 91: 713– 718

8. Mkandala C . The Effect of Malaria in Pregnancy on MaternalAnaemia and Birth Weight in Rural Malawi. A Master's paper submitted to the faculty of the University of North Carolina at Chapel Hill, 2003, Chapel Hill.

9. Adefioye O.A., Adeyeba O.A. , Hassan W.O. and Oyeniran, O.A. Prevalence of Malaria Parasite Infection among Pregnant Women in Osogbo, Southwest, Nigerian. American-Eurasian Journal of Scientific Research, 2007, 2 (1): 43-45.

10. Okwa O Omolade. The Status of Malaria among Pregnant Women: A
Study in Lagos, Nigeria *Afr J Reprod Health* 2003; 7[3]: 77– 83)

11. Huddle JM, Gibson RS, Cullinan TR: The impact of malarial infection
and diet on the anaemia status of rural pregnant Malawian women**.** *Eur J Clin Nutr* 1999, 53:792-801.

12. Brabin BJ. The risks and severity of malaria in pregnant women. In: Applied field research in malaria . Report no. 1 1991. Geneva: WHO:1-34.

13. Onwere S., Okoro O., Odukwu O. and Onwere A. Maternal malaria in pregnancy in Abia State University Teaching Hospital JOMIP Vol.7 March 2008.

14. Saute F, Menendez C, Mayor A, Aponte J.*et al*. Malaria in pregnancy in rural Mozambique: the role of parity, submicroscopic and multiple Plasmodium falciparum infections. Trop Med Int Health. 2002 Jan;7(1):19-28**.**

15. McGregor, LA . Epidemiology, malaria and pregnancy. Amer J Trop Med Hygiene 1984; 33: 517–525.

16. Idowu O A; Mafiana C F; Dapo S. Malaria among pregnant women in Abeokuta, Nigeria. Tanzania health research bulletin 2006;8(1):28-31.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)
AJCEM/200991/**21022** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 170-173

SEROPREVALENCE AND RISK FACTORS OF HEPATITIS C VIRUS IN PATIENTS AND BLOOD DONORS IN KANO, NIGERIA

Azeez-akande, O[1]. , Sarki, A[2]. Wokedi, E. E.[1], Olabode, A[3]and Alabi, P[4].Departments of Medical Microbiology andParasitology,[1] Haematology and Blood Tranfusion,[2] Medicine,[4] Faculty of Medicine/Aminu Kano Teaching Hospital, PMB 3011, Kano, Nigeria, and Federal College of Veterinary and Medical Laboratory Sciences,[3] Vom, Nigeria.

CORRESPONDENCE: Dr. O. Azeez-Akande, Department of Medical Microbiology and Parasitology, Faculty of Medicine, Bayero University, PMB 3011 Kano, Nigeria. E-MAIL: akadaze@yahoo.com

ABSTRACT

Hepatitis C virus (HCV) is a major cause of chronic liver disease resulting in cirrhosis and hepatocarcinoma. It is believed to be widespread in Africa but its epidemiology is incomplete and is yet to be determined in many areas of the sub-saharan Africa including Nigeria. Using third generation enzyme immuno-assay (EIA-3) and recombinant immunoblot assay (RIBA) technique as confirmatory test, we examined the prevalence of HCV antibodies in 226 blood donors and 226 patients attending Aminu Kano Teaching Hospital (AKTH) in Kano, Nigeria and evaluated the risk factors of HCV transmission in this environment. HCV antibodies were detected in 0.4% and 2.2% blood donors and patients respectively. The overall HCV seroprevalence was 1.3%. There was increased infection acquisition with increasing age; one (16.7%) HCV infection occurred in 25-34 years age group and 5 or 83.3% in subjects ≥ 45 years in age which was significant (P< 0.05). The ratio of infection in male to female was 1:5. Evidence of previous exposure via transfusion was common in HCV seropositive subjects and could be a major risk factor of acquisition in this environment. Adequate screening of blood products in sub-Saharan Africa (Nigeria inclusive) may minimize the risk of HCV transmission and associated health complications.

Key words: Hepatitis C virus, seroprevalence, patients, blood donors, risk factors.

INTRODUCTION

Hepatitis C Virus (HCV) is an enveloped, single-stranded, positive sense RNA virus. It is a member of flaviviridae family placed in genus, hepacivirus (1, 2). It is an important
cause of liver disease in the tropics; provoking chronic persistent infection progressively causing chronic hepatitis leading to liver cirrhosis and hepatocarcinoma (HCC) (3,5).

As at 1997, the World Health Organization (WHO)(6) estimated that about 170 million people were chronic carriers of HCV infection worldwide. Furthermore, an average of 14% of infected persons was at risk of developing liver

cirrhosis, HCC or both annually. There are about 3.2-4 million cases of chronic HCV in the United States (US) alone which results in at least 10,000 deaths each year (7,8). In Canada, the number of HCV cases was estimated to be as high as 270,000 (9).

Routes of HCV transmission include parenteral (including illicit drug abuse by injection), sexual, vertical (from mother to child) and through blood transfusion while tattooing and all forms of circumcision (ritual or traditional) have been suggested as modes of acquisition of the disease (10-13).

Data from various epidemiological studies globally indicated that HCV seroprevalence among the general population varies from < 1 % to 2% in industrialized countries of the west (14) and >20% in certain regions of Africa (15) and south-east Asia (16). However, among the blood donors, the HCV seroprevalence was 1.4% in the US, 0.5-1 %in the United Kingdom (UK) and 0.68% in France (17). In sub-saharan Africa (Nigeria inclusive), there is paucity of data on HCV infection while a few studies carried out in Nigeria were mainly from the southern part of the country, and none from our hospital location in Kano; a commercial centre in the north-west geopolitical zone of Nigeria. The study was therefore aimed 'to determine the seroprevalence of HCV among blood donors and patients attending Aminu Kano Teaching Hospital (AKTH), a tertiary health centre in Kana, Nigeria and evaluate the risk factors of HCV acquisition in this environment.

SUBJECTS, MATERIALS AND METHODS

The subjects screened for HCV antibodies comprised of 226 (215 males and 15 females) asymptomatic blood donors consecutively recruited for testing. Others were 226 (95 males and 127 females) patients from the general outpatient Department (GOPD) of

the hospital who were randomly selected for the study. In total, there were 310 or 68.6% males and 142 (31.4%) females. Their ages ranged from 15 to 59 years (median 37.5 years). Using a structured questionnaire and with informed consent, all the participants were interviewed to record history of surgery, blood transfusion, tattooing, use of illicit drugs like cocaine or heroin by injection, known previous infectious diseases, anaemia etc. All individuals who reported for blood re-donation for the blood bank during the investigation and the immunocompromised were excluded from the study. The study was conducted between February and August, 2007 and was approved by ethnical committee of the hospital.

TABLE 1: HCV ANTIBODY TEST RESULTS OF SUBJECTS AT AKTH

Subjects	No screened	No positive (%)
Patients	226	5 (2.2)
Blood donors	226	1 (0.4)
Total	452	6(1.3)

Serological assay

Serum samples were obtained by standard methods and tested for HCV antibodies. Briefly, 5 millilitres of blood was collected from each subject by venous puncture and centrifuged at 3000rpm for 5 minutes. Serum was separated from each blood sample and stored at - 20°C until analyzed. The sera were tested for anti-HCV antibodies by employing third generation enzyme immuno-assay technique (Caltech. Diagn. Incorp. Cal. USA). For assurance, all positively reactive samples from the initial test were retested with recombinant immunoblot assay (RIBA) or, western blot, (Diasorin, Italy) for confirmation. Testing Procedures and interpretation of results were as prescribed by the manufactures of test kits. These were methodically followed and strictly adhered to. All positive patients were informed of the test results for further medical evaluation and management. Data were analysed using simple proportion student's t-test or Wilcoxon signed rank was used to compare continuous variables and the chi-square test was used to compared proportions. A p value of $\leq$ 0.05 was considered significant.

RESULTS

The HCV diagnosis of the participants comprising blood donors and patients attending AKTH in Kano, Nigeria is shown in table 1. The overall HCV seroprevalence was 1.3% consisting of 2.2% and 0.4% seropositive patients and blood donors respectively. Of 452 subjects screened for HCV infection, 310 (68.6%) were males and 142 or 31.4% were females. The ages of patients ranged from 15-59 years (mean $\pm$ SD, 39.5 $\pm$ 3.5) while ages of the blood donors ranged from 18-49 (mean ± SD, 35.2 ± 4.1) (Table 2).

TABLE 2: SEX AND AGE RANGE OF PATIENTS AND BLOOD DONORS SCREENED FOR HCV ANTIBODY AT AKTH

Subjects	No screened (Male/female)	Age range (yr.) Mean $\pm$ SD
Patients	226 (95/127)	15 – 59 (39.5 $\pm$ 3.5)
]Total (%)	452(310)	(68.6)/142 (31.4)

The distribution of HCV seropositivity according to age range, sex and history of transfusion is shown in table 3. Out of 6 HCV seropositive subjects, 5 or 83.3% were females with all of them having history of blood transfusion and was found to be statistically significant (P<0.05) while the remaining one was a male who did not have any record of transfusion. However, the possible source or route of acquisition in this male subject could not be ascertained.

TABLE 3: HCV SEROPOSITIVITY OF SUBJECTS ACCORDING TO SEX AND HISTORY OF TRANSFUSION

Age range of Subjects (yr.)	No (% +ve)	sex M	F	No (% with history of transfusion)
15 – 24	-	-	-	-
25 – 34	1(6.7)	1	-	1
35 – 44	-	-	-	-
$\geq$ 45	5(83.3)	1	4	4
Total (%)	6	1(16.7)	5(83.3)	5(83.3)

There was increased infection acquisition with increasing age; five seropositive subjects were $\geq$ 45 years in age which was significant (P<0.05) while the remaining female subject was 27 years old.

DISCUSSION

In this study, the overall prevalence of HCV infection was found to be 1.3% comprising blood donors and patients attending our hospital (AKTH) in Kano, Nigeria. This prevalence is low compared with the rates reported elsewhere in the country viz: among some selected risk groups including 37.9% in patients attending STD clinics (18), 18.7% among patients with HCC (19) and 14% among blood donors and patients attending sickle-cell anaemia clinics (20) in Nigeria. The variations may be due to difference in study populations, and rate of exposure to risk factors of HCV transmission in those areas. However, this figure of 1.3% falls within the published data of 0.4 - 1.5% seroprevalence from Western Europe and North America (17) and corroborates the level of infection rate of HCV in those areas.

