

ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

APRIL 2018

VOLUME 19

NUMBER 2



Official Publication of the African Society for Clinical Microbiology



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

<https://www.ajol.info/index.php/ajcem> and <https://www.afrcjem.org>

Editorial Board

Chairman

Prof. Rasheed A. Bakare
Department of Medical Microbiology,
College of Medicine, University of Ibadan, Ibadan, Nigeria

Editor-in-Chief

Prof. Samuel S. Taiwo
Department of Medical Microbiology,
Ladoke Akintola University of Technology (LAUTECH)
Teaching Hospital, Ogbomoso, Nigeria

Editorial Advisers

Prof. Daniel Z. Egah
Department of Medical Microbiology,
College of Medicine, University of Jos

Prof. Uchenna C. Ozumba
Department of Medical Microbiology,
University of Nigeria Teaching Hospital, Enugu, Nigeria

Prof. Orikomaba K. Obunge
Department of Medical Microbiology,
College of Health Sciences, University of PortHarcourt, Nigeria

Prof. Adebola T. Olayinka
Department of Medical Microbiology,
College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria

Dr. Kenneth C. Iregbu
Department of Medical Microbiology,
National Hospital, Abuja, Nigeria

Prof. Galadima B. Gadzama
Department of Medical Microbiology,
College of Health Sciences, University of Maiduguri, Maiduguri, Nigeria

Foreign Editorial Advisers

Dr. Tolu Musa-Booth
University of Maryland, Baltimore 21206,
United States of America

Dr. Cecilia Bentsi (rtd)
Ministry of Health, Accra, Ghana

Prof. Adriano Duse
Clinical Microbiology and Infectious Diseases unit,
University of the Witwatersrand, Johannesburg, South Africa

Dr. Dickson Shey Nsagha
Department of Public Health and Hygiene,
Faculty of Health Sciences, University of Buea,
Box 63, Buea, Cameroon



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

<https://www.ajol.info/index.php/ajcem> and <https://www.afrcjem.org>

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, Molecular Genetics in relation to microorganisms and humans, Clinical Microbiology, Clinical Veterinary Microbiology, and Public Health Microbiology.

Subscription information

African Journal of Clinical and Experimental Microbiology is an OPEN ACCESS JOURNAL CC BY VERSION 4.0 INTERNATIONAL; and from 2016 will be published four times a year: January, April, July and October.

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> OR from the journal website <https://www.afrcjem.org>. Subscription is however, still open to individuals, libraries, University Departments, Research Institutes and other Multi-reader institutions who may still want to have hard copies of our Journal. For each volume (4 issues), subscription rate is £400 (United Kingdom), US \$800 (USA/Canada), US \$600 (African Countries), US \$800 (Other Countries), ₦28,000 (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 Samuel S. Taiwo (FMCPATH)
Editor-in-Chief,
African Journal of Clinical and Experimental Microbiology,
Department of Medical Microbiology,
LAUTECH Teaching Hospital, PMB 4007, Ogbomoso, Nigeria
Mobile Phone: +2348033436344
Email: afrcjem@gmail.com OR ajcem2019@gmail.com

Payments for subscription and any other payments are to be made online in favour of African Journal of Clinical and Experimental Microbiology

Guidelines to contributors

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> OR at the journal website: <https://www.afrcjem.org>

Responsibility for accuracy of manuscripts lies entirely with the authors. The original typescript in **Microsoft Word (default)** should be sent to afrcjem@gmail.com OR ajcem2019@gmail.com

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.

French abstracts:

As from May 2014, the Journal will be including French translations of abstracts. Authors should include French translations of their titles, their addresses and abstracts of their manuscripts. If they are not included, it will be done by the Journal at a nominal fee to be paid as part of handling charges.

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
3. Bakare, R. A., Oni, A. A., Okesola, A. A., et al. Efficacy of pefloxacin on acute uncomplicated gonococcal urethritis. *Nig. Qt. J. Hosp. Med.* 1996; 6: 335

For books:

4. 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:
5. 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

Peer Review Process

All manuscripts submitted to the Journal are first scrutinized by the Editorial Board for suitability of publication within the scope of the Journal and for manuscript preparation in line with the Journal guideline. Successful manuscripts are then sent to a minimum of two independent assessors for peer review in a blinded manner. Two assessors' reports must agree for the Board to make a decision concerning acceptance or rejection of a manuscript. The review process takes between 4 to 6 weeks for completion.

Article Processing Charge

African Journal of Clinical and Experimental Microbiology is open access, therefore authors are charged based on number of print pages (double column) of their published articles (not number of pages of their submitted manuscripts). The charge per print page is £10 (UK), €12 (Europe), \$15 (US/Canada) and ₦ 5000 (Nigeria).

Waiver on Article Processing Charge

Authors based in some countries may enjoy some percentage waiver on article processing charge for their accepted manuscripts. Waivers are not automatic but given at the discretion of the Editorial Board and according to the World Bank Atlas classification of countries based on Gross National Income (GNI) per capita. The following percentage waiver may be given; High Income Countries – 0% waiver; Upper Middle Income Countries – 0% waiver; Lower Middle Income Countries – up to 25% waiver; Low Income Countries – up to 40% waiver. Authors from countries entitled to waiver should request for this at the time of manuscript submission.

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 afrcjem@gmail.com OR ajcem2019@gmail.com OR can be submitted online through the Journal website at <https://www.afrcjem.org>
- 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 MAY 2018 ISBN 1595-689X VOL19 No.2

AJCEM/1811

<http://www.ajol.info/journals/ajcem>

COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.1>

AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 76-83

POLYMERASE CHAIN REACTION VERSUS ENZYME-LINKED IMMUNOSORBENT ASSAY IN DETECTION OF CHLAMYDIA TRACHOMATIS INFECTION AMONG GYNAECOLOGICAL PATIENTS IN SOUTH-WESTERN NIGERIA

Ajani¹ T. A.; Anaedobe² C.G.; Oluwasola³ T.A.; Ajani⁴ M. A.; Fayemiwo¹ S.A. & Bakare¹ R.A

¹Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Oyo State, Nigeria;

²Department of Medical Microbiology and Parasitology, University of Abuja, FCT, Nigeria;

³Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Oyo State, Nigeria;

⁴Department of Histopathology, Babcock University, Ilishan-Remo, Ogun State

Correspondence: Dr. Anaedobe Chinenye Gloria, Department of Medical Microbiology and Parasitology, University of Abuja, FCT, Nigeria. Phone: +234 806 784 4403 Email: chimedico@yahoo.com

ABSTRACT

Background: *Chlamydia trachomatis* (*C. trachomatis*), is the most common bacterial Sexually Transmitted Infection, a major cause of Pelvic Inflammatory Disease and female infertility. Since *C. trachomatis* infections are frequently asymptomatic with higher prevalence in developing countries, highly sensitive and affordable methods are desirable for routine screening and diagnosis. This study aimed to evaluate the performance of *C. trachomatis*-specific IgG antibody by ELISA as a screening tool for *C. trachomatis* infection, by comparing the performance of ELISA with the gold standard Polymerase Chain Reaction (PCR).

Method: In this cross sectional study, we enrolled 150 women attending infertility clinic at Ibadan between January and November, 2015. ELISA for detection of IgG antibodies specific to *C. trachomatis* major outer membrane protein (MOMP) was performed on the blood samples using third generation indirect Enzyme Linked Immunosorbent Assay (ELISA) and endocervical samples were analyzed for presence of *C. trachomatis* nucleic acid using PCR. Socio-demographic bio-data and gynaecological history were obtained with questionnaire; data was analyzed using SPSS version 20.0.

Results: Overall, 58 (38.7%) were positive for *C. trachomatis* specific IgG antibody by ELISA and 11 (7.3%) for *C. trachomatis* nucleic acid by PCR. Using PCR as the gold standard, ELISA had a sensitivity of 81.8% specificity of 64.8%, positive predictive value of 15.5% negative predictive value of 97.8% and accuracy of 66%.

Conclusion: The high sensitivity of the ELISA indicates that over 80% of patients identified as being positive in the screened population are truly infected. Also, the negative predictive value approaches 100% amongst those screened out as being negative. Thus its use as a screening tool for *C. trachomatis* infection is warranted particularly in developing countries where cheaper and easier to use alternatives to PCR are in dire need.

Key words: *C. trachomatis*, infertility, polymerase chain reaction, ELISA, sexually transmitted infections.

RÉACTION DE POLYMÉRISATION EN CHAÎNE PAR RAPPORT À DOSAGE IMMUNO-ENZYMATIQUE POUR LA DÉTECTION DE CHLAMYDIA TRACHOMATIS CHEZ LES PATIENTS GYNÉCOLOGIQUES DANS LE SUD-OUEST DU NIGERIA

Ajani¹ T. A.; Anaedobe² C.G.; Oluwasola³ T.A.; Ajani⁴ M. A.; Fayemiwo¹ S.A. & Bakare¹ R.A

¹ Département de microbiologie médicale et de la parasitologie, de l'University College Hospital, Ibadan, Oyo State, Nigeria ;

² Département de microbiologie médicale et de parasitologie, Université d'Abuja, Nigéria, FCT;

³ Département d'Obstétrique et gynécologie, University College Hospital, Ibadan, Oyo State, Nigeria;

⁴ Département de l'histopathologie, Babcock, Université de l'état d'Ogun, Ilishan-Remo

Correspondance: Dr. Anaedobe Chinenye Gloria, Département de microbiologie médicale et de parasitologie, Université d'Abuja, Nigéria, FCT. Téléphone : +234 806 784 4403 Email : chimedico@yahoo.com

ABSTRACT

Background: Chlamydia trachomatis (C. trachomatis), est la plus courante infection bactérienne transmise sexuellement, une cause majeure de maladie inflammatoire pelvienne et de l'infertilité féminine. Depuis les infections à C. trachomatis sont fréquemment asymptomatique avec une prévalence plus importante dans les pays en développement, particulièrement sensible et méthodes abordables sont souhaitables pour le dépistage de routine et le diagnostic. Cette étude visait à évaluer la performance de C. trachomatis anticorps IgG spécifiques par ELISA comme un outil de dépistage pour l'infection à C. trachomatis, en comparant le rendement d'ELISA à l'étalon de la réaction en chaîne par polymérase (PCR).

Méthode: Dans cette étude transversale, nous avons inscrit 150 femmes fréquentant une clinique d'infertilité à Ibadan, entre janvier et novembre 2015. ELISA pour la détection des anticorps IgG spécifiques à C. trachomatis grande protéine de la membrane externe (MOMP) a été effectuée sur les échantillons de sang à l'aide indirecte de troisième génération dosage immunoenzymatique (ELISA) et endocervical les échantillons ont été analysés pour la présence de C. trachomatis par PCR de l'acide nucléique. Bio-socio-démographiques et des données de l'histoire gynécologique ont été obtenus avec questionnaire ; les données ont été analysées à l'aide de SPSS version 20.0.

Résultats: au total, 58 (38,7 %) étaient positif pour C. trachomatis anticorps IgG spécifiques par ELISA et 11 (7,3 %) pour C. trachomatis par PCR de l'acide nucléique. Par PCR comme le gold standard, ELISA avait une sensibilité de 81,8 % de spécificité de 64,8 %, valeur prédictive positive de 15,5 % valeur prédictive négative de 97,8 % et la précision de 66 %.

Conclusion: La grande sensibilité de l'ELISA indique que plus de 80 % des patients identifiés comme étant positifs dans la population de dépistage sont vraiment infecté. En outre, la valeur prédictive négative de 100 % des approches parmi ceux éliminés comme étant négatif. Ainsi, son utilisation comme outil de dépistage pour l'infection à C. trachomatis est justifiée en particulier dans les pays où moins cher et plus facile d'utiliser des alternatives à PCR ont grandement besoin.

Mots clés: C. trachomatis, infertilité, réaction de polymérisation en chaîne, ELISA, les infections sexuellement transmissibles.

INTRODUCTION

C. trachomatis (serotypes D-K) are obligatory intracellular gram-negative bacteria; it is one of the most common bacterial causes of Sexually Transmitted Infections (STIs) and a major cause of Pelvic Inflammatory Disease (PID) in women [1]. Genital *C. trachomatis* infections are often asymptomatic in up to 80% of women causing a wide spectrum of diseases which can be complicated by tubal factor infertility, ectopic pregnancy and adverse pregnancy outcomes [2].

In 2010, sexually transmitted Chlamydia infection reportedly affected about 215 million people globally [2]. World Health Organisation estimates that 92 million new cases of genital *C. trachomatis* infection occur each year globally and more than two-thirds of these cases occur in resource-poor countries where diagnostic and treatment facilities are very limited [3-5]. In most developing countries, tubal factor infertility often results from pelvic infections and is frequently caused by sexually transmitted *C. trachomatis* infection [3]. In Nigeria, infertility is a major public health concern, with a prevalence of 20% to 40% compared with global prevalence of 15% [6]. Despite this high prevalence, *C. trachomatis* infection is not routinely screened for, even among patients being managed for infertility.

Diagnostic techniques for *C. trachomatis* infection include cell culture, cytology, conventional serology,

direct florescent antibody (DFA), enzyme-linked immunosorbent assay (ELISA), DNA hybridization techniques and polymerase chain reaction (PCR). [7]. Cell culture method, previously regarded as gold standard is expensive, technically demanding and difficult to standardize [7]. The conventional serological assays are not without limitations as it has been reported that serum antibody is not always consistent with active infection especially in regions of high prevalence of *C. trachomatis* [8]. The presence of antibodies cannot differentiate an acute, chronic or a resolved *C. trachomatis* infection and cross-reactive antibodies are sometimes produced in response to lipopolysaccharides of other *Chlamydia* species such as *C. pneumoniae* and Gram-negative bacterial lipopolysaccharides leading to high number of false positive results [9]. To address this limitation, direct florescent antibody (DFA), ELISA for the detection of IgG antibody specific for *C. trachomatis* infection and chlamydial antigens in clinical specimens have been made available. The DFA uses monoclonal antibodies directed against specie specific antigens on chlamydial MOMP while the ELISA detects IgG antibodies specific to *C. trachomatis* MOMP or lipopolysaccharide [10] as well as specific antigens extracted from chlamydial elementary bodies [9]. Both DFA and ELISA are reported to have a higher sensitivity and specificity with lower false positive results [9, 10].

Nucleic acid amplification tests, (NAAT), such as PCR and ligase chain reaction are more sensitive and specific than conventional immunoassays as they target unique nucleic acid (DNA or RNA) of the chlamydial organism [11, 12 13] and do not require viable organisms to detect infection [14]. PCR is considered to be more reliable for diagnosis of asymptomatic pelvic infection caused by *C. trachomatis* as these infections are often associated with low copy numbers and are often missed by less accurate microbiology diagnostic techniques [15].

While detection by PCR has better performance for the detection of *C. trachomatis* and now considered as a gold standard, they require special laboratories, equipment, and expertise which are apparently impracticable in most developing countries [7]. Hence, there is need for more affordable and easier to perform diagnostic assays with fast throughput of high number of specimen, such as *C. trachomatis*-specific IgG antibody detection assay. This assay has better performance than conventional serodiagnostic assays like the non- specific antibody detection immunoassays [9,10]. However, this specie- specific antibody assay can assist but cannot replace antigen detection immunoassays, molecular tests or isolation of the organism by the culture technique [7, 11-13]. *Chlamydia* screening programs in women of reproductive age facilitates prompt diagnosis and therapeutic intervention, thus reducing the morbidity from long term complications and the prevalence of the disease by controlling its transmission and future exposures.

In Nigeria, very few studies on genital Chlamydia infection and infertility have been performed using *C. trachomatis*-specific antibody detection immunoassay which has no cross-reactivity with other chlamydial species. Hence the performance of the *C. trachomatis*-specific antibody detection assays as a screening tool has not been ascertained. The objective of the study was to evaluate the performance of *C. trachomatis*-specific IgG antibody ELISA technique as a screening tool for *C. trachomatis* infection among infertile women, by comparing the performance of the ELISA with the gold standard Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

Study Design

This was a cross-sectional study, in which consenting women, attending infertility clinic at the University College Hospital in Ibadan, Nigeria were recruited

and their blood and endocervical specimens taken for analyses. Semi structured questionnaires which had been pretested and validated, were used to obtain socio-demographic characteristics and gynaecological history from the study participants.

Study Population: Sample size was calculated to give a 95% confidence level, a margin of error of 10%, using a prevalence of 9.8% reported in a previous survey on the prevalence of *C. trachomatis* infection among attendees of a fertility clinic in Abeokuta, Ogun state, Nigeria [16]. A total of a hundred and fifty (150) consenting women with ages ranging between 20 and 40 years were recruited between January and November 2015. A written informed consent was obtained after careful explanation, in a clear language, of the concept of the study to each participant before their inclusion in the study. Ethical clearance was sought and obtained from the Joint Ethical committee of the University of Ibadan and University College Hospital Ibadan before the commencement of the study.

Ethical Approval: The study protocol was approved by the Institutional Review Board of the University of Ibadan, and the University College Hospital both in Oyo State, Nigeria.

Consent: Written informed consent was voluntarily signed by all the study participants.

Specimen collection and handling: Venous blood and endocervical swab were taken from each woman recruited into the study. About 3mls of venous blood was collected aseptically by venipuncture into EDTA bottles. The plasma was separated by centrifugation at room temperature at 3000 rpm and stored in aliquots in the freezer at -20°C. This was done on every visit to the Gynaecology clinic.

Collection of the endocervical swab was done under aseptic condition for each recruited woman. The patients were placed in lithotomy position, and then sterile speculum was inserted into the vagina to expose the cervical os. A sterile copan eNat cervical swabs were introduced into the endocervix to collect endocervical specimen. Care was taken not to touch the walls of the vagina and the speculum. The swabs were subsequently put inside eNat preservation medium and stored at -20 °C in the freezer until processing.

Laboratory Investigations: All samples were analyzed, using an indirect third generation ELISA for the detection of *C. trachomatis*-specific IgG antibody in human plasma using microplates coated with immunodominant species specific polypeptide

derived from *C. trachomatis* MOMP (DIA-PRO by Diagnostic Bioprobes Milano Italy). Protocol for the measurement was done according to the manufacturer's instruction and reading was done at optical density (O.D.) of 450 nm with an Enzyme - linked Immunoassay (EIA) plate reader. The tests ran were validated and results were interpreted according to the manufacturer's instruction.

C. trachomatis nucleic acid was extracted from the endocervical specimen of all 150 study participants, using Jena Bioscience Bacteria DNA preparation kit. A thermocycler was used to run the polymerase chain reaction for the amplification of a 240 base pair sequence of the cryptic plasmid DNA (Biomers Germany), the amplified product was subjected to electrophoresis using 1.5% agarose gel and visualized under an ultraviolet (UV) transilluminator. All tests were carried out according to the manufacturer's instructions as outlined in the package inserts.

Data Analysis: Data collected were subjected to descriptive and inferential statistical analysis using SPSS version 20. (SPSS Inc. Illinois, USA). The Mean, standard deviation and test of comparison using student's t-test were calculated for continuous variables, while categorical variables were summarized as proportions, and further analyzed using Chi square and Fisher's exact test to assess association between the variables. Test of association using logistic regression was done to describe the relationship between the predictor variables. A P value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic characteristics of the participants: The 150 participants aged between 20 years to 40 years, had a mean age of 34.1 ± 5.6 years, with most of the women within the 30-39 year age range (64%). Majority of the respondents 89/150 (59.3%) had secondary infertility while the remaining 61/150 (40.7%) had primary infertility. The socio-demographic characteristics are as illustrated in Table 1.

Prevalence of *C. trachomatis* infection: Of the 150 infertile women enrolled in the study, *C. trachomatis* specific IgG antibody was detected in 58(38.7%) using ELISA, while the rate of *C. trachomatis* infection confirmed by PCR was 11(7.3%). Of the 58 positive results by ELISA, only 9 were true positive after confirmation by PCR, while the rest 49 were false positive. ELISA was able to detect 9 out of the 11 women confirmed with *C. trachomatis* infection.

TABLE 1: SOCIO-DEMOGRAPHIC FACTORS AND GYNAECOLOGICAL HISTORY OF THE STUDY POPULATION

Variables	Frequency	Percentage
Age groups (in years)		
20 – 29	29	19.3
30 – 39	96	64.0
≥ 40	25	16.7
Religion		
Christianity	92	61.3
Islam	58	38.7
Marital Status		
Single	11	7.3
Married	136	90.7
Divorced	3	2.0
Family Setting		
Monogamy	129	86.0
Polygamy	21	14.0
Residence		
Within Ibadan	132	88.0
Outside Ibadan	18	12.0
Education		
Primary uncompleted	2	1.3
Primary completed	8	5.3
Secondary uncompleted	7	4.7
Secondary completed	44	29.3
Post-secondary	27	18.0
Tertiary	62	41.3
Employment		
Self-employed	89	59.3
Employed by government /private	46	30.7
Unemployed	10	6.7
Housewife	1	0.7
Student	2	1.3
Others	2	1.3
Type of Infertility		
primary	61	40.7
secondary	89	59.3
Previous History of Abortion		
Yes	56	37.3
No	94	62.7

Performance evaluation of ELISA screening result in Comparison to PCR:

After confirmation test with PCR, the performance of ELISA screening result was evaluated for:

True positive (Number of participants that are ELISA positive and PCR positive),

False negative (Number of participants that are ELISA negative and PCR positive)

True negative (Number of participants that are ELISA negative and PCR negative)

False positive (Number of participants that are ELISA positive and PCR negative)

This is further illustrated in table 2 below

TABLE 2: PERFORMANCE EVALUATION OF ELISA SCREENING RESULT

Screening result by ELISA	Presence of disease confirmed by PCR		Total
	Present	Absent	
Positive	9 (true positive)	49 (false positive)	58
Negative	2 (false negative)	90 (true negative)	92
Total	11	139	150

The results of ELISA in comparison to PCR assay were statistically significant in the study population ($\chi^2=9.3$, $P<.01$), as illustrated in Table 3. The sensitivity and specificity of ELISA were 81.8%, 64.8% respectively. While the positive predictive value, negative predictive value and accuracy of the assay for the detection of *C. trachomatis* infection were 15.5%, 97.8%, and 66% respectively.

DISCUSSION

Genital *C. trachomatis* infection is a major public health concern with potentials for long term complications in women including infertility [2]. Due to its predominantly asymptomatic presentation;

mandatory screening of women attending infertility clinics is warranted. Also, early detection and prompt treatment are critical intervention strategies to curb the risk of transmission to sexual partners, prevent development of reproductive sequelae and reduce disease burden. In regions where it is impracticable to set up molecular laboratories for the detection of *C. trachomatis*, there is need for affordable, easier to perform and reliable screening tools to ensure early detection and management of infected patients. The prevalence of asymptomatic genital chlamydial infection among the study population using ELISA specific for *C. trachomatis* IgG antibody was 38.7%. Confirmation by PCR assay revealed 7.3% of the 150 women enrolled in this study had *C. trachomatis* nucleic acid in their endocervical specimen.

This finding on ELISA is comparable with the results of other studies; 32.1% reported among infertile women in Lagos, 32.4% in Benin and 31% in Zaria, Nigeria [11, 17, 18]. However, a lower rate was reported from Ibadan by Moharson-Bello *et al* [19] who researched largely among uneducated study population who were likely to have lower socio-economic status and decreased likelihood of presenting to hospitals for health-related problems. The disparity may also be due to the difference in the principle of action of the screening assay used; *C. trachomatis* specific IgG antibodies with no cross reaction with *C. pneumonia* was used in our study. Israel *et al* reported *Chlamydia* IgG antibodies in 74% of symptomatic women with history of recurrent or chronic infection in Port-Harcourt, Nigeria [20]. The difference in the prevalence rate can be attributed to the type of assay used. *C. trachomatis* specific IgG antibody detection by ELISA is more sensitive and specific than the regular IgG antibody detection assays and has fewer false positive results. This may also be a reflection of the absence of routine screening and treatment for *C. trachomatis* infections in this population.

TABLE 3: COMPARISON OF THE PREVALENCE OF CHLAMYDIA TRACHOMATIS INFECTION IN RESPONDENTS USING PCR AND ELISA (N = 150)

Variables	PCR			Df	X ²	p-value
	Positive/(%)	Negative/(%)	Total/(%)			
ELISA						
Positive	9 (81.8%)	49 (35.3%)	58 (38.7%)	1	9.3	0.002
Negative	2 (18.2%)	90 (64.7%)	92 (61.3%)			
Total	11 (100%)	139 (100%)	150 (100%)			

Sensitivity = True positive/ (True positive+ False negative); Specificity= True negative/ (True negative+ False positive)
 Positive predictive value =True positive/(True positive + False positive); Negative predictive value = True negative/ (True negative+ False negative); Accuracy = True positive + True negative/(total screened patients); The sensitivity of ELISA = $9/11 \times 100 = 81.81\%$; The specificity of ELISA = $90/139 \times 100 = 64.75\%$; The Positive predictive value = $9/58 \times 100 = 15.52\%$; The negative predictive value = $90/92 \times 100 = 97.83\%$; Accuracy of ELISA = $9+90/150 \times 100 = 66\%$; P value =0.002 (significant)

By principle of action, the specie specific antibody detection immunoassay used in our study is different

from other regular antibody detection immunoassays. It uses highly specific and immunogenic antigen

derived from *C. trachomatis* MOMP to detect chlamydial antibodies and has no cross reactivity with *C. pneumonia* antibodies. A major drawback of the regular IgG antibody detection assays is that it does not distinguish between species. Cross-reactive antibodies bound to lipopolysaccharides of other *Chlamydia* species including *C. pneumoniae* and Gram-negative bacterial lipopolysaccharides makes such assay less sensitive and specific as a screening tool for *C. trachomatis* infection. Invariably; there will be high false-positive test results if used in regions of high prevalence of Chlamydial infection [9].

In other Africa regions, prevalence rates of between 36% and 39.3% have been reported among women with unexplained infertility [4,21] although a lower prevalence of 18.5% was reported among symptomatic participants in Rwanda [22]. It was noted by the authors that vigorous treatment-seeking behaviour was common among women in infertile relationships when facing genital symptoms in that environment and this perhaps had resulted in early treatment before aetiologic diagnosis was achieved.

Findings from our study and in the literature, suggest a high prevalence of asymptomatic genital *C. trachomatis* infection among infertile women in most parts of Africa [23, 24]. The reasons for this might be attributed to the poor screening protocol for *C. trachomatis* infection among women of reproductive age, the near absent diagnostic and treatment facilities, poor health seeking behaviour of these women and asymptomatic nature of the infection. In order to effectively identify infected women, reliable, cheap and easy to use screening assays like the *C. trachomatis* specific IgG antibody detection by ELISA may be utilized.

In other parts of the world, lower prevalence rates have been reported; 9% in Iran, [25] 25% in Baghdad, [26] 21.1%, 15.20 %, and 9.84% in Netherlands, Romania and Saudi Arabia respectively [27, 28, 30]. Improved medical facilities for early detection and prompt treatment of *C. trachomatis* infection may account for these low prevalence rates.

The prevalence of *C. trachomatis* infection varies with different study population, specimen type, presence of clinical symptoms, previous or chronic infection, type and sensitivity of the microbiology diagnostic methods used and the laboratory expertise.

In the index study, using PCR as gold standard, ELISA has a sensitivity of 81%, specificity of 65%, positive and negative predictive values of 16% and 98% in that order, all approximated. Our findings of high sensitivity, moderate specificity, [29] low positive predictive value and high negative predictive

value [22] were in tandem from similar studies in India and Rwanda [22, 29]. In Iran, Batool *et al* reported a low sensitivity and high specificity of the ELISA but a low positive predictive value and high negative predictive value similar to that observed in our study [25]. The participants in the Iranian study were symptomatic with possible active infection and the regular IgG antibody detection immunoassay used is said to have low sensitivity in people with active infection and does not distinguish between species [9, 10].

Molecular assay for detection of chlamydial DNA are the preferred investigations for diagnosis of *C. trachomatis* infection in all specimen types. However, this is not practicable in resource poor regions and comparable alternatives are of utmost importance. The high sensitivity and moderate specificity of the *C. trachomatis* specific IgG antibody detection by ELISA suggests it can be a good screening tool particularly in regions of relatively high prevalence of *C. trachomatis* infection albeit the tendency to give false positive result. In other words, the high sensitivity of the ELISA indicates that over 80% of patients identified as being positive in the screened population are truly infected. Also, the negative predictive value approaches 100% amongst those screened out as being negative, indicating that the women with negative results were unlikely to be infected. The low positive predictive value indicates that ELISA is not without limitations, therefore positive test results should be confirmed by PCR especially amidst the asymptomatic patient. At this point, the screening tool has largely reduced the number of patients for confirmatory test thus saving cost.

ELISA for detection of chlamydial antibodies in clinical specimens using *C. trachomatis* MOMP has been studied extensively as a screening tool and has been reported to have good performance when compared with the gold standard [9,26-27,29-32]. Thus, the affordability, easy application and high throughput of the immunoassay, makes it a practical screening tool in regions of high *C. trachomatis* prevalence and a good substitution for the more sensitive and specific PCR especially low-income countries.

A limitation to this study is the different specimen type used; plasma for the ELISA and endocervical specimen for the PCR. To allow a scientifically valid comparison and evaluation of performance of ELISA using PCR results as gold standards, both tests should be performed on the same single specimen and the study should include a substantial number of true-positive results. It is important to point out that the diagnosis of acute *C. trachomatis* infection is based on

direct detection of the organism, in this case, serodiagnosis are of limited value.

Conclusion:

The high prevalence of asymptomatic *C. trachomatis* infection among infertile women documented in this study underscores the need for routine screening for chlamydial infection among them. *C. trachomatis* specific IgG antibody detection by ELISA gave comparable results in more than 80% of true positive samples detected by PCR. This suggests that it is a practical and cheaper alternative to the molecular

tests for routine screening and diagnosis especially in resource poor countries.

Competing interests: The authors declare no competing interest.

Acknowledgements We wish to appreciate the Head of Department and Staff of the Gynaecology Clinic of University College Hospital Ibadan, Oyo State, for their co-operation and support during the study.

REFERENCES

1. Meenakshi M, Seema S, Anjan M, Sumathi M, Manju B. Genital *Chlamydia trachomatis*: An update *Indian J Med Res.*2013; **138**:303-316.
2. Vos T, Flaxman A, Mohsen N, Rafael L, Catherine M, Majid E *et al.* Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010; *The lancet* **380**:2163-2196.
3. Cuffini C, Bottiglieri M, Kigwen X, Carlos E., Romina A, Deimundo V. Molecular Epidemiology of genital *Chlamydia trachomatis* infection in asymptomatic adolescent young people. *J Microbiol Research* 2012; **2**(4):114-117.
4. Paola M, Donatella U, Marina. Comparison of two Molecular Methods for diagnosis of *Chlamydia trachomatis*. *J infect Dev.countries*2012; **7**(1):64-66.
5. United Kingdom Health Protection Agency. Genital *Chlamydia trachomatis* <http://www.hpa.org.uk/topics/infectiousdiseases/infectionsaz/chlamydia/>. Cited 31st of August 2013
6. Nsonwu-Anyanwu A C, Charles-Davies MA, Oni A A, Taiwo V O, Bello FA. chlamydial infection, plasma peroxidation and obesity in tubal Infertility. *Ann.lbd.pg Med* 2011; **9**(2):82-88
7. Watson EJ, Templeton A, Russell I, Paavonen J, Mardh PA, Sary A, *et al.* The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol.* 2002;**51**:1021-31.
8. Salah A, Hesham A S, Nesrine F H, Huda B D. The detection of *Chlamydia trachomatis* antigen in cervical secretions and serum antibodies in infertile females undergoing ICSI and its impact on pregnancy success. *Life Scien J.*2011; **8**(2):259-263.
9. Frikha-Gargouri O., Gdoura R., Znazen A., Gargouri J., Rebai A and Hammami A. Diagnostic value of an enzyme-linked immunosorbent assay using the recombinant CT694 species-specific protein of *Chlamydia trachomatis*. *Journal of Applied Microbiology* 2009; **107**:1875–1882
10. Phillips; Nelson AL; Woodward JA; Current Clinical practise: Sexually Transmitted Diseases: A Practical Guide for Primary Care New. The Humana Press, Totowa, New Jersey 2006; 127-128.
11. Tayo AO, Akinola OI, Ottun TA, Babatunde A, Shakunle B. Chlamydia infection and infertility: Any Relationship The Lagos State University Teaching Hospital Experience. *Int J Med. Med. Sciences* 2011; **1**(1): 017-019.
12. Shatha T. A .Detection of *Chlamydia trachomatis* using Polymerase chain reaction. *AL-Mustanisiriya J scien* 2010; **23** (6):35-42.
13. Jawetz, Melnick, Adelbergs. Chlamydia. In: Geo. F. B, Karen C. C, Janet S. B, Stephen A. M, editors. Medical Microbiology 24th ed. U.S.A. The McGraw-Hill Companies, 2007.
14. Atalabi OM, Fayemiwo SA, Oladokun AA, Bakare RA. Pattern of Asymptomatic Sexually transmitted infections in Women undergoing hysterosalpingography for infertility evaluation in Ibadan Nigeria. *Trop.J. Obstet Gynaecol* 2013; **30**(2):91-98.
15. Ian S M. Epidemiology and diagnosis of genital *Chlamydia trachomatis* infection. *Culture* 2007; **23** (1):01-08.
16. Ogiogwa I O, Motayo B O, Okerentugba P O, Innocent-Adiele, H C, Tafeng Y, Onoh C C *et al.* Detection of *Chlamydia trachomatis* antigen among attendees of a fertility clinic in Abeokuta, Ogun State Nigeria. *Researcher* 2012; **4**(4):96-100.
17. Ibadin, K O, Onaiwu. I E, Eghafona O I, Aziken, M. E. Seroevidence of *Chlamydia trachomatis* antibody in infertile women in University of Benin Teaching Hospital (UBTH)