The prevalence of HCV infection in our study was 0.4% among blood donors and 2.2% among hospital patients. These results were consistent with the estimates of 0.3 - 1 among blood donors in Europe and Australia, (11) and 1-2.4% reported from parts of

Asia including Pakistan, India, China and Bangladesh (21). These prevalence rates were however, lower than 4% - 12.5% of HCV infection published from various regions of Africa (22), and as high as 47% reported in parts of Nigeria (23,24). The wide disparity in HCV prevalence may be due to profound diversity in demographic characters of populations and subpopulations in this continent. Moreso, the issue of quality and reliability of HCV infection diagnosis especially in poor-resourced developing economics due to inadequate facility and high cost of test kits may play a role in the present scenario (22).

The incidence of HCV infection was correlated with increasing age (one case in 25-34 years age range; 5 cases in $\geq$ 45 years age) and was significantly ($P<0.05$) higher in females than males by ratio 5:1. The scenario corroborates the report of Halim and Ajayi[23] who suggested that age could be a contributing risk factor in the acquisition of HCV infection especially in endemic areas. Similarly, the reason for increased rate of infection in females than their male counterparts may be explained by tendency of exposure to multiple risk factors in depressed economy with inadequate health care system in a country like Nigeria. These include variety of poorly conducted surgery-assisted treatments for gynaecological problems which may require blood transfusion. For instance, evidence of previous exposure to HCV through caesarian operation (CS) and transfusion (conducted outside of our hospital) during child delivery was given by 5 (83.3%) female subjects all of whom were HCV positive; a finding that is statistically significant ($P < 0.05$). This outlook corroborates the hypothesis that transfusion is a major risk factor in the transmission of HCV especially in poor-resourced areas of the developing world. In view of this outcome, it is imperative that adequate screening of blood and blood products for HCV be carried out in all blood transfusions to minimize the risk of HCV transmission in sub-Saharan Africa including Nigeria. There is need to provide necessary facility in this regard that will aid HCV control measures and prevent associated health complications.

ACKNOWLEDGMENT

We thank the laboratory scientists of pathology laboratory, AKTH for their technical support.

REFERENCES

1. Sattar, S.A., Tetro, J., Springthorpe, V.S. and Giulivi, A. Preventing the spread of hepatitis Band C viruses: Where are germicides *Am. J Infect. Control.* 2001; 29:18 - 197.
2. Gault, E., Patrick, S., Morice, Y., Sanders, L., Berrada, A., Rogers, B., and Paul, D. Evaluation of a new serotyping assay for detection of anti-Hepatitis C virus type - specific antibodies in serum samples: *J Clin. Microbiol.* 2003; 41 (5): 2084 - 2087.
3. Strickland, G.T. Hepatitis C in developing countries. *Postgrad. Doc. Africa* 2002; *24*(2): 26 - 28.
4. Lauer, G. M. and Walker, B. D. Hepatitis C virus infection. *N. Engl. J. Med.* 2001; 345: 41-52.
5. Satoshi, O. Florese, R. H., Nagano-Fuji, M., Rachmat, H., Lin, D., Yonson, K. *et al.* Identification of Hepatitis C virus (HCV) subtype 16 strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV N53 protein. *J Clin. Microbiol.* 2003; 41(7): 2835-2844.
6. Kane, A., Loyd, J., Zaffran, M., Simonsen, I., and Kane, M. Transmission of hepatitis B, hepatitis C and human immunodefiency viruses through unsafe injections in the developing world: model - based regional estimates *Bull. WHO* 1999; 77:801 - 807.
7. Armstrong, G. L., Wasley, A., Simard, E. P., McQuilan, G. M. Keuhneli, W. L. and Alter, M. J. The Prevalence of hepatitis C virus infection in the United States 1999 through 2002. *Ann. Intern. Med.* 2006; 144: 705 -714.
8. Center for Disease Control and Prevention (CDC). Viral hepatitis. Http:/ /www.cdc.gov/ncidod/diseases/hepatitis/index. htm.1999; retrieved April, 27, 2007.
9. Sherman, M. How significant is hepatitis C infection in Canada? Hepnet: The hepatitis information Network; http://www.hepnet.com/update.htm. 2005; retrieved August 19, 2005
10. Tibbs, C. I. Hepatitis C. *Trans. Roy. Soc. Trop. Med. Hyg.* 1997; 91: 121 - 124.
11. Diensteg, J. L. Sexual and perinatal transmission of hepatitis C virus. *Hepatol.* 1997; 26 (suppl. 1): 665 -705
12. Laument, C., Henzel, D., Mulanga-Kabeya, C., Maertens, G., Larouze, B and Delaporte, E. Seroepidemiological survey of Hepatitis C virus among commercial sex workers and pregnant women in Kinshasha, Democratic Republic of Congo. *Int. J.Epidemiol.* 2001; *30*: 872 - 877.
13. Thomson, J. A., Rodger, A. J., Thompson, S. C., Jolley, D., Byrne, A., Best, S. J. *et al.* The prevalence of hepatitis C in patients admitted

with acute hepatitis to Fairfield Infectious Diseases Hospital, 1971 – 1975. *Med. J. Aust.* 1998; *169:* 160 - 363

14. World Health Organization Hepatitis C *WHO Wkly. Epidemiol. Rec.* 1997; *72:* 65 - 69.

15. Abdul-Azeez, F., Habib, M., Mohammed, M. K. *et al* Hepatitis C virus (HCV) infection in a community in the Nile Delta: Population description and HCV prevalence. *Hepatol.* 2002; 32:111 - 115.

16. Greene, W. K., Cheong, Y. N, and Yap, K. W. Prevalence of hepatitis C virus sequence variants in South - East Asia. *J Gen. Virol.* 1995; 76: 211 - 215.

17. Chen, D.S., Kuo, G. C., Sung, J. I. *et al,* Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: The Taiwan experience. *J Infect. Dis.* 1990; 162: 817-822.

18. Oni, A. A., Odaibo, G. N., Ola, S. O., Olaleye, O. D., and Bakare, R. A. Seroprevalence of hepatitis C virus among patients attending STD clinic in Ibadan, Nigeria. *Afr J. Clin. Expt. Microbiol.* 2005; 6(1): 53 - 59.

19. 01ubuyide, 1. O., O1a, S. O., A1iyu, B. *et al.* Hepatitis Band C in doctors and dentists in Nigeria *Qt. J. Med.* 1997; 90: 417 - 422.

20. Mutimer, D. J., Olomu, A., Skidmore, S., Olomu, N., Ratcliffe, D., Rodgers, B., Mutimer, H. P., Gunson, B. K. and Elias, E. Viral hepatitis in Nigeria -sickle- cell disease and commercial blood donors *Qt. J. Med.* 1994; 87 (7): 407 - 411.

21. World Health Organization (WHO) Hepatitis C: Global Surveillance. *Wkly Epidemiol. Rec.* 1997*; 46*: 341- 344.

22. Tibbs, C. I., Palmer, S. I., Coker, R. *et al.* Prevalence of hepatitis C virus in tropical communities: the importance of confirmatory assays. *J. Med. Virol.* 1991; *34:* 143 - 147.

23. Halim, N. K. and Ajayi, O.I. Risk factors and seroprevalence of hepatitis C antibody in blood donors in Nigeria. *East Afr·. Med. J.* 2000; 77(8): 410 - 412.

24. Ola, S. O., Otegbayo, J. A., Odaibo, J. N., Olaleye, O. D. and Olubuyide, O. L. Serum hepatitis C virus and hepatitis B surface antigenanemia in Nigerian patients. *West Afri. J. Med.* 2002; 21 (3): 215 -217.

ORIGINAL ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)
AJCEM/201002/**21023** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 174-178

THE EFFECTS OF ANTIRETROVIRAL TREATMENT ON LIVER FUNCTION ENZYMES AMONG HIV-INFECTED OUT PATIENTS ATTENDING THE CENTRAL HOSPITAL OF YAOUNDÉ, CAMEROON

Lucien[1]*, K. F. H. , Clement[1] , A,N.J., Fon[1], N. P., Weledji[1] P. and Ndikvu,[2] C. P. [1] Faculty of Health Sciences, University of Buea, Republic of Cameroon and [2] Faculty of Science, University of Buea, Republic of Cameroon

*** Correspondence: Kamga Fouamno Henri Lucien (PhD), Faculty of Health Sciences, University of Buea, P.O Box, 63, Buea, Republic of Cameroon, Phone number (+237) 99721972. E-mail: henrikamga2002@yahoo.fr**

Abstract
The emergence of liver diseases as one of the major causes of death in people infected with HIV has paralleled the introduction of more effective antiretroviral therapies. This study was carried out with the aim of determining the effects of antiretroviral treatment on liver enzymes (SGOT and SGPT) in patients placed on antiretroviral therapy. A prospective cross-sectional 3 years study was carried out among patients confirmed to be HIV positive and who were to be placed on antiretroviral drugs at the HIV/AIDS out patient clinic of the Yaoundé Central Hospital, . Cameroon. Levels of transaminases of patients were measured in four phases using the International Federation of Clinical Chemistry (IFCC) protocol. Of the 150 patients who participated in the study, 54.0 %(81/150) presented with transaminitis at the final phase of the study with respect to aspartate aminotransferase (AST), 77.78% (63/81) of whose AST levels only increased after initiation of highly active antiretroviral therapy (HAART). 22.67% (34/150) presented with transaminitis with respect to alanine aminotransferase (ALT). At the final phase, 70.58% of whose ALT levels only increased after HAART initiation. Increase in blood transaminase levels was statistically independent on age group and the drug combinations. Increase in AST levels was associated with an increase in ALT levels upon treatment (r = 0.58). There was a significant positive linear relationship between duration of treatment and concentration of transaminases over the years (r= 0.9). We therefore concluded that highly active antiretroviral therapy (HAART) is associated with low level hepatotoxicity at therapy initiation, regardless of drug class or combination.
Keywords: Antiretroviral treatment, Liver function enzymes, Hepatotoxicity, Highly active antiretroviral therapy

Introduction

Antiretroviral drug-related liver injury (ARLI) is defined by elevations in liver enzymes in serum, with alanine aminotransferase (ALT) characteristically greater than aspartate aminotransferase (AST). It is one of the greatest causes of treatment discontinuation in HIV-infected patients [1]. Its prevention and management is therefore very important among HIV-infected patients who are to be placed on highly active antiretroviral therapy (HAART) [2]. Till date, there has been broad variability in the criteria used in clinical studies to categorize the severity of hepatotoxicity. Some studies have utilized ALT parameters as minimal as two times the upper limits of normal [3] while others have employed an absolute threshold (e.g., >100 IU/ml), regardless of baseline liver function tests [4]. Surveys have reported an increased incidence of hepatic injury in HAART-treated patients and identified life-threatening hepatotoxic events and end-stage liver disease in patients on antiretroviral [5].