18. Benin City, Nigeria. *Malaysian J. Microbiol.* 2011; 6(1) : 91-93.
19. Koledade A, Adesiyun1 A, Oguntayo A, Olayinka A, Randawa A. Prevalence Of *Chlamydia trachomatis* Infection Among Women Attending Gynaecological Clinic For Infertility In Zaria, Nigeria. *The Internet J. Gynecol. Obstet.* 2014 ; **19** (1)[published online] .
20. Morhason-Bello IO, Ojengbede OA , Oladokun A, Adedokun BO, Ajayi A. The Prevalence and Outcome of Asymptomatic Chlamydial Infection Screening Among Infertile Women Attending Gynecological Clinic in Ibadan, South West Nigeria. *Ann Med Health Sci Res.* 2014; **4**(2): 253-257.
21. Israel J, Ola O, Chris A. The Prevalence of Serum Immunoglobulin G antibody to *Chlamydia trachomatis* in Subfertile Women Presenting at the University of Port Harcourt Teaching Hospital, Nigeria. *Int J. Biomed Sci.* 2011; **2**(7) :120-124
22. Screening for *Chlamydia trachomatis* in Egyptian women with unexplained infertility, comparing real-time PCR techniques to standard serology tests: case control study. *BMC Womens Health.* 2015; 15: 45.[published online].
23. Claude M, Dhont N. *Chlamydia trachomatis* infection in fertile and sub fertile woman in Rwanda: Prevalence and diagnostic significance of IgG and IgM antibodies testing. *Hum. Repro Advance* 2011; **0**(0):1-8.
24. Oloyede OAO, Fakoya TA, Oloyede AA, Alayo AM. Prevalence and Awareness about Chlamydial infection in Women Undergoing infertility evaluation in Lagos, Nigeria. *Int J Health Research.* 2009; **2**(2): 157-162
25. Okonofua FE. Infertility in Sub Saharan Africa. In: Okonofua F, Odunsi K. (eds). Contemporary Obstetrics and Gyneacology for Developing Countries. Ed 1, Woman's Health and Action Research Center. Benin City, Edo State, Nigeria.2003; pp 129 -156
26. Batool H R, Leili CT, FedyeH H, Mahmood J T, Mohammed M N, Mamak Shariat *et al.* Effects of *Chlamydia trachomatis* infection on fertility, a case control study. *J Reprod. fertility*2013; **14**(55):67-72.
27. Ismail M K, Ali A S. Evaluation of *Chlamydia trachomatis* Antibodies in Women with Infertility. *Al- Mustansiriya J. Sci.* 2012; **23**(3):22-28.
28. Bax C J, Mutsaers E M, Jansen C L, Trimbos J B Do`rr P J *et al.* Comparison of Serological Assays for Detection of *Chlamydia trachomatis* Antibodies in Different Groups of Obstetrical and Gynecological Patients. *clini. diagnostic laboratory immunology* 2003;**1**(10):174-176.
29. Mareş M, Socolov D, Doroftei B, Botezatu A, Goia CD. The prevalence of some bacterial markers in female patients undergoing an initial infertility evaluation in north-east Romania. *Roum Arch Microbiol Immunol.* 2009;**68**(3):171-174.
30. Durgesh D G, Bajaj J, Damle S, Jayanti M P , Sonali D, Shilpa C. K . Study of *Chlamydia trachomatis* in Infertile Women. *Ind j research* 2013; **2**(3):260-263.
31. Remah M K. Screening for *Chlamydia trachomatis* infection among infertile women in Saudi Arabia. *Int J Womens Health* 2013; **5**: 277–284.
32. Menon S., Stansfield S.H., Walsh M., Hope E., Isaia L., Righarts A. A., et al. Sero-epidemiological assessment of Chlamydia trachomatis infection and sub-fertility in Samoan women. *BMC Infectious Diseases* 2016; **16**:175
33. Muvunyi CM, Dhont N, Verhelst R, Temmerman M, Claeys G, Padalko E. Chlamydia trachomatis infection in fertile and subfertile women in Rwanda: prevalence and diagnostic significance of IgG and IgA antibodies testing. *Hum Reprod.* 2011; **26**(12):3319-26.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X MAY VOL19 No.2
AJCEM/1812 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.2>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 84-91

IN-VITRO ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF DIOSPYROS MONBUTTENSIS

Ayepola O. O, Olasehinde G. I, Adedeji O. A, Adeyemi O. O, Onile-Ere, O.A

Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria

Correspondence: adedeji.olamide@yahoo.com

ABSTRACT

Diospyros species in folklore medicine are used as anti-inflammatory, antibacterial, antioxidant, anticancer and antiviral agents. The in vitro antimicrobial activity of crude extracts of the leaves of *Diospyros monbuttensis* were evaluated against three bacterial species (*Staphylococcus aureus*, *Escherichia coli* and *Micrococcus luteus*) and fungal strain (*Aspergillus niger*). Extraction was carried out using both polar and non-polar solvents (ethanol and water). The leaves were screened for phytochemical constituents and preliminary screening for antimicrobial activity carried out using the agar well diffusion method. The minimum inhibitory concentration (MIC) was determined using the agar well dilution method. The phytochemical screening revealed the presence of saponins, tannins, glycosides and alkaloids in the plant. The ethanolic leaf extract of *D. monbuttensis* had no activity against the test organisms, but antimicrobial activity was observed for the aqueous extract against *S. aureus* and *E. coli* at all concentrations tested. The MIC of the aqueous extract of *D. monbuttensis* on *S. aureus* and *E. coli* was 0.78 mg/ml. The results of this study indicate that *Diospyros monbuttensis* leaves may be used for treatment of infections caused by *S. aureus* and *E. coli*.

Keywords: *Diospyros monbuttensis*; Antimicrobial activity; phytochemical screening

L'ACTIVITÉ ANTIMICROBIENNE IN VITRO D'EXTRAITS BRUTS DE DIOSPYROS MONBUTTENSIS

Ayepola, O. O., Olasehinde G. I, Adedeji, O.A., Adeyemi O. O, Onile-Ere, O.A

Département des Sciences Biologiques, Faculté des sciences et de la technologie, l'université, Alliance Ota, l'état d'Ogun, Nigéria

Correspondance : adedeji.olamide@yahoo.com

RÉSUMÉ

Diospyros espèce dans la médecine folklorique sont utilisés comme anti-inflammatoire, Antibactérien, antioxydant, anticancéreux et d'agents antiviraux. L'activité antimicrobienne in vitro d'extraits bruts des feuilles de *Diospyros monbuttensis* ont été évaluées en fonction de trois espèces bactériennes (*Staphylococcus aureus*, *Escherichia coli* et *Micrococcus luteus*) et la souche fongique (*Aspergillus niger*). L'extraction a été réalisée en utilisant à la fois polar et les solvants non-polaires (éthanol et d'eau). Les feuilles ont été examinés pour constituants phytochimiques et contrôle préalable de l'activité antimicrobienne réalisée à l'aide de la méthode de diffusion et de l'agar. La concentration minimale inhibitrice (CMI) a été déterminé en utilisant la méthode de dilution en gélose bien. Le dépistage phytochimique a révélé la présence de saponines, tanins, glucosides cardiotoniques et d'alcaloïdes dans la plante. L'extrait de feuilles de éthanolique *D. monbuttensis* n'avait aucune activité contre les organismes à l'essai, mais l'activité antimicrobienne a été observée pour l'extrait aqueux contre *S. aureus* et *E. coli* à toutes les concentrations testées. Le MIC de l'extrait aqueux de *D. monbuttensis* sur *S. aureus* et *E. coli* était de 0,78 mg/ml. Les résultats de cette étude indiquent que *Diospyros monbuttensis* les feuilles peuvent être utilisées pour le traitement des infections causées par *S. aureus* et *E. coli*.

Mots-clés: *Diospyros monbuttensis* ; activité antimicrobienne ; dépistage phytochimiques

INTRODUCTION

Diospyros monbuttensis is commonly used as chewing sticks and also found varied uses in folklore medicine (1). It is widespread and can be found largely in West Africa and Asia. *Diospyros* species are used in folklore medicine as anti-inflammatory, antibacterial, antioxidant, anticancer, antiviral and termite resistant activities (2). *Diospyros monbuttensis* leaves have been reported to possess antibacterial properties (3). The antimicrobial activities of the plant leaves extract has been attributed to the presence of tannins, saponins, glycosides and alkaloids (3,4).

METHODS

Collection of Plant Materials: Leaves of *Diospyros*

monbuttensis were collected from Ota, Ogun state. The plants were identified in the Department of Pharmacognosy, Lagos University Teaching Hospital, Lagos state.

Plant Extraction

Plant materials for extraction were thoroughly washed and air dried at 37°C for 21 days and blended to powder using a laboratory blender (CentrifugeR (Model:CB8231-D) 1.75L). Ethanolic and aqueous extraction was carried out using the cold extraction method. The extracts were concentrated using a rotary evaporator and kept in tightly stoppered bottles in the refrigerator until the antimicrobial assay.

Microorganisms

The microorganisms used include Gram positive bacterial species (*Staphylococcus aureus*, *Micrococcus luteus*), Gram negative bacterial species (*Escherichia coli*) and one fungus (*Aspergillus niger*). They were obtained from the laboratory stock of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun state. The isolates were confirmed using conventional biochemical methods.

Determination of Antimicrobial Activity of Crude Extracts

Agar well diffusion method as earlier described with modification was used to carry out the antimicrobial activity testing of the crude extract (5). Three to five colonies of an overnight culture was transferred into test tubes containing 5ml of distilled water and adjusted to 0.5 McFarland standards. A sterile cotton swab was dipped into the adjusted suspension and used to streak the dried surface of Mueller Hinton agar plates (bacteria) and the potato dextrose agar plate (fungi). The inoculum was allowed to diffuse into the agar and a sterile cork-borer of 9mm diameter was used to bore uniform wells on the surface of the agar and after which the wells were filled with 0.4mls of the extract at concentrations of 100mgmL⁻¹, 75mgmL⁻¹, 50mgmL⁻¹ and 25mgmL⁻¹. The plates were incubated at 37 °C for 18-24 hours for bacteria. The fungal culture was kept at room temperature for 5 days after which the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (Mic): Different concentrations of the extracts were prepared to give a final concentration in the range of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.04875, 0.024375mgmL⁻¹. Two millilitre of each dilution of the extract was mixed with 18mL of molten Mueller Hinton agar, poured into petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. In all cases the lowest concentration at which there was no growth was recorded as the MIC.

Phytochemical Screening of Plant Samples

The freshly prepared aqueous extract of the plants was qualitatively tested for the presence of phytochemicals using standard procedures adapted from (6,7) [6,7].

RESULTS

The result for the antimicrobial activity of the ethanolic leaf extract of *Diospyros monbuttensis* was not active against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* at all concentrations that were tested (Table 1). However, the aqueous extracts showed activity at 25mgmL⁻¹, 50mgmL⁻¹, 75mgmL⁻¹, 100mgmL⁻¹ against *S. aureus* and *E. coli* (Table 2). The minimum inhibitory concentration (MIC) of the aqueous extract of *D. monbuttensis* was 0.78mgmL⁻¹ for *S. aureus* and *E. coli*. The results for the phytochemical screening of the leaf of *Diospyros monbuttensis* as shown in Table 3 revealed the presence of saponins, tannins, glycosides and alkaloids.

TABLE 1: ANTIMICROBIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF *DIOSPYROS MONBUTTENSIS* AGAINST SELECTED MICROORGANISMS

Isolates	25mg/ml	50mg/ml	75mg/ml	100mg/ml
<i>Micrococcus luteus</i>	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-

Zone of Inhibition measured in millimetres (mm)

TABLE 2: ANTIMICROBIAL ACTIVITY OF AQUEOUS LEAF EXTRACTS OF *DIOSPYROS MONBUTTENSIS* AGAINST SELECTED MICROORGANISMS

Isolates	25mg/ml	50mg/ml	75mg/ml	100mg/ml
<i>Micrococcus luteus</i>	-	-	-	-
<i>Escherichia coli</i>	15	16	25	25
<i>Staphylococcus aureus</i>	11	12	17	19
<i>Aspergillus niger</i>	-	-	-	-

Zone of Inhibition measured in millimetres (mm)

TABLE 3: PHYTOCHEMICAL SCREENING OF
D. MONBUTTENSIS LEAVES

Phytochemical tests	Result
Saponin	+
Flavonoids	–
Glycosides	+
Terpenoids	–
Tannins	+
Protein	–
Alkaloids	+

Keys: + Positive - Negative

DISCUSSION

The development of microbial resistance to the available antibiotics has informed the need to explore natural disease control options which has led to further investigation of antimicrobial activity of some medicinal plants (8,9). Studies have been carried out to discover useful antibacterial and antifungal compounds from plants (6,10). The results of this study showed that the ethanolic extracts of the plant leaves of *Diospyros monbuttensis* had no antimicrobial activities on the test organisms at all concentrations tested. The aqueous extracts of the plant showed promising antimicrobial activities. These results further validate the usefulness of this medicinal plant in traditional remedies among many tribes in the world in the treatment of bacterial infections.

D. monbuttensis is usually used as an antimalarial (3,11). The phytochemical screening of the plant revealed the presence of Saponins, Glycosides, Tannins and Alkaloids. The antimicrobial activities of this formulation may be due to its phytochemical

constituents as earlier reported for some plants (12). Various studies have demonstrated that the alkaloids present in the leaves have a potential antioxidant property. This antioxidant activity is due to the ability to trap the free radicals and to chelate metal ions (2). Some analgesics and anti-inflammatory activities of this plant has been assigned to tannins. Apart from this, the tannins contribute to healing of wounds (13). Tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes.

Alkaloids and glycosides have a number of biological activities and strong antibacterial potentials. Saponins act by altering the permeability of cell walls and hence exert toxicity on all organized tissues. They exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (14). This property explain the mechanisms of antibacterial action of the aqueous extract of *Diospyros monbuttensis* against *Staphylococcus aureus* and *Escherichia coli*.

CONCLUSION

The results obtained from this study support the use of these plant parts in the traditional treatment of diseases in Nigeria. The results of this finding could be very important to pharmaceutical industries in the development of new antimicrobial drugs in order to address therapeutic needs. The screening of various natural organic compounds and identification of active agents may provide cheaper drugs that will be available and affordable to everyone. Further studies need to be conducted using different solvents, and there is also need for further studies on the plant parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

REFERENCES

- Gbile ZO, Adesina SK. Nigerian flora and its pharmaceutical potential. Journal of ethnopharmacology [Internet]. 1987;19(1):1–16. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3295399>
- Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from edible legumes: chemistry, processing, and health benefits. Journal of medicinal food [Internet]. 2004;7(1):67–78. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15117556>
- Olasehinde GI, Ojurongbe O, Adeyeba AO, Fagade OE, Valecha N, Ayanda IO, et al. In vitro studies on the sensitivity pattern of Plasmodium falciparum to anti-malarial drugs and local herbal extracts. Malaria Journal [Internet]. 2014 Jan [cited 2017 Mar 20];13(1):63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24555525>
- Odelola HA, Okorosobo VI. Preliminary investigation of in-vitro antimicrobial activity of two Nigerian Diospyros species (Ebenaceae). African journal of medicine and medical sciences [Internet]. 1988 Sep;17(3):167–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2845757>
- Adeniyi BA, Ayepola OO. The Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of Eucalyptus camaldulensis and Eucalyptus torrelliana (Myrtaceae). Research Journal of Medicinal Plant [Internet]. 2008 Jan 1;2(1):34–8. Available from: <http://www.scialert.net/abstract/?doi=rjmp.2008.34.38>
- Sofowora A. Medicinal plants and traditional medicine in Africa [Internet]. 2nd ed. Spectrum Books; 1993 [cited 2017 Mar 9]. Available from: https://books.google.co.in/books/about/Medicinal_Plants_and_Traditional_Medicin.html?id=ZdaxQwAACAAJ
- Trease GE, Evans WC. Textbook of pharmacognosy. 12th Editi. London: Tindall

- and Co.; 1983.
8. Prakash NKU, Bhuvaneswa S, Jahnavi B, Abhinaya K, Rajalin AG, Kumar MP, et al. A Study on Antibacterial Activity of Common Weeds in Northern Districts of Tamil Nadu, India. *Research Journal of Medicinal Plant* [Internet]. 2012 Apr 1;6(4):341-5. Available from: <http://www.scialert.net/abstract/?doi=rjmp.2012.341.345>
 9. Singh S, Prasad R, Pathania K, Joshi H. Antifungal activity of Plumbagin & Isodiospyrin from *Diospyros kaki* root bark. *Asian Journal of Plant Science and Research*. 2012;2(1):1-5.
 10. Oluremi BB, Osungunna MO, Ogbole OO. Phytochemical and Antimicrobial Screening of the Leaf Extract of *Diospyros barteri*. *Gurke. Pharmacognosy Journal* [Internet]. 2010 Aug;2(11):405-8. Available from: <http://www.phcogfirst.com/article/340>
 11. Sylvain B, Karim T, Karamoko O, André O T, Souleymane M, Ako A, et al. Phytochemical screening of some medicinal plants used to treat malaria in Côte d'Ivoire (West Africa). *International Journal of Chemistry and Pharmaceutical Sciences*. 2014;2(6):919-25.
 12. Couliadiati TH, Millogo-Ko H, Lamien-Med A, Yougbaré-Z M, Millogo-Ra J, Nacoulma OG. Antioxidant and Antibacterial Activities of Two *Combretum* Species from Burkina Faso. *Research Journal of Medicinal Plant* [Internet]. 2011 Jan 1;5(1):42-53. Available from: <http://www.scialert.net/abstract/?doi=rjmp.2011.42.53>
 13. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*. 2006;5(4):357-61.
 14. Ayyanar M, Subash-Babu P. *Syzygium cumini* (L.) Skeels: a review of its phytochemical constituents and traditional uses. *Asian Pacific journal of tropical biomedicine* [Internet]. 2012 Mar;2(3):240-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23569906>

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X MAY 2018 VOL19 No.2
AJCEM/1813 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.3>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 88-92

SERUM AND SPUTUM SURFACTANTS -A AND -D IN MULTIDRUG-RESISTANT AND DRUG-SENSITIVE TUBERCULOSIS PATIENTS

Onifade, A.A, Edem, V.F and Arinola, O.G*

Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria

*Correspondence: Arinola O.G.

ABSTRACT

Abnormal production and function of surfactants are associated with pulmonary diseases. Also, pulmonary infections alter surfactant metabolism. Due to lack of information on the levels of surfactants A (SP-A) and D (SP-D) in Nigerian tuberculosis (TB) patients, this study assessed these surfactants in both sputum and serum of drug sensitive- and multidrug resistant- TB patients using ELISA. The aim is to explore the diagnostic or therapeutic potential of SP-A and SP-D in TB patients. Also, to find out appropriate sample for the analyses of SP-A and SP-D in TB patients.

The mean serum levels of SP-A and SP-D were not significantly reduced in MDR-TB (n=30) and DS-TB patients (n=30) compared with non-TB apparently healthy controls (n=30) ($p > 0.05$). Mean sputum levels of SP-A and SP-D were significantly reduced in DS-TB patients compared with the levels in MDR-TB patients ($p < 0.05$). These findings suggest that the sputum SP-D and SP-A levels but not serum SP-D and SP-A levels are useful indicators of the disease activity in pulmonary TB patients.

Keywords: Tuberculosis, Surfactants, Diagnosis, Nigeria

LES TAUX SÉRIQUES D'EXPECTORATIONS ET SANS TENSIOACTIFS- A ET D DANS LA TUBERCULOSE MULTIRÉSISTANTE ET LA TUBERCULOSE CHEZ LES PATIENTS SENSIBLES

Onifade, A.A, Edem, VF et Arinola, O.G*

Département de pathologie chimique, Collège de médecine, Université d'Ibadan, Nigeria

*Correspondance : Arinola O.G.

Résumé

production anormale et la fonction d'agents tensio-actifs sont associés à des maladies pulmonaires. En outre, les infections pulmonaires modifier le métabolisme du surfactant. En raison du manque d'informations sur les niveaux d'agents de surface A (SP-A) et D (SP-D) in Nigerian la tuberculose (TB) patients, cette étude a évalué ces surfactants dans les expectorations et le sérum de la multirésistance et sensible- patients atteints de tuberculose à l'aide d'ELISA. L'objectif est d'explorer les possibilités diagnostiques ou thérapeutiques de SP-A et SP-D dans les patients atteints de tuberculose. Aussi, nous avons voulu en savoir échantillon approprié pour l'analyse de SP-A et SP-D dans les patients atteints de tuberculose.

Les niveaux sériques de SP-A et SP-D n'ont pas été significativement réduite dans la TB-MR (n =30) et DS-TB patients (n =30) par rapport aux non-contrôles apparemment en bonne santé (n =30) ($p > 0,05$). Les niveaux d'expectorations de SP-A et SP-D ont été significativement réduits dans DS-tuberculeux par rapport aux niveaux de la multirésistance patients ($p < 0,05$). Ces résultats suggèrent que l'expectoration SP-D et SP-A mais pas la SP-D et SP-A sont des indicateurs utiles à l'activité de la maladie chez les patients atteints de tuberculose pulmonaire.

Mots clés : Tuberculose, surfactants, le diagnostic, le Nigeria

INTRODUCTION

Mycobacterium tuberculosis (*M.tb*) is a facultative intracellular pathogen that infects humans causing tuberculosis (1, 2). Inhalation of infectious aerosols is the common route of transmission and it is finally deposited in the terminal bronchioles and alveoli of the lungs (3). At this early stage of infection, interactions of *M.tb* with the innate immune environment of the lung have been proposed to determine the outcome and progression of *M.tb* infection (2). However, such interactions are

incompletely known. One of the first interactions of *M.tb* in the lung is the binding is pulmonary surfactant molecules, which are multi-molecular complexes composed of lipids and proteins secreted by alveolar type II cells and Clara cells in distal bronchioles (4, 5).

Four specific surfactant proteins have been identified viz: surfactant protein SP-A, SP-B, SP-C and SP-D. SP-B and SP-C have been characterized as hydrophobic polypeptides that enhance the adsorption of lipid to the surface of the alveoli (6).

On the other hand SP-A and SP-D are hydrophilic and participate in the innate host defense immune system. SP-D binds to macrophages and neutrophils and promotes phagocytosis and killing of bacteria, fungi, and viruses (7). SP-A and SP-D lines the lungs and are essential for pulmonary function by reducing surface tension (5-7). SP-A and SP-D also play important roles in innate immune responses to a wide range of respiratory pathogens including *M.tb* (8-15). Additionally, it has been shown that pulmonary SP-D contributes significantly to host control of infections by the parasitic helminth *Nippostrongylus brasiliensis* (16).

Binding of pathogens by SP-A and SP-D trigger various immune responses, including opsonisation leading to enhanced phagocytosis and killing by recruited macrophages and neutrophils via oxidative mechanisms, aggregation of pathogens thereby hindering their entry into host cells, and direct microbicidal activities by increasing cellular membrane permeability (17). SPs also assist in the clearance of apoptotic cells and in modulating inflammation (18). The interaction of SPs with immune cells to initiate clearance mechanisms is mediated by SP's collagen region with a number of proposed receptor molecules on these cells (19).

Considering the intricate relationship between SP-A and SP-D with innate immune cells necessary to control respiratory pathogens, the present study thus determined both serum and sputum levels of SP-A and SP-D in DS-TB and MDR-TB, which were not previously studied among Nigerian TB patients.

MATERIALS AND METHODS

A total of 90 participants were enrolled for this study. This comprised of thirty (30) multi-drug resistant TB (MDR-TB) patients, thirty (30) drug-sensitive TB (DS-TB) patients and thirty (30) non-TB apparently healthy controls. MDR-TB patients had been previously diagnosed as being infected with isoniazid and rifampicin resistant strains of *Mycobacterium tuberculosis* (Mtb) using clinical history, Chest X-ray and GENE Xpert. These patients were admitted into the MDR-TB centre,

University College Hospital (UCH) Ibadan, Nigeria for anti-TB treatment. DS-TB patients were recruited from the Medicine Out-patient Clinic, University College Hospital, Ibadan, Nigeria by a Consultant Chest Physician after confirmation with Microbiological test (sputum smear microscopy), chest X-ray and clinical history. Five milliliters (5 ml) of blood was drawn from the antecubital vein of each participant and dispensed into sterile plain sample tubes without anticoagulant. Blood was centrifuged at 1000g for 15mins and serum was obtained. Serum was stored at -20°C until analysis. Spot collection of sputum sample was carried out. Study participants were asked to rinse their mouth with water to reduce salivary contamination of sputum. Sputum produced was collected into 20ml sterile disposable polypropylene tubes and kept on ice from collection to arrival at the laboratory. Sputum sample was homogenized with an equal volume of phosphate buffered saline (PBS). Homogenate was centrifuged at 10,000g for 10 minutes to remove cellular debris and mucus, and supernatant obtained was stored at -20°C until analysis. Enzyme Linked Immunosorbent Assay (ELISA) method was used for the determination of serum and sputum concentrations of surfactant-associated protein A (SP-A) and Surfactant-associated protein D (SP-D) as previously carried out (20).

RESULTS

As shown in the Table, the mean serum levels of SP-A were not significantly reduced in MDR-TB patients and DS-TB patients compared with control while the mean serum levels of SP-D were not significantly raised in MDR-TB patients and DS-TB patients compared with control. The mean sputum levels of SP-A was significantly reduced in DS-TB patients compared with MDR-TB patients and control while the mean sputum levels of SP-D were significantly raised in DS-TB patients and MDR-TB patients compared with control. Also, the mean sputum level of SP-D was significantly raised in MDR-TB patients compared with DS-TB patients.

TABLE 1: COMPARISON OF MEAN LEVELS OF SPUTUM AND SERUM SP-A (NG/L) AND SP-D (PG/ML) IN MDR-TB, DS-TB AND CONTROL

Variables	MDR-TB	DS-TB	Control	p'	p''	p'''
Serum SP-A	306.90±133.32	344.66±139.87	368.72±210.37	0.361	0.725	0.455
Sputum SP-A	503.22±170.25	312.03±187.98	570.15±175.43	0.326	0.001*	0.007*
Serum SP-D	1709.38±665.56	1701.31±596.96	1547.34±324.58	0.448	0.430	0.972
Sputum SP-D	3596.45±636.45	2600.0±1085.04	1210.57±457.39	0.000*	0.000*	0.005*

p' MDR-TB compared with control; p'' DS-TB compared with control; p''' MDR-TB compared with DS-TB

*Significant at p<0.05

DISCUSSION

Tuberculosis which claims at least 3 million lives annually is caused by *M.tb* (1, 2). The interaction between *M.tb* and the pulmonary lining represents the initial contact of the bacterium with the host immune system (1-3), thus the factors which affect phagocytosis of *M.tb* by host immune cells are important to our understanding of disease pathogenesis. Surfactant and its associated proteins, in conjunction with alveolar macrophages, are important components of the pulmonary alveoli. Surfactant proteins (SP-A, SP-B, SP-C, and SP-D) are enriched with a relatively unique phospholipid (dipalmitoylphosphatidylcholine) (3, 4). The main functions of surfactant are lowering surface tension at the air-liquid interface and thus preventing alveolar collapse at end-expiration, interacting with and subsequent killing of pathogens or preventing their dissemination and modulating immune responses (5-7). Two major surfactant associated proteins, surfactant protein A (SP-A) and surfactant protein D (SP-D), contain carbohydrate recognition domains (CRDs) (3-7).

SP-A binds with lipopolysaccharides and peptidoglycan derived from *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Legionella pneumophila*, *Staphylococcus aureus* and *Streptococcus pneumoniae* as well as *Mycobacterium avium*, *Mycobacterium tuberculosis* and *Mycoplasma pneumoniae* which consequently result in agglutination, enhancement of pathogen uptake, and growth inhibition (8-15). The metabolism of SP-A in TB patients considered for this study might account for reduced levels of SP-A especially in the sputum which is a transmission medium for *M.tb*. This may be to agglutinate *M.tb* in local environment. This hypothesis is supported by a previous study (21) which reported that human SP-A enhances the uptake of the virulent Erdman strain of *M.tb* by human monocyte-derived macrophages and human alveolar macrophages through direct interaction with the macrophages. The study thus concluded that SP-A was reported to play a role in the pathogenesis of tuberculosis by enhancing the ability of *M.tb* to enter and survive within its local niche in the host. Another data suggested that SP-A has pleiotropic effects even at the low concentrations found in tuberculosis patients (22). SP-A was reported to augment inflammation in the presence of infection and inhibits inflammation in uninfected macrophages so as to protect uninvolved lung segments from the deleterious effects of inflammation (23). This further support reduced level of SP-A in TB patients as a result of continuous metabolism.

SP-D is synthesized and secreted into the airspaces of the lung by the respiratory epithelium (4, 5). At the alveolar level, SP-D is constitutively synthesized and secreted by alveolar type II cells. More

proximally in the lung, SP-D is secreted by a subset of bronchiolar epithelial cells, the non-ciliated Clara cells. Because SP-D is stored within the secretory granules of Clara cells (6, 9), it seems likely that SP-D is subject to regulated secretion via granule exocytosis at this level of the respiratory tract. In some species, SP-D is also synthesized by epithelial cells and/or submucosal glands associated with the bronchi and trachea (6). This might explain significantly raised sputum levels of SP-D especially in TB patients. SP-D has been reported to agglutinate bacteria, delay phagocytosis and facilitate phagolysosomal fusion (10-12). This explains why SP-D was higher in MDR-TB patients than DS-TB patients considered for this study.

The opposing trends of sputum SP-A (reduced level) and sputum SP-D (raised level) observed in TB patients considered for our study might be related to slight differences in their chemical composition and functions. SP-A preferentially binds monosaccharides and lipid ligands, while SP-D additionally binds disaccharides, complex carbohydrates and anionic phospholipids present on cell surfaces (18). This might be explained by subtle structural differences between the CRDs of trimeric SP-A and SP-D resulting in the flatter and more hydrophobic surface of SP-A preferentially binding to less polar substrates, while the more hydrophilic surface of SP-D displays a higher affinity for highly polar targets (24). Moreover, in SP-A, the Gly-X-Y repeats in the collagenous region are interrupted resulting in a kink (and hence the bunch-like structure) and a relatively smaller distance to the distal CRD compared to SP-D which displays more freedom to bind and aggregate target pathogens (25).

In the case of *M. tuberculosis*, SP-D has been shown to bind mannosylated lipoarabinomannan (ManLam) from the Erdman strain (26), while SP-A has been shown to bind a wider range of mycobacterial targets, including ManLam (from virulent and avirulent mycobacterial strains) (27), lipomannan (28), a 60-kDa glycoprotein (29), and the *M. tuberculosis* surface glycoprotein Apa (30). In addition to their carbohydrate specificities, SP-A and SP-D was also reported to differ with respect to several other structural features that determine their interactions with the innate immune system. SP-A forms a bouquet-like 18-mer that associates with surfactant lipids and tubular myosin and can bind to the C1qR receptor, toll-like receptors TLR2 and TLR4, the CD91/calreticulin complex, the signal inhibitory regulatory protein SIRP- α , and the unconventional myosin XVIII α receptor SPR210. SP-D, on the other hand, forms a cross-like dodecamer (12 chains) that resides in the aqueous phase of the alveoli and can bind microfibril-associated protein 4, CD14, defensins, decorin, C1q, TLR2, TLR4, and a 340-kDa glycoprotein of unknown function (31).

Exploration of surfactant A therapeutic potential is proposed by the present authors. Animal study and limited human data show that exogenous surfactant decreases DNA synthesis of inflammatory mediators (32, 33). Intratracheal administration of a surfactant-amikacin mixture to rats with *Pseudomonas pneumonia* showed improved anti-inflammatory effects compared with amikacin alone (33). There is also evidence that certain pharmacologic agents may enhance endogenous surfactant levels, although the current data are limited. Antenatal steroids accelerate development

of type 2 pneumocytes and thus increase the production of surfactant proteins and enzymes necessary for phospholipid synthesis. Corticosteroids also induce pulmonary β -receptors, which play a role in surfactant release and alveolar fluid absorption when stimulated (34). These observations suggest the possibility that surfactant or its stimulants be used to modulate immune responses during inflammatory lung disease like tuberculosis, but further studies are necessary.