With the widespread use of HAART and the availability of new antiretroviral medications, ARLI has gained prominent attention owing to its negative impact on clinical outcomes. Drug-associated hepatotoxicity also creates an economic burden on already strained medical budgets, since additional visits and hospital admissions are often required for appropriate patient care and management [1]. Furthermore, antiretroviral drug discontinuation hampers maintenance of HIV suppression. The severity of ARLI may range from the absence of symptoms to liver decompensation, and the outcome can range from spontaneous resolution to liver failure and death [6].

In August 2003, Cameroon defined and included first and second line drug regimens in the national treatment guidelines, including different HIV-drug combinations based on WHO recommendations and in 2005, the number of people living with HIV was estimated at 505.000, among which 61% were

females. An estimated number of 108,000 Cameroonians (0-49 years) were reported to be in need of antiretroviral therapy. This figure has been on the rise given that the number of people infected with the virus increased on daily basis and subsequently required treatment [7].

Several highly active antiretroviral therapy (HAART) regimens are hepatotoxic and the liver is one of the vital organs useful in the metabolism of these drugs as well as in detoxification. It is therefore important that the liver which is the main biochemical hub of the body be monitored and those HAART regimens that may be toxic to it identified so that changes or modifications can be made to enhance patient care.

Materials and Methods

Study Design

A cross-sectional study that involved newly diagnosed HIV positive individuals who reported for treatment at the outpatient clinic of the Yaoundé central Hospital in Cameroon was done. Information extraction forms were used to collect data from patients' files.

Study area

Yaoundé is the administrative capital of Cameroon with a population of 143000 inhabitants. It is centrally located and made up of immigrants from all the regions of Cameroon. The outpatient clinic of the Yaoundé Central Hospital is in the heart of the city.

Study population

The study was carried out on volunteer patients already diagnosed and confirmed to be HIV positive. Patients were made to understand that it was not a hospital obligation for them to participate in the research and neither was it a pre-requisite to accessing routine medical services. Written informed consent forms were distributed and explained to the participants one week prior to the beginning of the samples collection. Only HIV positive volunteers who returned their informed consent forms duly signed were recruited irrespective of their gender or clinical state of the disease. Only participants who were 20 years and above were recruited in the study

Phases of the study

The study was conducted for a period of three (3) years, from November 2006 to December 2009. Blood samples were collected in four (4) phases as follows.

- Phase 1: in November 2006, before patients were placed on antiretroviral therapy
- Phase 2: in November 2007 one year after commencement of therapy
- Phase 3: in November 2008, two years after commencement of therapy
- Phase 4: December 2009, final phase.

Laboratory procedure

About 4ml of blood samples were collected by venipuncture into labelled (coded) dry test tubes. After collection, samples were irreversibly de-linked from donor's information except that concerning the age. Blood samples were allowed to coagulate after which they were centrifuged at 3000 rpm for 5minutes to obtain sera.

Measurement of ALT (SGPT)

Measurement of ALT was done following a method that has been made according to the 2002 International Federation of Clinical Chemistry (IFCC) protocol [8]. Commercial kits produced by HOSPITEX DIAGNOSTICS Ltd were used. The test was carried out using the monoreagent procedure.

The working reagent was prepared by mixing 4 volumes of reagent one (R1) in 1 volume of reagent two (R2). Mixed by inversion and stored away from any light sources at 2-8°C.

The spectrophotometer was calibrated for ALT measurement. A series of labelled test tubes, i.e. Blank, normal control and patients from 1, 2….n^{th} according to the number of samples to be analysed.

	Working reagent	Sample
Blank	**1ml(1000ul)**	
Normal control	**1ml**	**100ul**
Patient	**1ml**	**100ul**

The preparation was mixed and the first reading of absorbance was executed after 90 seconds. Incubating at 37°C, 3 other readings were performed at 60 seconds interval. The change in absorbance per minute was then calculated.

The activities were obtained from the following calculations:

340nm: Activity (U/L) = change in absorbance / min. x 1769.

Normal values for ALT (SGPT) at 37°C: Women up to 34U/L. Men up to 45U/L.

Measurement of AST (SGOT)

Measurement of AST was done following a method that has been made according to the 2002 International Federation of Clinical Chemistry (IFCC) protocol [8]. Commercial kits produced by HOSPITEX DIAGNOSTICS Ltd were used. The test was conducted using the monoreagent procedure.

The working reagent was prepared by adding the vial of reagent two (R2) in the vial of reagent one (R1). The reagent was then stored at 2-8°C away from any light sources.

The spectrophotometer was calibrated for AST measurement. A series of labelled test tubes, i.e. Blank, normal control and patients from 1, 2….n^{th} according to the number of samples to be analysed.

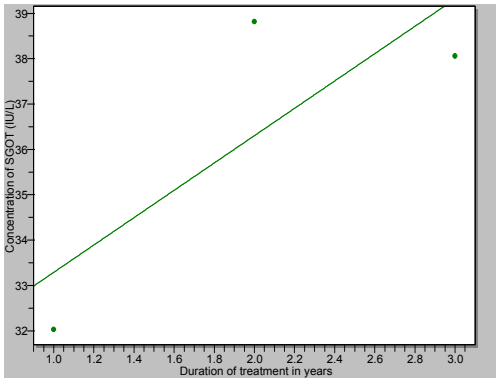
The spectrophotometer was brought to zero against distilled water.

	Working reagent	Sample
Blank	**1ml(1000ul)**	
Normal control	**1ml**	**100ul**
Patient	**1ml**	**100ul**

The preparation was mixed and the first reading of absorbance was executed after 90 seconds. Incubating at 37°C, 3 other readings were performed at 60 seconds interval. The change in absorbance per minute was then calculated as follows:
340nm: Activity (U/L) = Change in absorbance/ minute x 1769.
(Normal values for AST (SGOT): Women up to 31U/L and Men up to 35U/L).

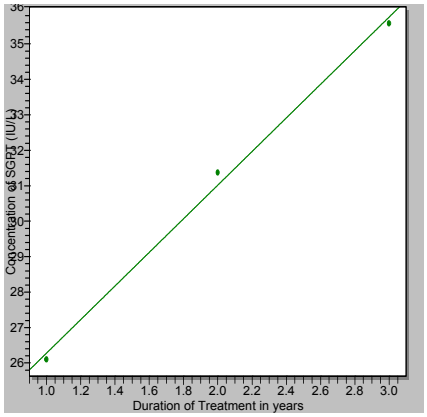
Statistical Analysis
Statistical tests such as Chi-square and Pearson's product moment correlation were used to test for the significance of the results obtained.

Results
Of the 150 patients who participated in this study, 81 (54.0 %) showed a transaminitis with respect to AST at the final phase of the study, 77.78% (63/81) of whose AST levels only increased after initiation of HAART. 22.67% (34/150) presented with transaminitis with respect to ALT at the final phase, 70.58% of whose ALT levels only increased at HAART initiation. Increase in AST levels was found to be is associated with an increase in ALT levels upon treatment (r = 0.58).
Figure 1 and Figure 2 show the relationship between the mean concentration of transaminases (SGOT and SGPT) and the duration of treatment. There was a linear increase of SGOT mean concentration in blood over time (r=0.81).



(r=0.81)

Figure 1: The relationship between the mean concentration of SGOT and duration of treatment



(r=0.99)

Figure 2: The relationship between the mean concentration of SGPT and duration of treatment

Also, there was a significant positive linear relationship between the concentration of transaminase (SGPT) with treatment duration (r=0.99).The level of transaminases concentration was found to be increasing over time.

Tables 1 shows the age groups and AST levels among the 150 patients. The both transaminase levels were highest among the patients aged above 50 years.
Table 2 shows the drug combinations and transaminase (AST and ALT) levels. Stocrin,Duovir was the combination showing the highest percentage of increased AST (55.17%) while LamivirS showed the highest percentage of increased ALT (35.29%).

Table1: Age groups and transaminase (AST and ALT) levels

Age group (year)	Total	Number(%)* showing high AST levels	Number(%)* showing high ALT levels
20-35	**73**	**32 (21.33)**	**19 (26.02)**
36-50	**57**	**29 (50.87)**	**13 (22.80)**
>50	**20**	**14 (70)**	**8 (40)**
Total	**150**	**75 (50)**	**40 (26.67)**

***percentage based on number of participant of the same age group $X^2 = 5.30$ $P>0.05$**

rug combination	Total	Number(%* showing high AST levels	Number(%)* showing high ALT levels
ZidolamN	24	9 (37.5)	6 (25)
LamivirS	17	7 (41.18)	6 (35.29)
Triomine	51	33 (64.7)	15 (29.41)
Stocrin, Duovir	58	32 (55.17)	7 (12.07)
Total	150	81 (54.0)	34 (22.67)

Table 2: Drug combinations and transaminase (AST and ALT) levels

***percentage based on number of participant using the same drug combination** $X^2 = 5.78$ $P > 0.05$

Discussion

In 2004 Cameroon was still in the context of generalized HIV epidemic, with a prevalence of 5,5% of which 6,8% were women and 4,1% were men. Groups identified to have high risk behaviours included mostly men and women in uniform, commercial sex workers, truck drivers and populations living along the Chad – Cameroon pipeline project area. Youths around 20 years of age were found to be the most vulnerable. The major mode of transmission was through non – protected heterosexual activity, even if mother to child transmission remained a preoccupation [9], [8]. These are reasons why the study population sample included only individuals above.

In the present study, the level of transaminases concentration was found to be increasing over time. These results tie with those obtained in a previous retrospective review of more than 10,000 adults living with AIDS in Boston, where researchers confirmed that long time antiretroviral therapy is associated with high rate of severe hepatotoxicity regardless of drug class or combination [5].

In other studies, a different pattern of drug injury with nevirapine use has emerged, with onset of liver enzyme elevations occurring beyond 16 weeks of therapy, consistent with direct or idiosyncratic host-mediated liver injury [10]. Rates of hepatotoxicity from various registration trials were ranged from 1% to 9.5%, but few patients have shown serious liver-related outcomes [11]

Also, based on the WHO toxicity scale, the greatest percentage of patients who presented with elevated levels of transaminases (76.81% for AST and 53.33% for ALT) were found to present with first degree hepatotoxicity which corresponds to low level liver toxicity. However, this does not tie with reports by spengler *et al*.[5] in which it was pointed out that longitudinal surveys have not only reported increased incidence of ARLI in patients but also identified life-threatening hepatotoxic events and end-stage liver disease in patients on antiretroviral therapy. The term hepatotoxicity may be misleading in the case of HIV drugs as some of the elevated liver enzymes may be due to other causes such as acute viral hepatitis, reactivation of chronic hepatitis B or C, alcohol ingestion as well as complementary drugs or medicines associated with HAART several of which have been noticed to be associated with clear-cut drug-induced hepatitis (DIH) for example used for the treatment of Tuberculosis [12]

We have found transaminase levels to be highest among the patients aged above 50 years. Although this finding was not statistically significant, it was in accordance with that of spengler *et al.* [5], but did not tie with a more recent report[13] in which younger diabetic patients had a higher tendency to have elevated ALT compared to those over 65 years. Our study shows significant relationship between the age of the patient and the level of transaminases concentration. Other studies have indicated that certain co morbidities, such as chronic hepatitis B (HBV) or hepatitis C (HCV) infection, may predispose patients to ARLI [10]. Highly active antiretroviral therapy (HAART) was found to be associated with low level hepatotoxicity at initiation, regardless of drug class or combination. Elevated transaminases due to antiretroviral therapy was independent on patient's age and increased levels of transaminases showed a significant positive linear relationship with increase in the duration of treatment. We therefore recommended that levels of transaminases should be followed up after initiation of antiretroviral therapy for all patients irrespective of their drug combination or age. The role of liver steatosis as a mechanism of, and as predisposing factor to anti retroviral drug-related liver injury (ARLI) should be investigated.