REFERENCES

1. Flynn, J. L., and J. Chan. 2001. Immunology of tuberculosis. *Annu. Rev. Immunol.* 19:93-129.
2. North, R. J., and Y. J. Jung. Immunity to tuberculosis. *Annu. Rev. Immunol.* 2004; 22:599-623.
3. Nicas, M., W. W. Nazaroff, and A. Hubbard. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *J. Occup. Environ. Hyg.* 2005; 2:143-154.
4. Voorhout WF, Veenendaal T, Kuroki Y, Ogasawara Y, van Golde LM, Geuze HJ. Immunocytochemical localization of surfactant protein D (SP-D) in type II cells, Clara cells, and alveolar macrophages of rat lung. *J Histochem Cytochem.* 1992;40:1589-1597.
5. Kalina M, Mason RJ, Shannon JM. Surfactant protein C is expressed in alveolar type II cells but not in Clara cells of rat lung. *Am J Respir Cell Mol Biol.* 1992;6:594-600.
6. Crouch E, Wright JR. Surfactant proteins A and D and pulmonary host defense. *Annu Rev Physiol.* 2001;63:521-554.
7. Crouch E, Hartshorn K, Ofek I. Collectins and pulmonary innate immunity. *Immunol Rev.* 2000;173:52-65.
8. Hartshorn KL, White MR, Shepherd V, Reid K, Jensenius JC, Crouch EC: Mechanisms of anti-influenza activity of surfactant proteins A and D: comparison with serum collectins. *Am J Physiol* 1997;273:L1156-L1166.
9. LeVine AM, Elliott J, Whitsett JA, Srikiatkachorn A, Crouch E, DeSilva N, Korfhagen T: Surfactant protein-D enhances phagocytosis and pulmonary clearance of respiratory syncytial virus. *Am J Respir Cell Mol Biol* 2004;31:193-199.
10. LeVine AM, Whitsett JA, Gwozdz JA, Richardson TR, Fisher JH, Burhans MS, Korfhagen TR: Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. *J Immunol* (Baltimore, Md: 1950) 2000;165:3934-3940.
11. LeVine AM, Whitsett JA, Hartshorn KL, Crouch EC, Korfhagen TR: Surfactant protein D enhances clearance of influenza A virus from the lung in vivo. *J Immunol* (Baltimore, Md: 1950) 2001;167:5868-5873.
12. Ferguson JS, Martin JL, Azad AK, McCarthy TR, Kang PB, Voelker DR, Crouch EC, Schlesinger LS: Surfactant protein D increases fusion of Mycobacterium tuberculosis-containing phagosomes with lysosomes in human macrophages. *Infect Immun* 2006;74:7005-7009.
13. Gaynor CD, McCormack FX, Voelker DR, McGowan SE, Schlesinger LS: Pulmonary surfactant protein A mediates enhanced phagocytosis of Mycobacterium tuberculosis by a direct interaction with human macrophages. *J Immunol* (Baltimore, Md: 1950) 1995;155:5343-5351.
14. Ghildyal R, Hartley C, Varrasso A, Meanger J, Voelker DR, Anders EM, Mills J: Surfactant protein A binds to the fusion glycoprotein of respiratory syncytial virus and neutralizes virion infectivity. *J Infect Dis* 1999;180:2009-2013.
15. Madan T, Eggleton P, Kishore U, Strong P, Aggrawal SS, Sarma PU, Reid KB: Binding of pulmonary surfactant proteins A and D to *Aspergillus fumigatus* conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. *Infect Immun* 1997;65:3171-3179.
16. Thawer S, Auret J, Schnoeller C, Chetty A, Smith K, Darby M, Roberts L, Mackay RM, Whitwell HJ, Timms JF, Madsen J, Selkirk ME, Brombacher F, Clark HW, Horsnell WG: Surfactant protein-D is essential for immunity to helminth infection. *PLoS Pathogens* 2016;12:e1005461.
17. Wright JR: Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 2005;5:58-68.

18. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, Bernal AL, Reid KB, Madan T, Chakraborty T: Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol Immunol* 2006;43:1293-1315.
19. Jäkel A, Qaseem AS, Kishore U, Sim RB: Ligands and receptors of lung surfactant proteins SP-A and SP-D. *Front Biosci (Landmark Ed)* 2013;18:1129-1140.
20. Ige O, Edem V.F and **Arinola O.G.** Plasma adenosine deaminase enzyme reduces with treatment of pulmonary tuberculosis in Nigerian patients: Indication for diagnosis and treatment monitoring. *Nigerian Journal Of Physiological Science*. 2016; 31(1): 49-53.
21. Manabe YC, Dannenberg Jr. AM, Tyagi, S.K, Hatem C.L, Yoder M, Woolwine SC, Zook BC, Pitt ML and Bishai WR. Different Strains of Mycobacterium tuberculosis Cause Various Spectrums of Disease in the Rabbit Model of Tuberculosis. *Infect Immun*. 2003; 71(10): 6004-6011.
22. Lin Z, deMello D, Phelps DS, Koltun WA, Page M, Floros J: Both human SP-A1 and Sp-A2 genes are expressed in small and large intestine. *Pediatr Pathol Mol Med* 2001;20:367-386.
23. Jeffrey A. Gold, Yoshihiko Hoshino, Naohiko Tanaka, William N. Rom, Bindu Raju, Rany Condos and Michael D. Weiden. Surfactant Protein A Modulates the Inflammatory Response in Macrophages during Tuberculosis. *Infect. Immun*. February 2004 vol. 72 no. 2 645-650
24. Head JF, Mealy TR, McCormack FX, Seaton BA: Crystal structure of trimeric carbohydrate recognition and neck domains of surfactant protein A. *J Biol Chem* 2003;278:43254-43260.
25. Zhang P, McAlinden A, Li S, Schumacher T, Wang H, Hu S, Sandell L, Crouch E: The amino-terminal heptad repeats of the coiled-coil neck domain of pulmonary surfactant protein D are necessary for the assembly of trimeric subunits and dodecamers. *J Biol Chem* 2001;276:19862-19870.
26. Hawgood, S., et al. Sequential targeted deficiency of SP-A and -D leads to progressive alveolar lipoproteinosis and emphysema. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2002; 283:L1002-L1010.
27. Hall-Stoodley, L., et al. Mycobacterium tuberculosis binding to human surfactant proteins A and D, fibronectin, and small airway epithelial cells under shear conditions. *Infect. Immun.* 2006; 74:3587-3596.
28. Sidobre, S., J. Nigou, G. Puzo, and M. Riviere. Lipoglycans are putative ligands for the human pulmonary surfactant protein A attachment to mycobacteria. Critical role of the lipids for lectin-carbohydrate recognition. *J. Biol. Chem.* 2007; 275:2415-2422.
29. Pasula, R., et al. Surfactant protein A (SP-A) mediates attachment of Mycobacterium tuberculosis to murine alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 1997. 17:209-217.
30. Ragas, A., L. Roussel, G. Puzo, and M. Riviere. The Mycobacterium tuberculosis cell-surface glycoprotein apa as a potential adhesin to colonize target cells via the innate immune system pulmonary C-type lectin surfactant protein A. *J. Biol. Chem.* 2007; 282:5133-5142.
31. Torrelles, J. B., A. K. Azad, L. N. Henning, T. K. Carlson, and L. S. Schlesinger. Role of C-type lectins in mycobacterial infections. *Curr. Drug Targets*. 2008; 9:102-112.
32. Antal JM, Divis LT, Erzurum SC, Wiedemann HP, Thomassen MJ. Surfactant suppresses NF-kappa B activation in human monocytic cells. *Am J Respir Cell Mol Biol* 1996;14:374-379.
33. Birkun AA, Kubyshkin AV, Novikov NY, Krivorutchenko YL, Fedosov MI, Postnikova ON, Snitser AA. Joint intratracheal surfactant-antibacterial therapy in experimental Pseudomonas-induced pneumonia. *J Aerosol Med Pulm Drug Deliv* [online ahead of print] 17 Dec 2014; DOI:10.1089/jamp.2014.1161
34. Ballard PL, Ballard RA. Scientific basis and therapeutic regimens for use of antenatal glucocorticoids. *Am J Obstet Gynecol* 1995;173:254-262.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

MAY 2018

ISBN 1595-689X VOL19 No.2

AJCEM/1814

<http://www.ajol.info/journals/ajcem>

COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.4>

AFR. J. CLN. EXPER. MICROBIOL. 19 (2):93-103

USE OF STOOL CULTURE AS A NON INVASIVE METHOD FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* FROM STOOL OF DIARRHOEIC CHILDREN IN WESTERN NIGERIA

Oyedepi¹, K. S., Odeniyi², O. M., Ola-Bello², O.I., Ushie², S., Mustapha³, A.A. Ogunbase³, O. A.

1. Department of Medical Laboratory Science, College of Medicine, University of Lagos, Nigeria; 2. Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria; 3. Department of Paediatrics, Mother and Child Centre, General Hospital, Gbaja- Surulere, Lagos, Nigeria.

Corresponding author: K.S .Oyedepi; Tel: +2348066117865; kolaremi903@gmail.com

ABSTRACT

Background: *Helicobacter pylori* has been associated with chronic diarrhoea, iron deficiency anaemia, growth retardation, gastric malignancies, peptic ulcer disease, and gastritis among children. Diagnosis of this infection has been invasive using biopsies while stool culture is not common or routinely practiced. This study was designed to detect and isolate *H.pylori* from stool of diarrhoeic children and highlight possible use of such for routine laboratory diagnosis of *H.pylori* infections.

Material and methods: Two hundred and sixty faecal samples obtained from diarrhoeic children were screened for *H. pylori* antigen, using *H. pylori* stool antigen test kit (HpSA) and cultured on modified DENTS medium. Cholestyramine and nitrobluetetrazolium salt were added to the stool and the medium respectively to aid isolation of *H. pylori*. Correlation of BMI and *H. pylori* infection of the children was also evaluated.

Results: Twenty-six (10%) samples showed growth on culture while 91 (35%) tested positive for *H. pylori* antigen. Of the 26 children with positive culture, 16 had a low BMI. HpSA has sensitivity and specificity of 11.5% and 62.4%. There was a significant association ($\chi^2 = 12.86$, $df=2$, $P\text{-value} = 0.004$) between age group of participants and use of HpSA kit.

Conclusion: Stool culture for recovery of *H. pylori* is feasible in our environment and diarrhoeic children should be screened for *H. pylori* using both HpSA and culture. *H. pylori* is suggested to be screened routinely especially among children having diarrhoea and are underweight. Albeit, other causes should be eliminated before concluding on the reason for the underweight.

Keywords: *Helicobacter pylori*, stool culture, HpSA, BMI, Cholestyramine, Nitrobluetetrazolium salt

L'UTILISATION DE CULTURE DES SELLES COMME UNE MÉTHODE NON INVASIVE POUR LE DIAGNOSTIC DE L'*HELICOBACTER PYLORI* DANS LES SELLES D'ENFANTS DIARRHÉIQUES DANS L'OUEST DU NIGÉRIA

Oyedepi¹, K. S., Odeniyi², O. M., Ola-Bello², O.I., Ushie², S., Mustapha³, A.A. Ogunbase³, O. A .

1. Ministère de la science de laboratoire médical, Collège de médecine, Université de Lagos, Nigeria;
2. Département de microbiologie médicale et de parasitologie, Faculté de médecine de l'Université de Lagos, Nigeria; 3. Département de pédiatrie, Centre de la mère et de l'enfant, Hôpital Général, Gbaja- Surulere, Lagos, Nigeria.

Correspondence: K.S .Oyedepi ; Tel : 2348066117865 ; kolaremi903@gmail.com

RÉSUMÉ

Contexte : *Helicobacter pylori* a été associée à la diarrhée chronique, l'anémie, un retard de croissance, de cancers gastriques, ulcère gastro-duodénal, gastrite et chez les enfants. Le diagnostic de cette infection ont été à l'aide de biopsies invasives tout en culture des selles n'est pas commune ou pratique courante. Cette étude a été conçue pour détecter et isoler *H. pylori* dans les selles d'enfants diarrhéiques et mettre en évidence l'utilisation possible d'une telle routine de diagnostic en laboratoire des infections à *H. pylori*.

Matériel et méthodes : Deux cent soixante échantillons de selles diarrhéiques obtenues à partir d'enfants ont été examinés pour l'antigène d'*H. pylori*, à l'aide de *H. pylori* antigènes selles test kit (HpSA) et cultivées sur milieu modifié des bosse

Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY s.

La cholestyramine et nitrobluetetrazolium ont été ajoutés à la selle et le support de l'isolement à l'aide respectivement de *H. pylori*. Corrélation entre l'IMC et l'infection à *H. pylori* des enfants a également été évaluée.

Résultats : Vingt-six (10 %) échantillons montrent une croissance de la culture tandis que 91 (35 %) ont été testés positifs pour *H. pylori* antigène. Des 26 enfants avec culture positive, 16 avaient un faible IMC. HpSA a la sensibilité et la spécificité de 11,5 % et 62,4 %. Il y avait une association significative ($\chi^2 = 12,86$, $dl = 2$, $P\text{-value} = 0,004$) entre le groupe d'âge des participants et l'utilisation de kit HpSA.

Conclusion : culture des selles pour récupération de *H. pylori* est possible dans notre environnement et les enfants diarrhéiques devraient être examinés pour *H. pylori* en utilisant à la fois les HpSA et de la culture. *H. pylori* est suggéré pour être contrôlés régulièrement en particulier chez les enfants ayant la diarrhée et une insuffisance pondérale. Bien que d'autres co-fondateurs doivent être éliminés avant de conclure sur la raison de l'insuffisance pondérale.

Mots clés: *Helicobacter pylori*, culture des selles, HpSA, BMI, la cholestyramine, sel de Nitrobluetetrazolium

INTRODUCTION

Helicobacter pylori is a motile, microaerophilic Gram negative curved rod bacterium that inhabits the gastric mucosa of human stomach. and has been recognized as class 1 carcinogen [1]. The demonstration of its involvement in gastro duodenal pathologies has basically changed the perception of people about the disease [2]. Infection with the organism causes peptic ulcers, gastritis, duodenitis and gastric cancers [3,4,5]. It has been reported that more than half of the world's population are infected with the organism in both developed and developing countries [5]. Factors that influence the acquisition of *H. pylori* in childhood is basically overcrowding and the socio - economic condition of the parents [6-8]. The mode of transmission of childhood infection has been found to be common among parents who premasticate food for their children [9]. Most of the available evidence supports person-to-person transmission by faecal-oral, oral-to-oral and gastric-to-oral routes [9-12]. In children, gastric inflammation could cause low gastric secretion resulting in impaired "gastric barrier" associated with increased susceptibility to enteric infections, which is a major public health concern linked to diarrhoea, malnutrition and growth retardation in developing countries [13,14]. *H. pylori* infection can be transmitted orally through faecal matter originating from ingestion of waste-tainted food or water [15]. It is also possible that *H. pylori* could be transmitted from the stomach to the mouth through belching or gastro-oesophageal reflux, with common symptoms of gastritis, when small amount of the stomach's contents is involuntarily forced up to the oesophagus. The bacterium could then be transmitted through oral contact [16]. More than half of the world's population is infected with *H. pylori*, which is acquired almost always within the first 5 years of life [17]. The possible routes are faeco-oral, oral-oral and gastro-oral [16,18]. Thomas and associates, were able to isolate *H. pylori* DNA and also culture *H. pylori* from human faeces thereby, suggesting faeco-oral transmission [19]. Oral transmission has also been proven because

H. pylori was isolated from dental plaques and saliva [20].

The prevalence of the disease varies depending on the method of diagnosis. Using serology 15% and 46% prevalence rates were recorded among Gambian children aged less than 20 months and 40-60 months respectively [21] and 45% among Indian children [22]. In Bolivia and Alaska, the seroprevalence were 70% and 69% among the 9 years old respectively [22]. While, the seroprevalence in preschoolers in Brazil was found to be 69.7% [23].

In developing countries, studies have suggested that, *H. pylori* may be associated with chronic diarrhoea especially among children [21] and that *H. pylori* at a young age may induce hypochlorhydria which interferes with the normal acidic barrier in the stomach. However more works need to be done among children in order to determine whether eradication of *H. pylori* reduces the prevalence of chronic diarrhoea [21]. Low socioeconomic status and overcrowding are some of the predisposing factors to the infection [24].

Diagnosis of *H. pylori* are divided into invasive and noninvasive techniques [25,26]. Invasive tests need an Upper Gastrointestinal Endoscopy (UGIE), while the noninvasive techniques do not require endoscopy, but involve methods that make use of stool using kits such as HpSA, stool culture and Urea Breath Tests (UBT) [27]. Generally, biopsy cannot be justified, especially in children, unless one wishes to isolate the organism for antibiotic sensitivity testing or there is a clear clinical indication for UGIE [28]. Moreover, if one opted to test for *H. pylori* by biopsies with UGIE, it requires specimen from multiple regions of the stomach, and this may be too stressful for the child. However, a noninvasive and diagnostic tool for detecting *H. pylori* infection is more desirable in pediatrics, because the upper gastrointestinal endoscopies in young children are usually performed in intubation anesthesia or conscious sedation [29] and paediatric endoscopy is very costly and not common [28,30,31]. Other non-invasive methods such as Urea Breath Test (UBT) are expensive, difficult to administer in young children and not available in all

countries though it is as reliable as the invasive methods [13]. Despite the fact that recovery of *H. pylori* from stool culture is laborious, difficult, hence not usually practiced [32] coupled with the fact that culturing of this fastidious organism is relatively difficult, expensive and needs special media for growth; culture still remains the Gold standard [33]. The culture of *H. pylori* has two major advantages: Firstly, it allows antimicrobial susceptibility testing and secondly, the isolates that are obtained by culture can be further studied for its characteristics [25, 34].