REFERENCES

1- Nuñez, M.J., Martin-Carbonero, L., Moreno, V., Valencia, E., Garcia-Samaniego, J., and Gonzalez-

Castillo, J. Impact of antiretroviral. AIDS 2006; 22: 825-829.

2- Palella, F., Baker, R., Moorman, A., Chmiel, J., Wood, K. and Brooks, J.(2006). Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. Journal of Acquired Immune Deficiency Syndrome 2006; 43: 27-34.

3- Hernandez, L., Gilson, I., Jacobson, J., Affi, A., Puetz, T. and Dindzans, V. Antiretroviral hepatotoxicity in HIV-infected patients. Alimentary Pharmacology & Therapeutics 2001; 15:1627-1632.

4- den Brinker, Marieke; Wit, Ferdinand W. N. M.; Wertheim-van Dillen, Pauline M. E.; Jurriaans, Suzanne; Weel, Jan; van Leeuwen, Remko; Pakker, Nadine G.; Reiss, Peter; Danner, Sven A.; Jan Weverling, Gerrit; Lange and Joep M. A. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV- infection. AIDS 2000 14: 2895-2902

5- Spengler, U; Lichterfeld, M.; Rockstroh, J.K. Antiretroviral drug toxicity-Therapy. Reuters Health 2002

6- Clark, S., Creighton, S., Portmann, B., Taylor, C., Wendon, J. and Cramp M. Acute liver failure associated with antiretroviral treatment for HIV: a report of six cases. Journal of Hepatology 2002; 36:295-301.

7- Ministry of Public Heath. 2006-2010 National Strategic Plan for the fight against AIDS in Cameroun. Republic of Cameroon.

8- International Federation of Clinical Chemistry (IFCC). www.ifcc.org

9- Organisation des Nations Unies pour la lutte contre le Sida (ONUSIDA). Rapport annuel 2003. Genève: ONUSIDA

10- Soriano, V., Puoti, M., Garcia-Gascó, P., Rockstroh, J. K., Benhamou, Y., Barreiro, P. and McGovern, B. Antiretroviral drugs and liver injury AIDS 2001; 22: 1-13

11- Sulkowski, M., Thomas, D., Chaisson, R. and Moore, R. Hepatotoxicity associated with antiretroviral therapy in adults infected with HIV and the role of hepatitis C or B virus infection. Journal of the American Medeical Association 2004; 283:74-80.

12- Lewis, J. H. The rational use of potentially hepatotoxic medications in patients with underlying liver disease. Informa Healthcare 2002; 1:159-172

.13- Layla Judi, Ala Toukan, Yousef Khader, Kamel Ajlouni and M Amer Khatib. Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus. Annals of Saudi Medicine 2010; 30: 25-32

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/201008/**21025** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 182-194

USERS' PERCEPTIONS AND EFFICACY OF INDIGENOUS ADJUNCT TEETH-CLEANSING AGENTS ON THE BACTERIAL FLORA OF HUMAN DENTAL CARIES

***Adenike A. O. Ogunshe PhD[1] and Olubukonla G. Odumesi ND[2]**

1 Applied Microbiology and Infectious Diseases, Department of Microbiology, University of Ibadan, IBADAN, Nigeria. and 2 Department of Science Laboratory Technology, School of Pure and Applied Sciences, Moshood Abiola Polytechnic, ABEOKUTA, Nigeria.

*** Correspondence: Applied Microbiology and Infectious Diseases, Department of Microbiology, University of Ibadan, IBADAN, Nigeria. Fax: (234)-2-8103043 E-mail: adenikemicro@yahoo.com**

RUNNING TITLE: INDIGENOUS TEETH-CLEANSING AGENTS

ABSTRACT

Background and Aim: The aim of this study was to conduct *in vitro* and *in vivo* studies for the evaluation of antimicrobial potential of four Nigerian indigenous chewing sticks and to compare the oral effects of the popular Nigerian chewing sticks with toothpastes and other teeth-cleansing agents on a total of 21 and 17 oral bacterial strains isolated from children and adult dental caries respectively.

Materials and Methods: Teeth cleansing agents were assayed *in vitro* against the isolated bacterial carries flora on de Man Rogosa and Sharpe (MRS) agar using modified agar well-diffusion methods. The simulated *in vivo* studies were carried out using twenty four human subjects.

Results: The results obtained indicated that the bacterial flora exhibited different degrees of *in vitro* inhibitions of between low and moderate susceptibility against the teeth-cleansing agents. Toothpastes recorded the highest rates of inhibition against the dental caries isolates, while H_2O_2 was the most inhibitory among the chemical teeth-cleansing agents against the dental caries isolates from children (47.6%) and adults (52.9%). No species differential susceptibility to the teeth-cleansing agents was observed among the dental caries isolates.

Conclusions: This study showed that combined teeth cleansing hygiene with toothpastes, chewing sticks and chemical teeth cleansing agent (H_2O_2) gave the best mouth feel and were found to be the most preferred.

Keywords: Chewing sticks, dental caries, indigenous, oral health and hygiene, teeth-cleansing agents.

INTRODUCTION

The human oral cavity harbours a complex microbial community of diverse range of microorganisms (1, 2, 3), and these comprise of several species of bacteria, fungi and protozoa. The oral cavity is normally relatively stable but under certain circumstances can cause major dental diseases such as caries and periodontitis because many of the commensal oral microorganisms may cause diseases if provided with appropriate conditions. Dental problem is known to be the most common health problem in the human communities (2), while dental diseases are of great economic importance throughout the world, hence, the environmental forces, which both affect and effect the microbial colonisation of the mouth have been the subject of considerable research (4). There is also a growing public recognition of the importance of oral health because once a permanent tooth falls off it cannot be replaced by another tooth. It is therefore, very important to take care of the teeth.

People in rural areas, especially in tropical forest region of the world have utilised plants as part of their primary health care system for millennia (5, 6, 7), and this medical dependence on plants continues till today for at least 80% of the world's rural population (8). Ethnomedicinal plants in form of chewing sticks used for the cure of dental problems include *Zanthoxylum zanthoxyloides*, *Zanthoxylum nitidum*, *Jatropha curaas*, *Azadirachta indica* and *Salvadora persica*, *Acacia senegal* var. Senegal, *Eriosema psoraleoides*, *Ocimum suave*, *Opilia celtidifolia* and *Xerophyta suaveolens*, *Lamium tenuiflorum* etc., *kikar* (*Acacia arabica*) from Tanzania and *arak* (*Salvadora persica*) from Pakistan, as well as *miswak*

(*Salvadora persica*) from Saudi Arabia, among several others (9, 10, 11, 12, 13, 14, 15). In Nigeria, the roots of such plants and others (*Massularia acuminate*, *Sorindeia warneckei*) are traditionally used as chewing sticks for maintaining healthy teeth among indigenous populations (16, 17, 18).

Zanthoxylum xathoxyloides, belonging to the family Rutaceal and the order- Rutales consists of 9 genera, and about 28 species occur in the forest savanna mosaic of the lowland rainforest and sometimes, also abundant in coastal areas of Nigeria, where the roots are traditionally used as chewing sticks for toothache and for maintaining healthy teeth among the indigenous populations (19). The roots and twigs of *Aayan* (*Prosopis africana*), which belongs to the Family Compositae are widely used as tooth-cleaners. Namibian chewing stick, *Diospyros lycioides* commonly known as *mathala* was also found to contain antibacterial compounds against oral pathogens like *S. mutans* and *S. sanguinis* and some periodontal pathogens (20). The purpose of this study is therefore, to investigate the potentials of some of the most popular, local Nigerian chewing sticks and chemical agents as natural tooth cleansing agents or adjuncts to toothpastes in oral hygiene.

Materials and methods

Collection of chewing sticks samples
Orin ata (*Zanthoxylum xathoxyloides*) (Engl.). Zepernick & Timter, *Fagara zanthoxyloides* Lam, common name (candle wood); *Aayan* (*Prosopis africana*), common name (mesquite); *ewuro* (*Vernonia amygdalina*) Del., common name (bitter leaf); were all obtained from a local herbs market in Ibadan metropolis, while *dogoyaro* (*Azadirachta indica*), common name (Neem) was obtained from Moshhod Abiola Polytechnic, Abeokuta, Ogun State, Nigeria.

Isolation of bacterial species:
Dental caries specimens were aseptically transferred into 100ml MRS (de Man, Rogosa and Sharpe agar; Lab M, Lab M Ltd., Lancashire, UK; batch no. 092898) broth at pH 5.3 - 5.5 and incubated overnight at 32^0C for the selective isolation of *Streptococcus* and *Lactobacillus* species. 1ml volumes of the overnight broth cultures were separately transferred to sterile Petri dishes by the pour-plate method before incubation aerobically and microaerophically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid, England) at 32^0-35^0 C for 24h.

Characterisation of the* Streptococcus *and* Lactobacillus *strains

Streptococcus and *Lactobacillus* isolates were characterised based on standard phenotypic taxonomic tools (21, 22, 23, 24, 25). The purity of the strains was checked and the cells were washed twice in 0.9% sterile NaCl solution after centrifuging. The isolates were then stored at 4^oC in Hogness freezing buffer (3.6mM K_2HPO_4; 1.3mM KH_2PO_4; 2.0mM Na-citrate; 1.0mM $MgSO_4$; 12% glycerol) and kept frozen at -12^0C.

Determination of antimicrobial activities of the teeth-cleansing agents against the dental caries flora.
Agar well-diffusion method: Holes, 6 mm in diameter were aseptically punched out of the MRS agar plates, followed by surface flaming of the agar plates. The agar plates when cool were then seeded by streaking the entire surface of the culture plates with the indicator strains isolated from the dental caries. The seeded plates were then incubated at 32^0C for 30 minutes, after which 500µl of each of the chewing stick extracts, toothpastes, and chemical agents were separately dispensed into the agar wells, followed by incubation at 32^0C for 24h (26). The demonstration of antagonism depends on the release of diffusible inhibitory metabolites from the chewing stick extracts, toothpastes and chemical agents into the assay medium during incubation. Inhibitory zones surrounding the agar wells were noted and recorded in mm diameter, while zones less than 10.0 mm diameter and absence of zones of inhibition were recorded as resistant.

Simulated *in vivo* study:
Twenty four adults (Male = 17; Female = 7) who participated in the study were divided into two groups and taught on how to evaluate their oral hygiene by usage of chewing sticks, toothpastes and oral chemical agents, making mouth feel the selection criterion. Twelve of the participants were to use each of the 3 chewing sticks (*Orin ata*, *ewuro* and *dongoyaro* alone for one week each (Group 1). The other twelve participants (Group 2) were to use each of the toothpastes, Close-up [Unilever Nigeria Plc.. with active ingredients – sodium monofluorophopsphate, PEG SCMC synthetic cleaning agent]; Dabur herbal toothpaste with basil [Dabur India Ltd., NEPZ with ingredients – calcium carbonate, glycerin, sodium lauryl sulfate, gum carageenan, liquonine extract, bullet wood extract, *Acacia arabica*, oak galls, tumeric, *Patharman sarsapartilla*, lotus bark, nut grass, etc.,]; Florish gel [PZ, Industries, Nigeria with active ingredients (0.76% sodium monofluorophosphate), Holdent calcium fluoride with menthol [Charmanuel Industries Ltd., London, UK with active ingredients – 0.76% sodium monofluorophosphate, 0.10% sodium fluoride PL 0049/0020, calcium (Ca^{2+}) (1196)] and [Macleans (SmithKline Beecham Nigeria Plc., with active ingredients – 0.8% monofluorophosphate and 0.13% calcium glycorophosphate)] toothpastes alone for one week each. Informed consents were obtained from the subjects prior to the simulated *in vivo* studies.

The 2 groups were to reverse the oral treatments after the third week until the sixth week, when the two groups were to combine chewing sticks and toothpastes in the teeth cleansing study for one week and the chemical agents (ash and H_2O_2) were added for an additional week, making a total of 8 weeks. Mouth feel involves feeling the teeth with the tongue,

especially at the inner teeth surfaces for any feeling of teeth coating, especially on the molars and premolars; breath smell; presence or absence of saliva at the lip sides or minimal spitting while talking for about 15 minutes or more, as well as lightness of the tongue. During the pre-simulated training, the subjects were allowed to use the preferred toothpastes and chewing sticks in avoid bias. Children could not participate in this study because of the oral technicalities involved.

Results

A total of twenty-one oral bacterial strains consisting of *Streptococcus mutans* (7; 33.3%), *Strep. salivarius* (4; 19.0%), *Strep. sanguis* (1; 4.8%), *Lactobacillus acidophilus* (3; 14.3%), *L. casei* (5; 23.8%) and *L. fermentum* (1; 4.8%) were isolated from children caries; while the seventeen bacterial strains obtained from adult dental caries were *Streptococcus mutans* (6; 35.3%), *Strep. salivarius* (4; 23.5%), *L. casei* (5; 29.4%) and *L. fermentum* (2; 11.8%).

The oral bacterial flora were screened for their susceptibility to various teeth-cleansing agents that were commonly used by the rural and urban dwellers in most part of the country as obtained from the results of questionnaire administration (results not shown). The bacterial flora exhibited variable inhibitions to the teeth-cleansing agents, with toothpastes recording the highest rates. The inhibition zones of between 12.0 and 14.0 mm in diameter were considered as being of moderate inhibitory effects, while inhibition zones less than 10.0 mm in diameter were considered as negative (resistant) in this study. Florish (52.5%), Holdent (57.1%) and Close-up (61.9 %) were the most inhibitory among the toothpastes, with the bacterial flora displaying zones of inhibition of between 12.0 and 14.0 mm in diameter against the dental caries isolates from children. Florish (47.0%), Close-up (58.7%) and Holdent (76.4%) exhibited the highest inhibitions against the dental caries isolates from adults, with the bacterial flora also displaying zones of inhibition of between 12.0 and 14.0 mm in diameter against the dental caries isolates.

Table 2 shows the antagonistic effects of the four local chewing sticks on dental caries isolates from children and adults. *Dongoyaro* (47.6 %) and *ayan* (38.1%) were

TABLE 1: ANTAGONISTIC EFFECTS OF TOOTHPASTES ON DENTAL CARIES BACTERIAL ISOLATES

ORAL ISOLATES	TOOTHPASTES MACLEANS	DABUR	FLORISH	CLOSE-UP	HOLDENT
Children					
Streptococcus salivarius *984*	*12.0*	*14.0*	*14.0*	*14.0*	*14.0*
Streptococcus mutans *987*	*12.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus mutans *989*	*10.0*	*10.0*	*14.0*	*14.0*	*14.0*
Streptococcus sanguis *9812*	*12.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus casei *9816*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus casei *9819*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Streptococcus mutans *9820*	*12.0*	*12.0*	*14.0*	*14.0*	*14.0*
Lactobacillus acidophilus *9823*	*10.0*	*12.0*	*12.0*	*12.0*	*12.0*
Lactobacillus casei *9829*	*14.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus fermentum *9833*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Streptococcus mutans *9834*	*14.0*	*12.0*	*10.0*	*12.0*	*10.0*
Streptococcus salivarius *9839*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus salivarius *9841*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus mutans *9849*	*10.0*	*10.0*	*12.0*	*12.0*	*12.0*
Streptococcus mutans *9851*	*14.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus casei *9856*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus acidophilus *9861*	*10.0*	*12.0*	*12.0*	*12.0*	*12.0*
Streptococcus salivarius *9865*	*12.0*	*08.0*	*08.0*	*12.0*	*12.0*
Streptococcus mutans *9870*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus acidophilus *9880*	*12.0*	*14.0*	*14.0*	*12.0*	*12.0*
Lactobacillus casei *9882*	*12.0*	*10.0*	*14.0*	*12.0*	*14.0*
Adults					
Lactobacillus casei *A01*	*10.0*	*12.0*	*10.0*	*12.0*	*12.0*
Streptococcus mutans *A02*	*12.0*	*08.0*	*12.0*	*12.0*	*10.0*
Streptococcus mutans *A03*	*10.0*	*10.0*	*10.0*	*10.0*	*12.0*

Lactobacillus casei *A04*	*14.0*	*14.0*	*10.0*	*14.0*	*14.0*
Streptococcus salivarius *A05*	*10.0*	*08.0*	*10.0*	*10.0*	*12.0*
Streptococcus mutans *A06*	*14.0*	*12.0*	*14.0*	*10.0*	*10.0*
Lactobacillus fermentum *A07*	*10.0*	*10.0*	*10.0*	*12.0*	*14.0*
Lactobacillus fermentum *A08*	*08.0*	*10.0*	*12.0*	*12.0*	*12.0*
Streptococcus mutans *A09*	*10.0*	*14.0*	*10.0*	*12.0*	*12.0*
Streptococcus salivarius *A10*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus salivarius *A11*	*14.0*	*08.0*	*10.0*	*10.0*	*12.0*
Lactobacillus casei *A12*	*10.0*	*10.0*	*14.0*	*10.0*	*14.0*
Lactobacillus casei *A13*	*10.0*	*14.0*	*10.0*	*14.0*	*14.0*
Lactobacillus casei *A14*	*10.0*	*08.0*	*14.0*	*14.0*	*12.0*
Streptococcus salivarius *A15*	*12.0*	*10.0*	*12.0*	*10.0*	*12.0*
Streptococcus mutans *A16*	*10.0*	*10.0*	*12.0*	*12.0*	*10.0*
Streptococcus mutans *A17*	*12.0*	*08.0*	*14.0*	*14.0*	*14.0*

+14.0	*3 (14.4)*	*5 (23.8)*	*8 (38.1)*	*6 (28.6)*	*7 (33.3)* **}**
+12.0	*7 (33.3)*	*4 (19.0)*	*3 (14.4)*	*7 (33.3)*	*5 (23.8)* **}**
+10.0	*6 (28.6)*	*6 (28.6)*	*4 (19.0)*	*3 (14.4)*	*5 (23.8)*
< 10.0	*5 (23.8)*	*6 (28.6)*	*6 (28.6)*	*5 (23.8)*	*4 (19.0)*
+14.0	*3 (17.6)*	*3 (17.6)*	*4 (23.5)*	*4 (23.5)*	*5 (29.4)* **}**
+12.0	*3 (17.6)*	*2 (11.8)*	*4 (23.5)*	*6 (35.2)*	*8 (47.0)* **}**
+10.0	*10 (47.6)*	*7 (41.1)*	*9 (52.9)*	*7 (41.1)*	*4 (23.5)*
< 10.0	*1 (5.9)*	*5 (29.4)*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*

moderately inhibitory against the children dental caries isolates while *dongoyaro* (41.1 %) and *ewuro* (35.2%) were also moderately inhibitory against the children and adult dental caries isolates. According to Table 3, H_2O_2 was the most inhibitory among the chemical teeth-cleansing agents against the dental caries isolates from children (47.6 %) and adults (52.9 %). The results of the simulated *in vivo* studies were as shown in Table 4, with the combined teeth cleansing hygiene being the most preferred.

Discussion

A complex ecosystem is formed in the oral cavity, which changes constantly throughout life but some bacteria that are easily recovered from oral cavity upon routine culture have been found to be potentially pathogenic (3, 27, 28, 29, 30). The results obtained in this study in which *Streptococcus mutans* was the most recovered of the culturable bacterial species implicated in children and adult dental caries, followed by

Lactobacillus casei and *S. salivarius* is similar to the findings of the earlier workers; although, the slight observed inter-species differences may be due to differences in diet and geographical location. Franz *et al*. (31), for example, reported that *E. faecium* and *E. faecalis* in humans are dependent on geographical location, while Petti *et al*. (32) and other workers also found different variable affecting the oral microflora.

Since the invention of the first toothpaste, as far back as nearly 4000 years, the market of the toothpaste has never been slowed down (3, 33). Modern toothpaste was invented to aid in the removal of foreign particle and food substances in addition to cleaning of tooth, while fluoride is added to toothpaste to aid in protection from tooth decay; however, many of the innovations made in toothpastes after the fluoride break involve the addition of ingredients with special abilities to toothpaste and toothpaste packaging (34, 35). Almost all the toothpastes in Nigeria claim to contain certain active ingredients, especially calcium and fluoride, which prevent teeth against decay, and also responsible for the total hygiene by cleaning teeth thoroughly down to the gums. Inherent antiseptic properties of some of the toothpastes were claimed to prevent bacterial decomposition of food particles adhering to teeth and gaps between the teeth, hence preventing mouth odour.