During culture of *H. pylori*, patients are advised to stay off antibiotics because it is often more difficult to isolate the organism [35]. The addition of Nitroblue tetrazolium (NBT) salts to Columbia agar base (Oxoid) and horse serum (Oxoid), aid in the identification of *H. pylori* colonies cultured on agar media [25,35] while the addition of cholestyramine to the stool help in dissolving the bile in the stool to aid the isolation of *H.pylori* if present[36] .

In Nigeria, the first work done on *H.pylori* was by Coker and Akande [37] and it employed invasive method using biopsy. However, the current trend employed in detection and isolation of *H. pylori* infection is to move from an invasive diagnostic methods to non-invasive method[38] . *H. pylori* stool antigen (HpSA) test is non-invasive method which only detects the antigen in the faecal sample, and does not include the isolation of the organism[39] The limitation to the use of this method is that antibiotics susceptibility testing cannot be done without isolation of the organism. Hence culture of the organism from the faecal sample needs to be established in routine practice to enable effective management of the infection. This study was therefore designed to detect and isolate *H. pylori* from stool of diarrhoeic children in Lagos using HpSA test and culture respectively.

MATERIALS AND METHODS

Sampling Technique:

The stool samples were collected with a sterile bottle, wide open with attached screw-capped spoon plastic universal containers. A total of 260 stool samples were obtained from diarrhoeic children attending Paediatric Clinics in some health facilities in Lagos metropolis. Consent and assents from parent, guardian and children was sort for respectively depending on age. The parents helped in filling the questionnaires, detailing the weights and heights of the children to enable calculation of their Body Mass Index (BMIs), using the BMI Percentile graph. There was no criterion for hospital selection as samples were collected with only the prognosis of diarrhoea. The inclusion criteria were children within the age range of ≤ 1 to 16 years having diarrhoea and whose

parents consented and the child assented as the case may be before recruitment into the study. Children who have been on antibiotics for two weeks prior to sample collection were excluded.

Sample Processing: The stool samples were processed using detection method- *H.pylori* stool antigen (HpSA) test kit (SD^{BIOLINE} *H.pylori* Ag, Germany) and culture method on modified Dent's medium. The stool antigen assay was carried out according to the manufacturer's instructions. Three mls of the assay diluents was transferred into the desired collection tube for use and one gram of faecal sample was emulsified into it using sample collection swab stick provided. The swab was swirled for at least 10 times. This collection swab was discarded after squeezing it against the wall of the collection tube. The resulting suspension was allowed to stand for 5 minutes., then three drops of the suspended supernatant was added into the sample well(s) of the cassette test device and the assay was left to run for fifteen minutes. Appearance of two test lines i.e. "C" and "T" which are the Control and Test lines in the result window, indicates a POSITIVE RESULT. While one test line "C", NEGATIVE RESULT.

Stool culture

The stool sample was emulsified in phosphate buffered saline and 1gram of cholestyramine was added to the suspension[40]. This is to dissolve the bile in the stool. The emulsion was filtered using sterile muslin cloth to remove stool debris. Filtrate further filtered using membrane filter of pore size $0.45\ \mu\text{m}$ as it is expected to retain *H. pylori* if present in the stool. The membrane filter was now cultured for a period of 3 to 12days in a microaerophilic atmosphere (5% O₂, 10%CO₂ and 85% N₂) using the anaerogen gas pak (Oxoid-England) at 37°C on modified Dent's medium (Oxoid, England) [41,42] into which Nitroblue tetrazolium salt (NBT) was added[43] , the latter was supposed to aid the appearance of *H. pylori* on the culture plate, if there is any growth [41] . Plates were checked intermittently for sub culture after the first 3 days through to the 12th day before discarded as no growth. Colonies appearing very tiny, pin head size, with a shining grey features were sub cultured for further testing to characterize *H. pylori*. The isolates were Gram negative spiral rods and produce urease, oxidase, and catalase enzymes during preliminary characteristic reactions.

Antibiotics Susceptibility

The antibiotic susceptibility testing was done using the modified Kirby Bauer [44] technique using the disk diffusion method. Two to three colonies of the

Testing:

H. pylori isolates were emulsified with physiological saline dispensed into sterile universal container. This suspension was standardised to 0.5 MacFarland standard. Isosensitest agar (Oxoid- England) was employed for this test with the addition of 10% laked horse blood (Oxoid-England). This has been reported to be suitable for carrying out antibiotic susceptibility testing on *Helicobacter pylori* isolates [45]. The antibiotics used were; Metronidazole, Ciprofloxacin, Clarithromycin, Amoxicillin, Erythromycin, Gentamicin with a herbal extract usually used for peptic ulcer. The seeded plates were incubated under microaerophilic condition overnight at 37°C [44,49].

Calculation of the Body Mass Index

The Body Mass Index of the participants were calculated by taking the weights (kg) against heights in squared (m²). The BMIs were extrapolated from BMI Percentile graph (Children BMI scale) for the actual body mass. These were classified into the categories of normal weight, low weight, overweight or obese depending on the BMI of the participants [46] (Figure 1).

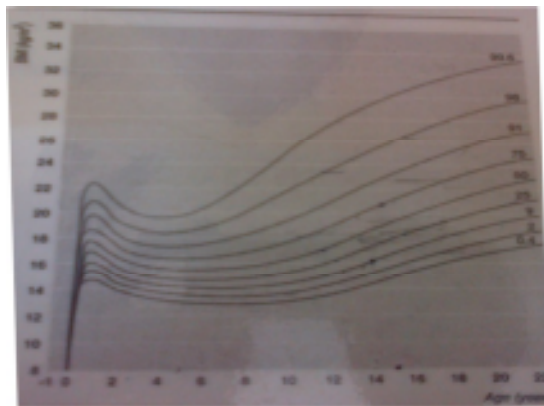


FIGURE1: BMI PERCENTILE GRAPH FOR CHILDREN
BMI PERCENTILE GUIDE (Percentile)
 Underweight: Below 5; Normal weight: 5 – 85; Overweight: 86 – 96; Obese: 97- 99

Questionnaire Analysis

Information regarding the predisposing factors to *H.pylori* infection among the population studied was collected through the questionnaires administered to the parents or guardian of the participants. The questionnaires were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 to get the information concerning the possible predisposing factors to *H. pylori* infection.

Ethical Considerations: The ethical approval for this study was obtained from Lagos University Teaching Hospital Health Research Ethics Committee. The consent of the participants was sought before recruitment into the study while confidentiality of all participants' information was maintained.

RESULTS

Socio-demographic characteristics of participants:

Of the 260 participants recruited for this study, 143 (55.0%) were males and 117 (45.0%) were females, with a male: female ratio 1: 1.2. The age ranges between 27 days to 16.1 years while the mean age and standard deviation (SD) are 1.45 ± 0.498 years (Table 1).

Frequency distribution of detection and isolation of *H. pylori* from stools of diarrhoeic children:

H. pylori antigen was detected in 91 (35.0%) participants using HpSA test kit while the organism was isolated from 26 (10%) of the stool samples using culture (the Gold standard). There was no statistical significant relationship ($\chi^2 = 6.98$, $df = 1$, $P\text{-value} = 0.085$) between the use of culture method and HpSA in diagnosing *H. pylori* infection from diarrhoea stool samples in this study (Table 2).

Comparison of performance characteristics of HpSA test kit and culture

The performance characteristics for HpSA show a sensitivity of 11.5%, specificity of 62.4% while the positive predictive value and negative predictive value were recorded as 3.3% and 86.4% respectively (Table 3).

Correlation of *H. pylori* infection with Body Mass Index (BMI) of the studied participants

Of the 26 (10%) *H. pylori* positive stool samples, 15 (57.7%) were underweight (< 5 percentile), 8 (30.8%) had a healthy weights (5 – 85 percentile) while 3 (11.5%) were obese based on the percentile of 96 – 99 (Figure 2).

Correlation of *H. pylori* antigen detection with Body Mass Index (BMI) of the studied participants

Out of the 91 (35.0%) *H.pylori* positive participants, 73 (80.2%) were underweight (< 5 percentile), 8 (8.8%) had a BMI percentile range of 5- 85, 7(7.7%) had a BMI percentile between (86 – 95) while 3(3.3%) had a BMI percentile range of (96 -99), which was an indication of obesity(Figure 3).

Prevalence of *H. pylori* infection among the studied population

From the 260 participants enrolled for this study, only 26 (10%) showed growth of *H. pylori*. after culture for 3-12days (Figure 4).

TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

Variables	Attributes	Respondents	
		Number	Percentage
1. Sex	Male	143	55.0
	Female	117	45.0
2. Age group	≤ 1 year	91	35.0
	2-5 years	111	42.6
	> 5 years	57	21.9
3. Number of members living together at home	3-5 persons	216	83.1
	6-9 persons	44	16.9
4. Type of apartment	One room apartment	60	23.1
	Room and parlor	60	23.1
	Flat	130	50.0
	Duplex	10	3.8
5. Source of water supply	Borehole	255	98.1
	Well	3	1.1
	Spring	3	1.1
6. BMI Percentile	< 5 percentile	156	60.0
	5-85 percentile	78	30.0
	96-99 percentile	26	10.0
7. Washing of hand after toileting	Yes	257	98.8
	No	3	1.1
8. Feeding habit (Chewing food for infant)	Yes	26	10.0
	No	234	90.0

Antibiotics susceptibility patterns of *H. pylori* isolates from the stool samples

Majority of the *H. pylori* isolates 21 (80.8%) were sensitive to Ciprofloxacin with zone of inhibition size of 35mm. Eight (30.8%) were sensitive to Gentamicin

with a zone sizes of 4mm while only 5 (19.2%) with a zone sizes of 10mm were sensitive to Erythromycin . However, *H.pylori* showed moderate sensitivity to the other antibiotics used in this study (Table 4).

TABLE 2: FREQUENCY DISTRIBUTION TABLE SHOWING ISOLATION AND DETECTION OF *H. PYLORI* AMONG THE STUDIED POPULATION

Variables	Attributes	Participants	
N=260		Number	Percentage
<i>H. pylori</i> stool antigen (HpSA) test	Positive	91	35.0
	Negative	169	65.0
Stool culture status	Positive (<i>H. pylori</i> -isolated)	26	10.0
	Negative (<i>H. pylori</i> not isolated)	234	90.0

TABLE 3 : COMPARISON OF PERFORMANCE CHARACTERISTICS OF HPSA RAPID TEST TECHNIQUE AND THE USE OF CULTURE METHOD (GOLD STANDARD)

N = 260	Culture of <i>H.pylori</i>		Total
H.pylori Ag. detection using HpSA	Number Positive (%) (<i>H.pylori</i> isolated)	Number Negative (%) (<i>H.pylori</i> not isolated)	
Number Positive (%) (<i>H. pylori</i> Ag Present)	3 (3.3)	88 (96.7)	91(100)
Number Negative (%) (<i>H. pylori</i> Ag absent)	23 (13.6)	146 (86.4)	169 (100)
Total	26 (10.0)	234 (90.0)	260 (100)

$\chi^2 = 6.98$, df = 1, P = 0.085; Sensitivity (%) = 11.5; Specificity (%) = 62.4; Positive Predictive Value(%) = 3.3; Negative Predictive Value(%) = 86.4

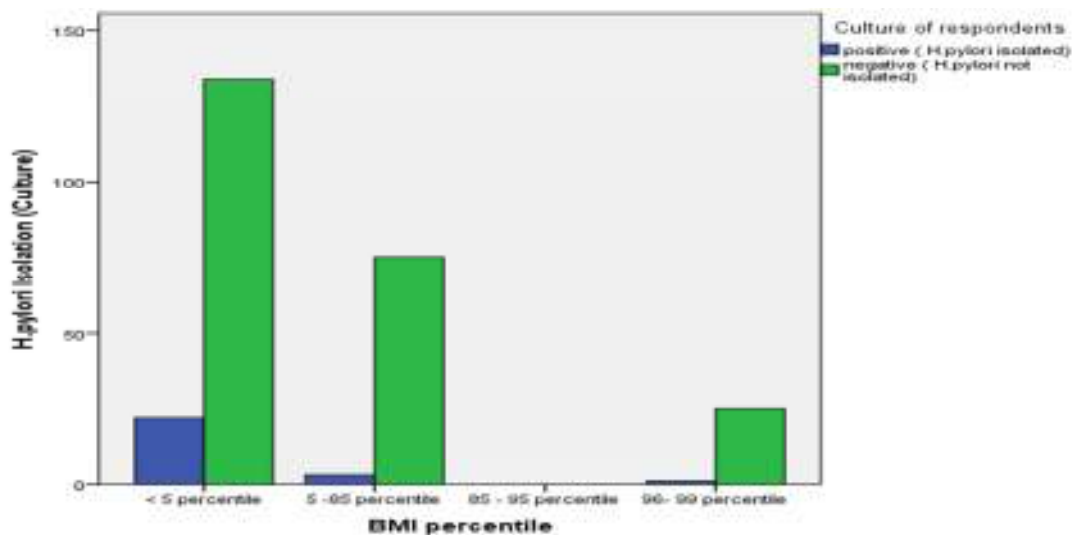


FIGURE 2: SHOWING CORRELATION OF *H. PYLORI* ISOLATION OF DIARRHOEIC CHILDREN AGAINST BMI PERCENTILE

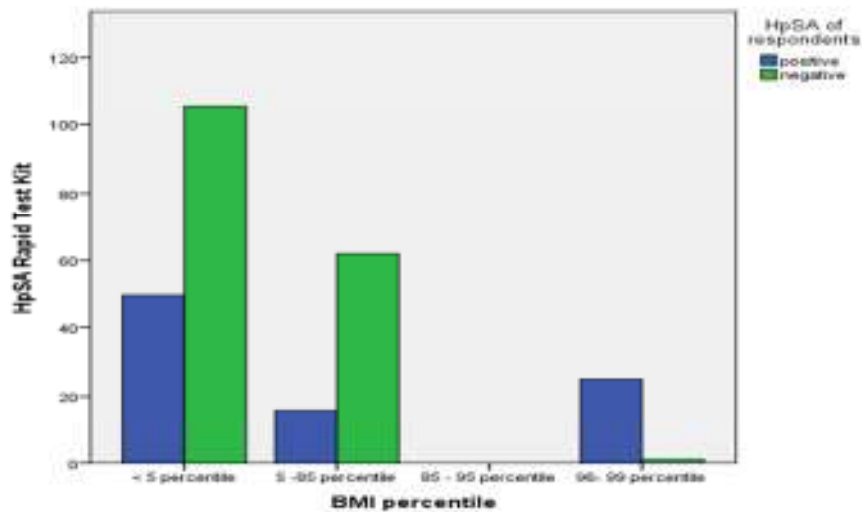


FIGURE 3: SHOWING HPSA RAPID ANTIGEN DETECTION TEST STATUS OF DIARRHOEIC CHILDREN AGAINST BMI PERCENTILE

TABLE 4: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE *H. PYLORI* ISOLATES

Antibiotics	Resistance (N=26) (%)	Sensitive (N=26) (%)	Zone size Range (mm)
Amoxicillin (10 µg)	11 (42.3)	15 (57.7)	15-23
Ciprofloxacin(5µg)	5 (19.2)	21 (80.8)	34-42
Clarithromycin(15µg)	8 (30.8)	18 (69.2)	25-31
Metronidazole(50µg)	11 (42.3)	15 (57.7)	6-8
Gentamicin(10µg)	18 (69.2)	8 (30.8)	15-18
Erythromycin (15 µg)	21 (80.8)	5 (19.2)	25-30
Herbal extract A(20µg)	26 (100)	0 (0)	6-8)
Herbal extract B(20µg)	26 (100)	0(0)	6-8) Metronidazole zone range adapted

DISCUSSION

Helicobacter pylori has been found to be associated with several disease conditions among which includes gastritis, gastric ulcer, iron deficiency anaemia, gastric cancer, stunted growth, diarrhoea etc among children [13,47]. In this study *H. pylori* has been incriminated as one of the aetiological agents of diarrhoea among children in Western Nigeria with the prevalence of 10.0% using culture method. This result corroborates similar works done elsewhere [19,36,38]. Thus suggesting the implication of *H. pylori*

as gastrointestinal pathogen and the need for the routine screening of diarrhoeic stools for *Helicobacter pylori* infection rather than reporting no pathogens found especially when *Salmonella* or *Shigella* species were not isolated.