Out of twelve toothpastes assayed in this study (based on the results of questionnaires administration, which tested the popularity of the tootthpastes), only the results of the most inhibitory toothpastes were reported in this study. Considering the zones of inhibition, Close-up, Darbur, Florish and Holdent toothpastes had higher *in vitro* inhibitory effects against the dental caries flora. In a similar study carried out in Nigeria (36) and Nepal, only few of their locally available toothpastes (especially, those that have triclosan as a major ingredient), were found to posses efficient antimicrobial properties (35). In spite of the major chemical ingredient(s) in toothpastes used in this study, the total number of the toothpastes with the moderate inhibitory activities was quite low compared with the total number of toothpastes in the country, more especially, the locally manufactured products.

Hydrogen peroxide is an oxidising agent that has effective antiseptic, disinfectant and deodorant properties, with antiseptic action due to its ready release of oxygen when applied to tissues. It was reported that though the effect lasts only as long as the oxygen is being released, it's of short duration and has weak antibacterial activity (37). According to Atlas (38), it was not effective against Gram-positive bacteria but was found in this study to be the most inhibitory teeth-cleansing chemical agent against dental caries isolates from children (47.6 %) and adults (52.9 %). Atlas (38) had also earlier documented that anaerobic bacteria have been found to be particularly sensitive to peroxides because they do not have catalase, an enzyme that degrades peroxides. This concept may therefore, be responsible for the high inhibitory activities of hydrogen-peroxide on the oral isolates which were mostly anaerobes.

Chewing sticks had been the traditional means of teeth cleansing among Nigerians, although Nigeria is not the only nation with traditional inclination for chewing sticks, and different plants are being used to treat different types of oral ailments like toothache, dental plaques, dental caries, pyorrhea and aphthae (9, 10, 13, 14, 16, 39, 40, 41, 42, 43). Various researchers have advocated the use of chewing sticks in community oral health programme because they are readily available, cheaper than the toothbrush and paste, and are found to possess antiplaque properties (16) but there are various findings on the efficacy of chewing sticks on oral health. While some findings reported their efficacy, some did not. The results of the *in vitro* inhibitory effects of four Nigerian local chewing sticks on dental caries bacterial isolates from children and adults in this study indicated moderate inhibitory activities among the children and adults isolates.

Additionally, a follow-up *in vivo* experiment on the teeth-cleansing agents to evaluate the oral hygiene on habitual chewing stick and toothbrush was conducted in this present study. The *in vivo* results showed the

TABLE 2: **ANTAGONISTIC EFFECTS OF LOCAL CHEWING STICKS ON DENTAL CARIES BACTERIAL ISOLATES**

	Local chewing sticks			
Isolates	**Orin ata**	**Ayan**	**Ewuro**	**Dongoyaro**
Children				
Streptococcus salivarius *984*	*08.0*	*10.0*	*14.0*	*10.0*
Streptococcus mutans *987*	*10.0*	*10.0*	*10.0*	*14.0*
Streptococcus mutans *989*	*12.0*	*10.0*	*10.0*	*12.0*
Streptococcus sanguis *9812*	*10.0*	*12.0*	*08.0*	*08.0*
Lactobacillus casei *9816*	*12.0*	*12.0*	*10.0*	*10.0*
Lactobacillus casei *9819*	*10.0*	*10.0*	*08.0*	*14.0*
Streptococcus mutans *9820*	*08.0*	*10.0*	*14.0*	*10.0*
Lactobacillus acidophilus *9823*	*12.0*	*12.0*	*10.0*	*12.0*
Lactobacillus casei *9829*	*12.0*	*10.0*	*10.0*	*12.0*
Lactobacillus fermentum *9833*	*08.0*	*10.0*	*08.0*	*10.0*
Streptococcus mutans *9834*	*08.0*	*10.0*	*12.0*	*12.0*
Streptococcus salivarius *9839*	*12.0*	*10.0*	*12.0*	*10.0*
Streptococcus salivarius *9841*	*10.0*	*12.0*	*10.0*	*12.0*
***Streptococcus mutans* 9849**	**10.0**	**12.0**	**10.0**	**14.0**
***Streptococcus mutans* 9851**	**10.0**	**12.0**	**10.0**	**14.0**
***Lactobacillus casei* 9856**	**08.0**	**08.0**	**12.0**	**10.0**
***Lactobacillus acidophilus* 9861**	**10.0**	**10.0**	**08.0**	**10.0**
***Streptococcus salivarius* 9865**	**12.0**	**10.0**	**12.0**	**10.0**
***Streptococcus mutans* 9870**	**10.0**	**12.0**	**10.0**	**10.0**
***Lactobacillus acidophilus* 9880**	**08.0**	**10.0**	**10.0**	**12.0**
***Lactobacillus casei* 9882**	**08.0**	**12.0**	**10.0**	**10.0**
Adults				
***Lactobacillus casei* A01**	**10.0**	**10.0**	**10.0**	**12.0**
***Streptococcus mutans* A02**	**10.0**	**08.0**	**12.0**	**12.0**
***Streptococcus mutans* A03**	**08.0**	**10.0**	**10.0**	**10.0**

Lactobacillus casei *A04*	***10.0***	***10.0***	***12.0***	***12.0***
Streptococcus salivarius *A05*	***12.0***	***10.0***	***08.0***	***10.0***
Streptococcus mutans *A06*	***12.0***	***12.0***	***10.0***	***12.0***
Lactobacillus fermentum *A07*	***10.0***	***10.0***	***10.0***	***10.0***
Lactobacillus fermentum *A08*	***12.0***	***10.0***	***12.0***	***08.0***
Streptococcus mutans *A09*	***10.0***	***08.0***	***10.0***	***10.0***
Streptococcus salivarius *A10*	***10.0***	***10.0***	***08.0***	***10.0***
Streptococcus salivarius *A11*	***08.0***	***10.0***	***10.0***	***10.0***
Lactobacillus casei *A12*	***10.0***	***10.0***	***10.0***	***12.0***
Lactobacillus casei *A13*	***10.0***	***12.0***	***12.0***	***12.0***
Lactobacillus casei *A14*	***10.0***	***10.0***	***10.0***	***10.0***
Streptococcus salivarius *A15*	***12.0***	***10.0***	***12.0***	***09.0***
Streptococcus mutans *A16*	***10.0***	***10.0***	***12.0***	***10.0***
Streptococcus mutans *A17*	***12.0***	***10.0***	***08.0***	***12.0***

+14.0	***- (0.0)***	***- (0.0)***	***2 (9.5)***	***4 (19.0) }***
+12.0	***6 (28.6)***	***8 (38.1)***	***4 (19.0)***	***6 (28.6) }***
+10.0	***8 (38.1)***	***12 (57.1)***	***11 (52.3)***	***10 (47.6)***
<10.0	***7 (33.3)***	***1 (4.76)***	***4 (19.0)***	***1 (4.76)***
+14.0	***- (0.0)***	***- (0.0)***	***- (0.0)***	***- (0.0) }***
+12.0	***5 (29.4)***	***2 (11.8)***	***6 (35.2)***	***7 (41.1) }***
+10.0	***10 (46.6)***	***13 (76.4)***	***8 (38.1)***	***8 (47.0)***
<10.0	***2 (11.8)***	***2 (11.8)***	***3 (17.6)***	***2 (11.8)***

local chewing stick, *orin ata* (*Zanthoxylum zanthoxyloides*) to be the most preferred, based on the mouth-feel effect by the adult study group. The simulated experiment among the control adult group indicated that the combined cleansing effect of *Zanthoxylum zanthoxyloides* before or after brushing with any of the toothpastes, followed by rinsing with

hydrogen peroxide gave the best mouth-feel effect for a period of 12-18 hours after the oral hygiene treatments.

The combined cleansing effect of *ewuro* (*Vernonia amygdalina*) and then brushing with any of the toothpastes followed by rinsing with hydrogen peroxide also gave a better mouth feel effect for a period of 8 hours. The antimicrobial property of *Zanthoxylum zanthoxyloides* has also been investigated by Muhammad and Shinkafi (44), and the extracted essential oils from this chewing stick were found to posses antiseptic and anti-carcinogenic actions.

TABLE 3: *ANTAGONISTIC EFFECTS OF CHEMICAL AGENTS ON DENTAL CARIES BACTERIAL ISOLATES*

	Chemical agents		
Isolates	***Ash***	***NaCl***	***H_2O_2***
Children			
Streptococcus salivarius ***984***	***10.0***	***12.0***	***10.0***
Streptococcus mutans ***987***	***12.0***	***10.0***	***10.0***
Streptococcus mutans ***989***	***10.0***	***10.0***	***10.0***
Streptococcus sanguis ***9812***	***NDG***	***NDG***	***NDG***
Lactobacillus casei ***9816***	***10.0***	***10.0***	***14.0***
Lactobacillus casei ***9819***	***10.0***	***10.0***	***12.0***
Streptococcus mutans ***9820***	***08.0***	***10.0***	***12.0***
Lactobacillus acidophilus ***9823***	***12.0***	***10.0***	***12.0***
Lactobacillus casei ***9829***	***10.0***	***10.0***	***12.0***
Lactobacillus fermentum ***9833***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***9834***	***12.0***	***12.0***	***10.0***
Streptococcus salivarius ***9839***	***10.0***	***12.0***	***14.0***
Streptococcus salivarius ***9841***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***9849***	***10.0***	***08.0***	***10.0***
Streptococcus mutans **9851**	**12.0**	**12.0**	**10.0**
Lactobacillus casei **9856**	**08.0**	**10.0**	**10.0**
Lactobacillus acidophilus **9861**	**10.0**	**14.0**	**14.0**
Streptococcus salivarius **9865**	**12.0**	**10.0**	**12.0**
Streptococcus mutans **9870**	**10.0**	**10.0**	**14.0**
Lactobacillus acidophilus **9880**	**12.0**	**12.0**	**12.0**
Lactobacillus casei **9882**	**10.0**	**10.0**	**10.0**
Adults			
Lactobacillus casei **A01**	**10.0**	**12.0**	**14.0**
Streptococcus mutans **A02**	**12.0**	**10.0**	**12.0**
Streptococcus mutans **A03**	**10.0**	**10.0**	**10.0**
Lactobacillus casei **A04**	**10.0**	**10.0**	**12.0**
Streptococcus salivarius **A05**	**12.0**	**08.0**	**10.0**
Streptococcus mutans **A06**	**08.0**	**12.0**	**12.0**
Lactobacillus fermentum **A07**	**10.0**	**10.0**	**10.0**
Lactobacillus fermentum **A08**	**12.0**	**10.0**	**08.0**
Streptococcus mutans **A09**	**10.0**	**08.0**	**10.0**
Streptococcus salivarius **A10**	**10.0**	**12.0**	**12.0**
Streptococcus salivarius **A11**	**10.0**	**10.0**	**12.0**
Lactobacillus casei **A12**	**08.0**	**10.0**	**10.0**

Lactobacillus casei ***A13***	***10.0***	***12.0***	***12.0***
Lactobacillus casei ***A14***	***08.0***	***10.0***	***10.0***
Streptococcus salivarius ***A15***	***12.0***	***08.0***	***14.0***
Streptococcus mutans ***A16***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***A17***	***08.0***	***12.0***	***12.0***
+14.0	***- (0.0)***	***1 (4.76)***	***4 (19.0) }***
+12.0	***6 (28.6)***	***5 (23.8)***	***6 (28.6) }***
+10.0	***12 (57.1)***	***13 (61.9)***	***10 (47.6)***
<10.0 / NDG	***3 (14.2)***	***2 (9.5)***	***1 (4.76)***
+14.0	***- (0.0)***	***- (0.0)***	***2 (11.8) }***
+12.0	***4 (23.5)***	***5 (29.4)***	***7 (41.1) }***
+10.0	***9 (59.4)***	***9 (52.9)***	***7 (41.1)***
<10.0	***4 (23.5)***	***3 (17.6)***	***1 (5.88)***

Keys: NDG = No determinable growth

The observed *in vitro* results of the teeth cleansing agents are not exclusively the same results obtained *in vivo* and this can be easily explained by the fact that the oral bacterial pathogens usually form a layer (biofilm) over the teeth enamel, and the time interval for the teeth cleansing agents to come in contact with them and brush them off are usually about 5-10 minutes brushing periods. In the determination of the mouth feel (checking the teeth with the tongue, especially at the inner teeth surfaces for any feeling of teeth coating, especially on the molars and premolars; breath smell; presence or absence of saliva foam at the lip sides or minimal spitting while talking for about 15 minutes or more, as well as lightness of the tongue), and it was generally accepted by the subjects that the combined teeth cleansing hygiene is the most preferred. Continuous slight spitting and or accumulation of foamy or non-foamy but thick whitish saliva at the corners of the lips were prevented or very minimal among the subjects during the study. It was also agreed that teeth cleansing, at least twice a day is the best mode of good oral hygiene.

In addition to the teeth cleansing effects, the subjects used in the present study also supported the usage of local chewing sticks as natural means of aiding mastication, being the only oral hygiene agent that can be daily chewed for periods of time. It was also advocated that chewing sticks aid in inducing salivation and thereby can also be responsible for cleansing of the salivary organs. Further studies on the effect of the teeth cleansing agents on the microbial loads of saliva are also on-going in our laboratories.

Since there could be varied human oral bacterial species among different nations, it is necessary that differentiation in oral bacterial flora associated with dental carries be put into consideration when preparing teeth-cleansing agents such as toothpastes. According to Tiwari *et al*. (3), fluoride and triclosan are the most commonly used and recommend active compounds by WHO, ADA and FDI, and regular evaluation of the efficacy of the fluoridated toothpaste by the private laboratory have been recommended by the WHO (45) but excess use of the fluoride can cause dental fluorisis, so the recommended amount of the fluoride was advised to be used as the ingredients in the toothpaste.

TABLE 4: SIMULATED IN VIVO *EFFECTS OF TEETH CLEANSING AGENTS ON MOUTH FEEL OF SUBJECTS*

Period of study	Best preferred	Better preferred	Preferred	
Weeks 1 & 4	***orin ata* [14]**	***ewuro* [7]**	***dongoyaro* [3]**	**Grp. 1**
Weeks 2 & 5	***orin ata* [12]**	***ewuro* [8]**	***dongoyaro* [4]**	**Grp. 2**
Weeks 3 & 6	***orin ata* [12]**	***ewuro* [9]**	***dongoyaro* [3]**	**Grp. 3**
Weeks 1 & 4	**Close up [10]**	**Holdent [9]**	**Florish [5]**	**Grp. 1**
Weeks 2 & 5	**Holdent [11]**	**Close up [9]**	**Florish [5]**	**Grp. 2**
Weeks 3 & 6	**Close up [12]**	**Holdent [9]**	**Florish [3]**	**Grp. 3**
Week 7	**Close up + *orin ata* [14]**	**Holdent + *orin ata* [6]**	**Close up + *ewuro* [4]**	**All Grps.**
Week 8*	**NaCl + toothpastes + Chw stck [14]**	**H_2O_2 + toothpastes + Chw stck**	**Ash + toothpastes +Chw stck**	

Keys: **Chw. stck = chewing sticks; Grp. = group; Grps. = groups**

Abiding by regulations, even in the production of household toilet products like toothpastes has been a running battle in the country, it is therefore, strongly recommended that all toothpastes in the country, including the locally produced ones be subjected to regular evaluation of their efficacy. Similarly, results on alternative teeth cleansing agents such as chewing sticks or herbal regimens would also have to consider this concept. This will aid in investigating into the indigenous chewing sticks of various countries, and there may ultimately be internationally recognised broad spectrum chewing sticks that cut across many nations. The active ingredients of these chewing sticks can then be extracted and prepared into industrial herbal toothpastes just as in the case of Darbur, an Indian toothpaste and a Korean toothpaste known as Herb & Bio.Salt, a gum care toothpaste consisting of natural herbal extracts from *Lonicera japonica*, *Taraxacum platycarpum*, green tea and sodium chloride.

REFERENCES

1. Black JG. Microbiology: Principles and Application, 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, USA. 1993; pp. 778.
2. Loesche WJ. Microbiology of dental decay and periodontal disease. In: Baron's Medical Microbiology. 4th Ed. University of Texas Medical Branch. 1996. pp. 1273.
3. Tiwari KB, Shrestha UT, Acharya AM. Subedi B, Paudyal B, Jnawali M, Shakya PUKC, Agrawa VP. Antibacterial activities of locally used toothpastes against dental pathogens. Braz Dent J. 2002; 13: 155-61.
4. Hogg SD. The lactic microflora of the oral cavity. In: The lactic acid bacteria vol. 1, Wood, B.J.B. (Ed.). Elsevier Science Publishers Ltd., England 1992; 115-147.
5. Farmsworth NR, Saejarto DD. The global importance of medicinal plants. In: The conservation of medicinal plants. *Proceedings of an International Consultation*. Akerele, O. Heywood, V. and Synge, H. (Eds.). Chiang Mai, Thailand. Cambridge University Press, Cambridge. 1991; 25-51
6. Abu-Rabia A: Urinary diseases and ethnobotany among pastoral nomads in the Middle East. *Journal of Ethnobiology and Ethnomedicine* 1:4: http://www.ethnobiomed.com/content/1/1 4
7. Busia K. Medical provision in Africa - past and present. Phytoth Res. 2005; 19 (11):919-23.
8. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *J. Ethnobiol Ethnomed*. 2006; **2:**43 .
9. van Palenstein H. WH, Munck L, Mushendwa S, Mrema FG. Cleaning effectiveness of chewing sticks among Tanzanian schoolchildren. J Clin Periodontol. 1992; 19(7): 460-463.
10. Almas K. The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatol Trop. 2001; 24(96): 17-20.
11. Wu CD, Darout IA, Skaug N. Chewing sticks: timeless natural toothbrushes for oral cleansing. J Periodontal Res. 2001; 36(5): 275-284.
12. Al-Bagieh NH, Idowu A, Salako NO. Effect of aqueous extract of *miswak* on the *in vitro* growth of *Candida albicans*. Oral Health Prev Dent. 2003; 1(4): 301-307.
13. Al-Otaibi M, Al-Harthy M, Söder B, Gustafsson A, Angmar-Månsson B. Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. Oral Health Prev Dent. 2003; 1(4): 301-307.
14. **Al-Otaibi M. The *miswak* (chewing stick) and oral health: studies on oral hygiene practices of urban Saudi Arabians. Swed Dent J Suppl. 2004; (167): 2-75.**
15. **Dulger, B. Antifungal activity of *Lamium tenuiflorum* against some medical yeast**

***Candida* and *Cryptococcus species*. Pharm Biol 2009; (5): 467 – 470.**

16. ote EO. Oral prophylactic procedures and gingival health among Nigerian school children. Afr Dent J. 1991; 5: 15-20.

17. Adesina SK, Olugbade TA, Akinwusi DD., Bergenthal D. Extractives from *Zanthoxylum lemairie* root and stem. Pharmaz. 1997; 52: 720-724.

18. Ndukwe KC, Lamikanra A, Okeke IN. Antibacterial activity in plants used as chewing sticks in Africa. Drugs Future, 2004; 29(12): 1221.

19. Adesina SK. The Nigerian *Zanthoxylum*: Chemical and biological values. Afr J Med. 2005; 2 (3): 282-301.

20. Sharpe M.E. Lactobacilli in meat products. Food Manuf. 1962; 37: 582-589.

21. Vera HD, Power DA. Culture media. In: Manual of Clinical microbiology, (Eds.), Lennette, E.H., Balows, A., Hausler, W.J. and Truant, J.P. Washington: American Society for Microbiology 3rd edition, 1980; 992-993.

22. Molin G, Jeppsson B, Ahrné S, Johansson ML, Nobaek S, Ståhl M, Bengmark S. Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. J Appl Bacteriol. 1993; 74: 314-323.

23. Schleifer KH, Ludwig W. Phylogenetic relationships of lactic acid bacteria. In: *The genera of Lactic Acid Bacteria* ed. Wood, B.J.B. and Holzapfel, W.H. Blackie Academic and Professional. 1995; Vol. 2, 7-18.

24. Hardie JM, Whiley RA. Classification and overview of the genera *Streptococcus* and *Enterococcus*. J Apppl Microbiol Symp Suppl. 1997; 83: 1S-11S.

25. Ammor S, Rachman C. Chaillou S. Prévost H. Dousset X. Zagorec M. Dufour E. Chevallier I. Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. Food Microbiol. 2005; 22 (5): 373-382.

26. Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of Gram-positive bacteria. Bacteriol Rev. 1976; 40: 722-756.

27. Schupabach P, Osterwalder V, Guggenheim B. Human root caries: microbiota in plaque covering sound, carious and arrested carious root surfaces. Carries Research, 1995; 29(5): 382-95

28. Lopez L, Berkowitz R, Zlotnik H, Moss M, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries. Paed Dent 1999; 21: 9-11. 20. Lopez L, Berkowitz R, Zlotnik H, Moss M, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries. Paed Dent 1999; 21: 9-11.

29. Saini S, Mahajan A, Sharma JK, Arora O, Saini OP. Polymicrobial aetiology of dental caries. Ind J Path Microbiol. 1999; 42: 25-29.

30. Vivacqua-Gomes N, Gurgel-Filho ED, Gomes BPFA, Ferraz CCR, Zaia AA, Souza-Filho FJ. Recovery of *Enterococcus faecalis* after single- or multiple-visit root canal treatments carried out in infected teeth *ex vivo*. Int Endotonl J. 2005; 38 (10): 697-704.