Before now there had been emphasis on invasive methods for diagnosis of *H. pylori* using biopsies. However, this study has detected and isolated *H. pylori* from stool - a non-invasive method using *H. pylori* stool antigen (HpSA) kit and culture technique. The HpSA stool antigen kit is convenient

especially when dealing with children as it does not involve surgery nor discomfort when using Urea Breath Test, serology or endoscopy. Therefore the inconveniences caused by process of endoscopy, anaesthesia, cost of paediatric endoscopes is reduced or virtually inexistent as stool samples can be used in place of biopsies for the diagnosis of *H. pylori* infection.

In this study a detection rate of 35.0% using HpSA kit was recorded and this compares with work of Smith *et al.*[27] where, a detection rate of 36.7% was recorded among dyspepsia patient undergoing upper gastrointestinal endoscopy. There was a statistical significant relationship between the age of participants and their *H. pylori* infection status as previously inferred by Tahereh *et al.*[48] where incidence of *H. pylori* was synonymous with increasing age of the studied population. This may be ascribed to increasing biomass of the organism as they multiply in their host with increasing age. Among the participants that were positive by cultural method for *H. pylori* infection, some of them were underweight and when tested statistically there was a significant association between the presence of *H. pylori* and low body mass index of these children. This result is in consonance with the result of Oderda *et al.*[13] implicating *H. pylori* in stunted growth of children in Italy.

The antibiotics susceptibility result obtained tallies with those recorded by Oyediji *et al.* [34] and this implies that the quinolones such as ciprofloxacin still shows good activity against *H. pylori* strains. However, it recorded lower resistance to amoxicillin and metronidazole than what was previously obtained in some studies [34,49]. Though, increase in resistance to metronidazole has also been reported by Henriksen and his associates [50]. This may be explained based on age difference of the study population compared, possible differences in exposure to antibiotics of these populations and antibiotics abuse, where one antibiotic may be used for treating different ailments. Therefore, one can conclude that the *H. pylori* isolates in this study may isolation is desired for possible antibiotics susceptibility testing. It should however, be noted that, prior to the use of HpSA, researcher should alert participants, parent/guardian on the need to abstain from antibiotics, as this may give a false negative result due to the effect of the antibiotics on the organism. Evaluating the culture method, though it has low sensitivity, it is highly specific and desirable when considering antibiotics sensitivity testing. However, there are some challenges posed with this method such as incessant and erratic power outage coupled with the fact that it is cumbersome, despite

probably be from supposedly antibiotic-naive children compared to antibiotics exposed adults with subsequent drug abuse and possible self-medication which may be responsible for the discrepancy observed [34,42]. Other factors could be the fact that, the result of antibiotics sensitivity testing of *H. pylori* is determined and or dependent on the type of media, sufficient incubation timing and as well the growth condition.

Current treatment strategies to eradicate *H. pylori* in children have been developed primarily by using data from adults [51]. This is not good enough as it is scientific to always perform antibiotics susceptibility testing for different strains of organisms before determining the best regimen for the treatment of the infection caused by these organisms [57]. This is because organisms from different age groups, location in the body and from different samples may exhibit different sensitivities as is observed in this study [52,53]. A triple therapy has been considered to be the standard treatment for children; a proton pump inhibitor combined with two antibiotics has been shown to be very effective in clearing *H. pylori* from the stomach [54]. The current recommendation is treatment with amoxicillin, clarithromycin and a proton pump inhibitor for 2 weeks [55]. However, another triple therapy regimen that are effective in children has been shown to include a proton pump inhibitor combined with Clarithromycin and Metronidazole [55], or amoxicillin and metronidazole combined with Bismuth that would be given for a duration of 2 weeks [56]. This treatment regimen seems to agree with the result of the antibiotics susceptibility testing obtained in this study.

Expatriating on the comparative advantage of the non-invasive method of diagnosis used in this study, it can be suggested that HpSA is easy to perform, patient friendly as no anaesthetics is involved and it is also cheaper compared with Urea Breath Test (UBT). Though the latter may be classified as non-invasive, its cost is similar to those of endoscopy test and not as patient friendly as HpSA stool antigen kit[57]. However, the limitation of HpSA is that, it is a qualitative based test and may not be useful if this it is the most advocated method for diagnosis of *Helicobacter pylori* based on its comparative advantages over other methods.

On the possible predisposing factors to the transmission of the infection through overcrowding, there was no significant association between number of occupant and *H. pylori* infection in this study. Other possible mode of transmission as postulated by [58,59] Mohammed *et al.*, and Ramy *et al.*, [59,60] such as oral-oral and faeco-oral routes may be implicated as corroborated in this study.

CONCLUSION

This study revealed a prevalence of 10% *H. pylori* infection among children having diarrhoea disease using culture method. This suggests that *H. pylori* may be one of the incriminating pathogens in diarrhoea disease among children. It also imply that, we may need to routinely screen for *H. pylori* in the stool of children having gastrointestinal problems, especially if no other supposed pathogens such as *E. coli*, *Salmonella* and *Shigella* are found.

The diagnosis of *H. pylori* infection using stool culture is more accurate method for diagnosis of the organism, though it has low sensitivity compared with *H. pylori* stool antigen (HpSA) [57]. Despite the fact that culture is laborious, it is advocated because of its importance in the effective antibiotics management of the infection. This is based on ability

to do the *in vitro* susceptibility testing of the organism involved. Furthermore, because of the expertise required in culture procedure, incubation time especially *H. pylori* (12days) and its cumbersome nature, *H. pylori* stool antigen (HpSA) rapid test kit may be used instead in detecting the *H. pylori* antigen which means treatment of such cases will be empirical, since no isolation is involved.

Finally, this study has shown that *H.pylori* is culturable from stool in our environment; it is also involved in other gastrointestinal problems aside peptic ulcer disease and gastritis and may also be a co-factor in stunted growth in children. It is therefore imperative that samples from gastrointestinal tract relating to gastrointestinal problems may also be screened for *H.pylori* especially when children are involved.

REFERENCES

1. Kawaguchi K., Matsuo J., Osaki T., Kamiya S., Yamaguchi H. Prevalence of *Helicobacter pylori* and *Acanthamoeba* in natural environment. *Letters in Applied Microbiology*. 2009; Volume 48, Issue 4, 48: 465-471.
2. Marshall MJ., Warren RJ. Unidentified curved bacilli on gastric epithelium active chronic gastritis. *Lancet*, 1983; 1: 1273-1275.
3. Fritz LE., Slavik T., Delpont W. Incidence of *Helicobacter felis* and the effect of coinfection with *Helicobacter pylori* on the gastric mucosa in the African population. *J. Clin. Microbiol.* 2006;44(5): 1692-1696.
4. Figueroa G., Troncoso M., Toledo M. Prevalence of serum antibodies to *Helicobacter pylori* *VacA* and *CagA* and gastric diseases in Chile. *J. M. Microbiol.* 2002;51: 300-304.
5. Ahmed KS., Khan AA., Ahmed I . Impact of household hygiene and water source on the prevalence and transmission of *H. pylori*: a South Indian perspective. *Singapore Med. J* 2007;48(6): 543-549.
6. Feldman, RA. Epidemiologic observations and open questions about disease and infection caused by *Helicobacter pylori*. In Achtman M and Suerbaum S (eds) *Helicobacter pylori*: molecular and cellular biology. Wymondham, United Kingdom, Horizon Scientific press, 2001; pp. 29-51.
7. Ndip RN., Malange EA., Akoachere TKJF . *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limber health districts of Cameroon: A pilot study. *Trop. Med. Int. Health*, 2004;9(9): 1036-1040.
8. Dube C., Nkosi TC., Clarke AM. *Helicobacter pylori* in an asymptomatic population of Eastern Cape Province, South Africa: Public health implication. *Rev. Environ. Health*, 2009b; 24(3): 249-255.
9. Mladenova I., Durazzo M., Pellicano R. Transmission of *Helicobacter pylori*: are there evidences for a fecal-oral route? *Pub Med*. 2006; 97(1):15-8.
10. Hardin FJ., Wright RA. *Helicobacter pylori*: review and update. *Arch. Hosp. Physician* 2002; 38(5): 23-31.
11. Asrat D., Kassa E., Mengistu Y. Antimicrobial susceptibility pattern of *Helicobacter pylori* strains isolated from adult dyspeptic patients in Tikur Anbassa University Hospital, Addis Ababa. *Ethiop. Med. J.* 2004; 42: 79-85.
12. Dube C., Tanih NF., Ndip RN. *Helicobacter pylori* in water sources: A global environmental health concern. *Rev. Environ. Health*, 2009a; 24(1): 1-14.
13. Oderda G., Rapa A., Ronchi B. Detection of *Helicobacter pylori* in stool specimens by noninvasive antigen enzyme immunoassay in children: multicentre Italian study. *BMJ*, 2000; 320 : 347-348.
14. Thomas JE., Dale A., Bunn JE. Early *Helicobacter pylori* colonization: the association with growth faltering in The Gambia. *Arch. Dis. Child.* 2004;89(12): 1149-1154.
15. Santiago p., Moreno Y., Ferrus MA. Identification of Viable *Helicobacter pylori* in Drinking Water Supplies by Cultural and Molecular Techniques. *EPublications*. 2015; 20(4): 252 - 259.
16. Mulchandani R., Hans-Olof N., Wadström T ., Joshi, BR. *Helicobacter pylori* : Is it Transmitted Through Faecal-oral or Oral-oral Route? *Journal of Pure and Applied Microbiology*. 2013; 7(3). p.2429-2438.
17. Megraud, F., Lehours, P., Vale, F.F. The history of *Helicobacter pylori*: from phylogeography to paleomicrobiology. *Journal of Clinical*

- Microbiolgy and Infection.2016; Volume 22, Issue 11, Pages 922-927.
18. Gold, BJ. *Helicobacter pylori* infection in children. Curr Prob Pediatr. 2001;31:247-66.
19. Guetarni H., Bensoltane A. Isolation And Characterization Of *Helicobacter pylori* Strains From Gastric Biopsies Of Algerian Patients . OnLine Journal of Biological Sciences.2013; 13 (2): 41- 49.
20. Goosen C., Theron J., Ntsala M. Evaluation of a novel heminested PCR assay based on the phosphoglucosamine mutase gene for detection of *Helicobacter pylori* in saliva and dental plaque. J Clin Microbiol. 2002; 40:205-209.
21. Dulciene MMQ., Andreia MCR., Jean EC. Unintended consequences of *Helicobacter pylori* infection in children in developing countries . Iron deficiency, diarrhea, and growth retardation.. Journal of Gut Microbes. 2013; Volume 4, Issue 6Pages 494-504 .
22. Kate V., Ananthakrishnan N., Ratnakar C. Anti-*H.pylori* IgG seroprevalence rates in asymptomatic children and adults from south India. Indian J Med Microbiol. 2001;19:20-25.
23. Rocha GA., Rocha AM., Silva LD. Transmission of *Helicobacter pylori* infection in families of preschool-aged children from Minas Gerais, Brazil. Trop Med Int Health, 2003;8:987-991.
24. Wizla-Derambure N., Michaud L., Ategbo S. Familial and community environmental risk factors for *Helicobacter pylori* infection in children and adolescents. J Pediatr Gastroenterol Nutr. 2001;33:58-63.
25. Wang YK., Kuo FC., Liu CJ., Wu MC., Shih HY., Wang SS., Wu JY., Ku CH., Huang YK., Wu DC. Diagnosis of *H. pylori* Infection : Current Option And Developments. World J Gastroenterol.. 2015; 21 (40):11221 - 11235.
26. Smith SL, Oyediji KS., Odeniyi OA. Diagnosis of *Helicobacter pylori* infection among patients with dental caries by stool antigen test. Br J Biomed Sci. 2006;63(3): 144-145.
27. Smith SL, Omonigbehin EA., Goodluck HA . Diagnostic methods for detection of *Helicobacter pylori* in Nigeria. Journal Tropical Gastroenterology, 2010;31(2):113-115.
28. Shaman R., Niranga MD., Janaka de Silva H. *Helicobacter pylori* infection in children. Sri Lanka Journal of Child health, 2009; 38: 86-88
29. Braden B., Posselt H., Ahrens P . New immunoassay in stool provides an accurate noninvasive diagnostic method for *Helicobacter pylori* screening in children. Pediatrics; 2000; 106: 115-117.
30. Tom, RO . Upper gastrointestinal endoscopic findings in adolescents at Lacor hospital, Uganda Afr Health Sci. 2006; 6(1): 39-42.
31. Miller V., Doig C M . Upper gastrointestinal tract endoscopy Personal practice Archives of Disease in Childhood, 1984;59, 1100 -1102
32. Dore MP., Osato MS., Malaty HM . Characterization of a culture method to recover *Helicobacter pylori* from faeces of patients. *Helicobacter* ,2000;5: 165- 168
33. Takahiro U., David YG. Diagnosis of *Helicobacter pylori* using the rapid urease test Ann Transl Med. 2015; 3(1): 9.
34. Oyediji KS., Smith SL, Coker AO. Antibiotic susceptibility patterns in *Helicobacter pylori* strains from patients with upper gastrointestinal pathology in Western Nigeria. Br. J.Biomed Sci. 2009;66 (1): 10-3.
35. Wang WH ., Wong WM. , Dailidienne D. Aspirin inhibits the growth of *Helicobacter pylori* and enhances its susceptibility to antimicrobial agents Gut 2003;52:490-495 doi:10.1136/gut.52.4.490
36. Tahereh F., Nargess V., Mehri N. Culture of *Helicobacter pylori* from stool samples in children. Canadian Journal of Microbiology, 2007; 53(3): 411 - 416, 10.1139/W06-144
37. Coker A.O and Akande, B. Isolation of *Campylobacter pyloridis* from Nigerian patients with gastroduodenal pathology. West African Journal of Medicine 1989; 8 (2) :106 - 110.
38. Mohammad K ., Fereshteh S ., Hossein A ., Naser E.D., Mohammad R.P., Mostafa H., Amir G., Mohammad H.S. Comparison of five diagnostic methods for *Helicobacter pylori*. Iran J Microbiol. 2013; 5(4): 396 - 401.
39. Abolfazl I., Mohammad - Reza G., Saeed S., Hosein S., Akram H., Mohadeseh M. Stool Antigen Tests for the Detection of *Helicobacter pylori* in Children. Iran J Pediatr. 2013; 23(2): 138 - 142.
40. Ndip R. N., MacKay W. G., Farthing M.J. G., Weaver L. T. Culturing of *Helicobacter pylori* from clinical specimens. Journal of Pediatric Gastroenterology and Nutrition. 2003;_Volume 36 - Issue 5 - pp 616 - 622
41. Bury-Moné S., Kaakoush NO Asencio C., Mégraud F., Thibonnier M., De Reuse H., Mendz GL. Is *Helicobacter pylori* a true microaerophile? 2006; 11(4):296-303.
42. Dulciene M. M.Q., Edilberton N. M., Gilfone A. R. Indicator Medium for Isolation of *Campylobacter pylori*. Journal of Clinical Microbiology.1987;; p. 2378-2379.
43. McNulty C., Robert O., David T., Peter H., Kenneth M., Ashley P., Gillian S ., Louise T., and the PHLS *Helicobacter* Working Group. *Helicobacter pylori* susceptibility testing by disc diffusion . Journal of Antimicrobial Chemotherapy. 2002; Volume 49, Issue 4, Pages 601-609.
44. Michael S ., Ina T., Cosima L., Jan B., Julia M., Arne K., Mariya V., Thomas W., Dirk S., Peter M. Different antibiotic susceptibility between antrum