31. Franz CAP, Holzapfel WH, Stiles MEM. Enterococci at the crossroads of food safety? International J Food Microbiol. 1999; 47: 1-24.

32. Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices results of an *in vitro* diffusion method study. J Am Dent Ass. 2004; 135: 1135-41.

24. Clarke JK. On the bacterial factor in the etiology of dental caries. Br J Exp Path. 1942; 5: 141-7.

33. Clarke JK. On the bacterial factor in the etiology of dental caries. Br J Exp Path. 1942; 5: 141-147.

34. Hawkins R, Locker D, Noble J, Kay EJ. Professionally applied topical fluorides for caries prevention. Br Dent J. 2003; 6: 313-317.

35. Okpalugo, J. Ibrahim, K. Inyang, US Toothpaste formulation efficacy in reducing oral flora. *Trop J Pharm Res*. 2009; 8 (1): 71-77.

36. Reynolds JEF. Disinfectants. In: Martindale - The Extra Pharmacopoeia, 30th ed. The Pharmaceutical Press, London, 1993; 781-805.

37. Atlas RM. Microorganisms in our world. JM. Smith (Ed.). St Louis: Mosby-Year Book, Inc. USA. 1995; 667-670.

38. Olsson B. Efficiency of traditional chewing sticks in oral hygiene programs among Ethiopian schoolchildren. Community Dent Oral Epidemiol. 1978; 6(3):105-109.

39. Etkin NL. A Hausa herbal pharmacopoeia: Biomedical evaluation of commonly used plant medicines. J Ethnopharmacol. 1981; 4 (1): 75-98.

40. Aderinokun GA, Lawoyin JO, Onyeaso CO. Effect of two common Nigerian chewing sticks on gingival health and oral hygiene. Odontostomatol Trop. 1999; 22 (87):13-18.

41. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. Ethnomedicine of Dharwad district in Karnataka, India--plants used in oral health care. J Ethnopharmacol. 2004; 94(2-3):261-266.

42. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmacol. 2006; 8: 104(1-2):68-78.

43. Muhammad S, Shinkafi MA. *In vitro* activity of the extracts of some Nigerian plants used as chewing sticks on human odontopathogens. Int J Pure Appl Sc. 2007; **1**(2): 25 –31.

44. Peterson PE. World Oral Health Report: Oral Health Programme Non-communicable Disease Prevention and Health Promotion, world Health Organization, Geneva, Switzerland; 2003.

LETTER TO THE EDITOR

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/201005/**21024** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 179-181

IN-VITRO RESISTANCE OF GRAM-NEGATIVE ENTERIC BACILLI FROM WOUND INFECTIONS TO HONEY

[1]D. Olusoga Ogbolu, [2]O. A. Daini, O. A. [1]Terry Alli, [3]O. M. Adeladan, [3]Ademola O. Salako, [4]Felicia F. Olusoga-Ogbolu and [3]Oni A. A.

[1]Department of Biomedical Sciences, College of Health Sciences, (Osogbo Campus), Ladoke Akintola University of Technology, Ogbomoso, Nigeria; [2]Department of Biochemistry, College of Health Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria; [3]Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria; [4]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria.

Correspondence: D. Olusoga Ogbolu. E-Mail: olusogadave@yahoo.com

RUNNING TITLE: RESISTANCE OF ENTERIC BACILLI TO HONEY

Honey was found by some workers to possess antibacterial activity. It has never been reported to cause any tissue damage, and appears to actually promote the healing process. Our work however showed that all the aerobic Gram-negative bacilli tested produced an *in-vitro* resistance to the honey used.

Sir,

The concept of prophylactic antibiotics was established in the 1960s when experimental data established that antibiotics had to be in the circulatory system at high enough dosage at the time of incision to be effective (1). With the use of antibiotics, a new era in the management of wound infections commenced. Unfortunately, eradication of the infective plague affecting surgical wounds has not ended because of the insurgence of antibiotic–resistant bacterial strains and the nature of more adventurous surgical intervention in immunocompromised patients and in implant surgery (2). Honey was found by some workers to possess antibacterial activity where antibiotics were ineffective (3, 4, 5). Much of the effectiveness of honey as a dressing appears to be due to its antimicrobial properties. Honey is reported to cause no tissue damage, and appears to actually promote the healing process (6).

Since honey is used extensively in Nigeria, even at University College Hospital, Ibadan (a premier hospital in Nigeria), we felt it desirable to reassess its effectiveness in view of the prevailing multiple antibiotic resistance reported by many authors (7, 8, 9). We isolated aerobic Gram-negative enteric bacilli from wound specimens at the University College Hospital (Out-patient and in-patient department) (Table 1) and were identified by standard procedures (10, 11). The minimum inhibitory concentration of honey for each isolate was determined using punch-hole agar diffusion method. Sensitivity pattern was compared with the standard *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All isolates flourished at 70%$^{v}/_{v}$ and below, very poor inhibition of the bacteria was noticed at 80%$^{v}/_{v}$, 90%$^{v}/_{v}$ and 100%$^{v}/_{v}$ (Table 1). The water activity of the honey (a_w) was 0.6 and the pH is 3.6.

All the aerobic Gram-negative bacilli isolated showed an *in-vitro* resistance to the honey used. This study is similar to the finding of Adefule *et al* (12) in which the disc assay method employed showed no zone of inhibition for the undiluted honey (100%$^{v}/_{v}$). Nzeako and Hamdi (3) and Subrahmanyam *et al* (13) reported *in-vitro* antibacterial activity of honey at various concentrations against Gram-negative bacilli isolated from wound infections. The honey used in this study was obtained from Saki in Oyo State and Pharmacy department, University College Hospital, Ibadan. Saki is known to produce a very high quality honey in south-western Nigeria, and that used in the study of Adefule *et al* (12) was obtained from Edo State, south-south region of Nigeria. It is possible that the honey obtained from these regions may be synthesized from the same family of flower nectar that shows less antibacterial activity unlike the honey in the study of Nzeako and Hamdi (3) and Subrahmanyam *et al* (13) which were obtained from Saudi-Arabia and India, respectively. Allen *et al* (14) showed that there are many types of honey with and without antibacterial activity and postulated that the type of the flower that

was the source of the nectar determines the nature of the antibacterial activity of honey.

The water activity (a_w) and pH of honey are also important factors in their antimicrobial properties; a_w (0.6) of this honey showed it is ripe enough to inhibit bacteria growth (11). Similarly, honey is characteristically quite acidic, the pH 3.6 obtained in this study is low enough to be inhibitory to many of these pathogens (6). It is also very possible that aerobic Gram-negative enteric bacilli have developed resistance to honey which was hitherto sensitive. In a related study carried out by Ogbolu *et al* (15); Candida isolates had better susceptibility to coconut oil than fluconazole and their results elucidated the use of coconut oil in complementary and alternative medicine especially in this era of emerging drug-resistant *Candida* species.

TABLE 1: Number and percentage of strains inhibited on MH with different concentrations of honey

Organisms	5%-70% x (%)	80% x (%)	90% x (%)	100% x (%)
Escherichia coli n =10	0 (0)	0 (0)	0 (0)	3(80)
Klebsiella species n=15	0 (0)	0 (0)	0 (0)	0 (0)
Proteus vulgaris n=2	0 (0)	0 (0)	0 (0)	2 (25)
Pseudomonas aeruginosa n=21	0 (0)	1 (4.8)	1 (4.8)	2 (9.5)
Key: - n = number of strains	x= number of	strain that show	inhibition	

Having observed a lesser antibacterial activity of honey against the aerobic gram-negative enteric bacilli *in-vitro* further studies to investigate the *in-vivo* antibacterial activity of honey against the enteric bacteria from wound infections will be fundamental to the well being of the populace, as treatment of wound with honey is still widely practiced in Nigeria. Essentially, study into the antibacterial activities of honeys produced by various flowers that are the sources of the nectar is also imperative to further clarify the ambiguity in the use of honey as an antimicrobial agent.

References

1. Burke JF. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. *Surgery* 1961; 50:161-168

2. Hemant S, Charles Z. Wound Infection. *eMedicine Journal* 2002; 3(3):4-7

3. Nzeako BC and Hamdi J. Antimicrobial potential of honey on some microbial isolates *J Appl Bacteriol* 2000; vol 2, pp 75-79.

4. Efem SEE. Clinical observation on the wound healing properties of honey. *Br. J. Surg*. 1998; 75: 679-681

5. Branemark PI, Ekholm R, Albrektsson B, Lindstrom J, Lundborg G. Lundskog. Tissue injury caused by wound Disinfectants *J. Bone Joint Surg. Am* 1967; 49: 48-62

6. Molan PC. "Honey as an antimicrobial agent". In: Mizrahi, A and Lensky Y. (eds) Bee Products: products: Properties, Applications and Apitherapy. *Plenum Press*, New York 1997; Pages 27-37

7. Oni AA, Bakare RA, Arowojolu OA, Kehinde OA, Toki RA, Fashina NA. Comparative in-vitro activities of commonly available quinolones and other antibiotics in bacterial isolates in Ibadan, Nigeria. *Afr. J. Med. Sci.* 2001; 30: 35-37.

8. Ozumba UC: Antibiotic Sensitivity of isolates of *Pseudomonas aeruginosa* in Enugu, Nigeria *Afr J Clin Exp Microbiol* 2003; vol 4 No 1; 48 – 51.

9. Daini OA, Ogbolu DO, Ogunledun. Quinolones Resistance and R-plasmids of some Gram-Negative Enteric Bacilli. *Afr J Clin. & Exp Microbrol* 2005; vol 6(1):14-20.

10. Barrow GI, Feltham RKA. Characters of Gram-negative bacteria. In: Cowan and Steel Manual for Identification of Medical Bacteria. 3rd edition, Cambridge University Press, 1993; 94-149.

11 Ewing WH. Edwards and Ewings Identification of Enterobacteriaceae. 4th edition. Elsevier Science Publishing, New York, 1986.

12. Adefule AK, Idowu GO, Iquot IS. In-Vitro Antimicrobial activity of undiluted Crude Honey: *Afr. J. Med and Pharm. Sci* 2003; 7: 118-21.

13. Subrahmanyam M, Archan H, Pawar SG. Antibacterial activity of Honey on Bacterial isolated from wounds. *Annals of Burns and Fire Disasters* vol xiv n.1 March.

14. Allen KL, Molan PC, Reid GNI. A survey of the antibacterial activity of some New Zealand honeys. *J.Pharm. Pharmacol* 1991; 43:817-22.

15. Ogbolu DO, Oni AA, Daini OA and Oloko AP. Invitro antimicrobial properties of coconut oil on Candida species in Ibadan, Nigeria. *J Med Food* 2007; 10 (2) 384-387.