- and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. *World J Gastroenterol.* 2014; 20(43):16245 – 16251.
45. Apostolopoulos P., Kousmoutsos I., Ekmektzoglou K.. Concomitant versus sequential therapy for the treatment of *Helicobacter pylori* infection: a Greek randomized prospective study *Scand J Gastroenterol.* 2016; 51(2):145- 151
 46. Mahir G., Ahmet O., Hande O K.. Impact of *Helicobacter pylori* on growth : Is The Infection or Mucosal Disease Related to Growth Impairment? *J Digestive Diseases and Sciences.* 2010;Vol. 55 Issue 10, pp 2878 -2886.
 47. Dulciene MMQ., Andreia MCR., Jean EC. Unintended consequences of *Helicobacter pylori* infection in children in developing countries. *Gut Microbes.* 2013; 4(6): 494–504.
 48. Tahereh F., Nargess V., Shayesteh S . Application of a stool antigen test to evaluate the incidence of *Helicobacter pylori* infection in children and adolescents from Tehran, Iran. *Clin. Diag. Lab. Immunol.* 2005; p 1094-1097.
 49. Clinical and Laboratory Standards Institute(January,2013). Antimicrobial susceptibility testing standards.
 50. Henriksen TH., Lerang F., Lia A., Schøyen R., Thoresen T., Berge., Ragnhildstveit E., Tveten Y., Berstad A. Laboratory handling of *Helicobacter pylori* critically influences the results of in-vitro metronidazole resistance determination. *Clinical Microbiology and Infection.* 2004; Volume 10, Issue 4, Version of Record online: 10.1111/j.1198-743X.2004.00779.x
 51. O'connor A., Taneike I., Nami A., Fitzgerald N., Murphy P., Ryan B., O'connor H., Qasim A., Breslin N., O'moráin C. *Helicobacter pylori* resistance to metronidazole and clarithromycin in Ireland. *Eur J Gastroenterol Hepatol.* 2010; 22(9):1123-1127.
 52. Shaman R., Niranga MD., Hithanadura J. de Silva. *Helicobacter pylori* Infection in Children. *Saudi J Gastroenterol.* 2009; 15(2): 86–94.
 53. Muhammad M., Ari F S., Iswan AN., Dadang M., Langgeng AW., Lukman HZ., Fardah A., Willy BU., David S., I Dewa NW., Jimmy BW., Alexander MJS., Fauzi Y., Syifa M., Pangestu A., Umami M., Hasan M., Yudith AAR., Phawinee S., Nasronudin DR., Rumiko S., Junko A., Yoshio Y. Surveillance of *Helicobacter pylori* Antibiotic Susceptibility in Indonesia: Different Resistance Types among Regions and with Novel Genetic Mutations. *PLoS ONE.* 2016;11(12)
 54. Kim MM., Kim N., Lee SH. The effects of probiotics on PPI triple therapy for *Helicobacter pylori* eradication. *Helicobacter.* 2008;13:261–268.
 55. Rocco MZ., Gabriele B-P., Roberto F., Giovanni G., Enrico R., Franco B. Comparison of 1 and 2 weeks of omeprazole, amoxicillin and clarithromycin treatment for *Helicobacter pylori* eradication: the HYPER Study. *Gut.* 2007; 56(4): 475–479.
 56. Khurana R., Fischbach L., Chiba N . Meta-analysis: *Helicobacter pylori* eradication treatment efficacy in children. *Aliment Pharmacol Ther.* 2007;25:523–536.
 57. Malfertheiner P., Megraud F., O'Morain C . Current concepts in the management of *Helicobacter pylori* infection: The Maastricht III Consensus Report. *Gut.* 2007; 56(6):772-781.
 58. Miftahussurur M., Yamaoka Y. Diagnostic Methods of *Helicobacter pylori* Infection for Epidemiological Studies: Critical Importance of Indirect Test Validation. *Biomed Res Int.* 2016; Volume 2016, Article ID 4819423, 14 pages <http://dx.doi.org/10.1155/2016/4819423>
 59. Mohammed MK., Radwa RS., Ramy KA. *Helicobacter pylori*: A poor man's gut pathogen? *Gut Pathogens.* 2010; 2:2.
 60. Ramy KA., Mohammed MK., Radwa RS. Contaminated water as a source of *Helicobacter pylori* infection: A review. *Journal of Advance Research.* Volume 6, Issue 4.2015; Pages 539 – 547.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2018 ISBN 1595-689X VOL19 No.2
AJCEM/1815 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.5>
AFR. J. CLN. EXPER. MICROBIO. 19 (2)104 -112

PATHOGENIC POTENTIAL OF *LISTERIA MONOCYTOGENES* ISOLATED FROM CATTLE FAECES IN ADO-EKITI

*Oyinloye^{1,2}, M. A., David¹, O. M and Famurewa¹, O.

¹Ekiti State University^{1,2}, Ado-Ekiti, Ekiti State; ²Afe Babalola University, Ado-Ekiti, Ekiti State

*correspondence: oyinloyema@abuad.edu.ng, +234 705 0455 585

ABSTRACT

Listeria monocytogenes is an opportunistic food-borne pathogen causing listeriosis especially among immune-compromised persons. Its high rate of morbidity and mortality has classed the organism among the top watch list in foods. It is known to produce several virulence factors which aid its survival in harsh conditions and its dissemination within host cells. The pathogenicity of *L. monocytogenes*, isolated from cattle faeces in Ado-Ekiti, was determined in Wister albino rats for two weeks and the relative virulence was calculated. Rats were challenged with isolates producing listeriolysin O and phospholipase orally, intraperitoneally and subcutaneously. Biochemical parameters and haematoxylin and eosin (H and E) stained sections of selected organs were examined for significant changes ($p < .05$) and histopathological effects post-experiment. Relative virulence was recorded at 0% with rats showing no signs of infection or death. However, significant changes in total protein, lipid profile and some selected antioxidant enzymes, as well as cytological changes in the examined H and E sections of organs showed that an infection had occurred. Bacteria may have however been eradicated by the immune-competent rats. This study therefore concludes that isolates may be pathogenic especially for persons tagged 'high risk' due to low immunity.

Keywords: *L. monocytogenes*, listeriosis, pathogenicity, histopathology, cattle faeces

POTENTIEL PATHOGÈNE DE *LISTERIA MONOCYTOGENES* ISOLÉES DE FÈCES DE BOVINS DANS ADO-EKITI-

*Oyinloye^{1,2}, M. R., David¹, O. M. et Famurewa¹, O.

¹L'état d'Ekiti, Université de l'état d'Ekiti, Ado-Ekiti ; ²Afe Babalola University, Ado-Ekiti, l'état d'Ekiti

*Correspondance : oyinloyema@abuad.edu.ng, 2347050455585

ABSTRACT

Listeria monocytogenes est un agent pathogène d'origine alimentaire opportuniste causant la listériose en particulier chez les personnes immunodéprimées. Le taux élevé de morbidité et de mortalité a classé l'organisme parmi les top liste de surveillance dans les aliments. Il est connu pour produire plusieurs facteurs de virulence dont l'aide sa survie dans des conditions difficiles et sa diffusion dans les cellules hôtes. La pathogénicité de *L. monocytogenes*, isolées de fèces de bovins à Ado-Ekiti, était déterminé à Wister albino rats pendant deux semaines et la virulence relative a été calculée. Les rats ont été remis en question avec les isolats produisant la listériolysine O et la phospholipase, oralement et par voie sous-cutanée. Les paramètres biochimiques et l'hématoxyline-éosine (H et F) les articles tachés de certains organes ont été examinés pour des changements significatifs ($p < .05$) et des effets histopathologiques post-expérience. Virulence relative a été enregistré à 0 % avec les rats ne montrant aucun signe d'infection ou de la mort. Cependant, des changements importants dans les protéines totales, le profil lipidique et certains enzymes antioxydantes, ainsi que des changements cytologiques dans les sections H et E d'organes ont montré qu'une infection s'est produite. Des bactéries peuvent cependant avoir été éliminé par le système immunitaire des rats-compétents. Cette étude conclut donc que les isolats peuvent être pathogènes en particulier pour les personnes avec le tag 'risque' en raison d'une faible immunité.

Mots-clés: *L. monocytogenes*, listériose, pathogénicité, histopathologie, bovins faeces

INTRODUCTION

One of the earliest recorded large outbreak of listeriosis was traced to locally made coleslaw (contaminated cabbage via manure) and the strain isolated was the same as the outbreak/epidemic strain, *L. monocytogenes* serotype 4b (1). Fleming *et al.*

(2) reported an outbreak of listeriosis which occurred in Massachusetts in 1983. This outbreak involved non pregnant adults who had immunosuppressive condition.

Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

At the period of the outbreak, there were simultaneous diagnostic cases of listeriosis in fetuses and neonates with fatality rate of 29% in both adults and neonatal groups. Post pasteurisation contamination of milk was concluded as the likely explanation for the outbreak. In the United Kingdom, McLaunchlin *et al.* (3) reported an unusual strain, *L. monocytogenes* serotype 1/2a, in an outbreak in Carlisle, East Columbia, with 11 cases of listeriosis. Similarly, another listeriosis outbreak implicated raw vegetables in Boston (4). Linnan *et al.* (5) reported one of the largest outbreaks of listeriosis in the USA. In this outbreak, majority of the cases were in pregnant Hispanic mothers and their neonates. Out of 93, 81(87%) cases were pregnant-associated resulting to 29 foetal or neonatal deaths. In recent reports, the Centres for Disease Control and Prevention (CDC) have recorded outbreaks in multiple States of the USA. In 2011, cantaloupes contaminated with the bacteria *L. monocytogenes* caused the deadliest food-borne disease outbreak in the United States in nearly 90 years. A total of one hundred and forty seven illnesses were recorded across 28 States with thirty three deaths and a miscarriage (6). The year 2012 also witnessed another multistate outbreak of listeriosis involving 14 States, linked with an imported cheese, recording twenty two illnesses, twenty hospitalizations and four deaths (7). Another cheese-infected outbreak involving 5 States recorded six illnesses, six hospitalizations, one death and a miscarriage (8). In 2014, eight cases of listeriosis linked with a contaminated dairy product across 2 States were reported. Five of the cases involved two mothers and their newborns, and a third newborn, leading to seven hospitalizations and one death (9). Very recently, the health implication of *L. monocytogenes* presence in milk was reported by CDC in a multistate infection of listeriosis outbreak in old people (73 to 81 years). The outbreak resulted in 50 % mortality of persons infected (10).

Listeria monocytogenes is a remarkable bacterium that has evolved over a long period to acquire a diverse collection of molecules, each with unique properties and functions, and each contributing to the success of *L. monocytogenes* as an intracellular pathogen (11). Upon ingestion by the host via contaminated food, *L. monocytogenes* withstands exposure to host proteolytic enzymes, the acidic stomach environment (pH 2.0), bile salts and non-specific inflammatory attacks,

largely through the actions of several stress-response genes (*opuCA*, *lmo1421* and *bsh*) and related proteins (11). Having survived this initial stage, *L. monocytogenes* adheres to and is internalized by host cells with the assistance of a family of surface proteins called internalins (12). The most notable internalins are internalin A (InlA) and internalin B (InlB). Following its uptake by host cells, *L. monocytogenes* is primarily located in single-membraned vacuoles. Two virulence associated molecules are responsible for lysis of the primary single-membraned vacuoles and subsequent escape by *L. monocytogenes*: listeriolysin O (LLO) and phosphatidylinositol-phospholipase C (PI-PLC) (13, 14). After lysis of the primary single-membraned vacuoles, *L. monocytogenes* is released to the cytosol, where it undergoes intracellular growth and multiplication. The intracellular mobility and cell-to-cell spread of *L. monocytogenes* require another surface protein, ActA (a 67 kDa protein encoded by *actA*), which is cotranscribed with PC-PLC and mediates the formation of polarized actin tails that propel the bacteria toward the cytoplasmic membrane. At the membrane, bacteria become enveloped in filopodium-like structures that are recognized and engulfed by adjacent cells, resulting in the formation of secondary double-membraned vacuoles. A successful lysis of the secondary double-membraned vacuoles signals the beginning of a new infection cycle, which is dependent on PC-PLC upon activation by Mpl (a 60 kDa metalloprotease encoded by *mpl*) (14).

The primary manifestations of listeriosis in humans include septicaemia, meningitis (or meningoencephalitis) and encephalitis, usually preceded by influenza-like symptoms including fever. Gastroenteric manifestations with fever also occur. Although the morbidity of listeriosis is relatively low, the mortality can reach values around 30% (15). These rates exceed those from other bacteria such as *Salmonella* and *Clostridium*, making listeriosis a leading cause of mortality among food-related infections (15). In pregnant women, infection may result in abortion, stillbirth or premature birth (16, 17), while infection is characterised by septicaemia and meningitis among adults (18). Listeriosis induced meningitis is often exacerbated by encephalitis which is very unusual for a bacterial infection (18). Listeriosis affecting the central nervous system accounts for around 55-75% of cases whereas septicaemia accounts for 15-20% with non-typical infections making up the remainder (14). Despite reports from different countries around the world of the involvement of this pathogen in different food-borne outbreaks, little is reported of its medical importance in Nigeria. Njoku-Obi and Njoku-Obi (19) reported the first evidence of *Listeria* through serological evidence among blood donors, staff and

students of the Lagos University Teaching Hospital. The study reported the presence of *L. monocytogenes* by somatic agglutination and complement fixing antibodies. The first case of isolation of the organism in Nigeria from an adult female patient was reported by Eyo *et al.* (20). The first neonatal case was reported in a neonate showing signs of listerial meningitis, haven been infected from the mother. Both mother and child were effectively treated with ampicillin (21). A 26.32% mortality rate from 19 patients presenting with meningitis and meningoencephalitis due to *L. monocytogenes* in a one-year prospective study was reported in another study (22). Results from other reports are as follow: in 420 faecal samples from patients presenting with gastro-enteritis, none yielded a positive result to *L. monocytogenes*; 0.4% positive samples from 1097 cerebrospinal fluid (23); 5 positive cases of *L. monocytogenes* out of 66 septicaemic neonates (24); from 33 septicaemic neonates screened, none was positive to *L. monocytogenes* (25); a microbiological screening of 162 donated blood samples for transfusion showed none positive to *L. monocytogenes* (26); and in a three-year retrospective study of 1500 paediatric patients, none was positive to listerial infection (27). Several reports have been made on the occurrence and spread of *L. monocytogenes* in Nigeria in food, food-drinks, and environmental samples (28). This study therefore aimed at evaluating the pathogenic potential of *L. monocytogenes* isolates from cattle faeces in Wister albino rats, challenged through the oral, peritoneal and subcutaneous routes.

METHODS

A total of thirty (30) faecal samples were gotten from the intestines of different slaughtered cows into sterile universal bottles and were transported on ice-packs within 2hrs to Afe Babalola University Microbiology laboratory for bacteriological analysis.

The method of Food and Drug Administration (FDA) bacteriological and analytical method (BAM) described by Hitchins (29) was used using a combination of selective enrichment broth (University of Vermont Medium Modified *Listeria* Enrichment Broth, UVM-MLEB; Alpha Biosciences, USA) and selective Brilliance *Listeria* Agar Base (BLAB; Oxoid, Basingstoke, Hampshire, UK). Cattle faeces were collected from the intestine after slaughter. One gram (1g) of sample was homogenised in 9 ml of Tryptone Soya broth. A volume of 1 ml of this homogenate was then inoculated into 9 ml of UVM-MLEB (Alpha Biosciences, USA), to which selective supplement (Oxoid, Basingstoke, Hampshire, UK) was added after 4 hours of incubation at 37°C, and subsequently incubated at 37°C for 44 hours. The enriched culture was streaked at 24 and 48 hours on Brilliance *Listeria* agar with differential and selective supplements

(Oxoid, Basingstoke, Hampshire, UK). After incubation at 37°C, blue-green colonies on the medium were presumed to be *Listeria* species. These isolates were subjected to Oxoid Biochemical Identification (OBIS-mono kit) reagents to differentiate between *L. monocytogenes* and non-*monocytogenes* species; confirmed *L. monocytogenes* were further subjected to biochemical characterization (Gram stain, catalase, oxidase, sugar fermentation, and motility at 25°C).

Histopathological study: Isolates were examined for the production of listeriolysin O and phospholipase using 7% sheep blood agar and differential supplement (Oxoid, Basingstoke, Hampshire, UK) respectively. Positive isolate for virulence factors were used to challenge Wister albino rats. National guidelines on the use of animals in experimental research was followed and approved by the University's Research Ethical Board. Inoculum density was determined using the method described by Stelma *et al.* (30). Four groups of five rats each were challenged orally, intraperitoneally and subcutaneously, while the fourth group served as the control and were challenged with sterile normal saline, and were observed for two weeks for signs of infection. Serum and organs (liver, spleen and kidney) were collected post-experiment; blood and faeces were cultured for re-isolation of bacteria; biochemical parameters were determined using commercial diagnostic kit (Crumlin, Co. Antrim, UK); and haematoxylin and eosin stained sections of organs were examined.

RESULT

A total of 176 bacteria were isolated from the samples having different colonial colours on the selective agar. However, blue-green colonies (Plate 1), *Listeria* species were recorded at 87 (49.43%) as green-yellow (GYC, 30/30), sky-blue (SBC, 30/30) and green colonies (GC, 27/30). *Listeria monocytogenes* was identified by OBIS-mono kit at 27 (90%), which were the green colonies (GC) with halo on the chromogenic selective agar and biochemical reactions also identified the 27 isolates as *L. monocytogenes* (Table 1). All isolates of *L. monocytogenes* demonstrated the production of listeriolysin O and phospholipase (Plate 2), known virulence factors. The pathogenic prowess of the isolate recorded a relative virulence of 0% and the bacteria were not re-isolated among all inoculated groups. However, different biochemical parameters revealed varying levels of infection signs. A significant elevated level ($p < .05$) of total protein seen among the intraperitoneal and subcutaneous groups revealed inflammation or infection among the groups

and the liver showed more signs of infection by *L. monocytogenes* than spleen and kidney (Table 2). Changes in the lipid profiles had no significant relationship to signs of infection (Table 3). Also an

elevated level of alanine aminotransferase (ALT) observed among oral and subcutaneous groups indicated acute hepatocellular damage (Table 4).

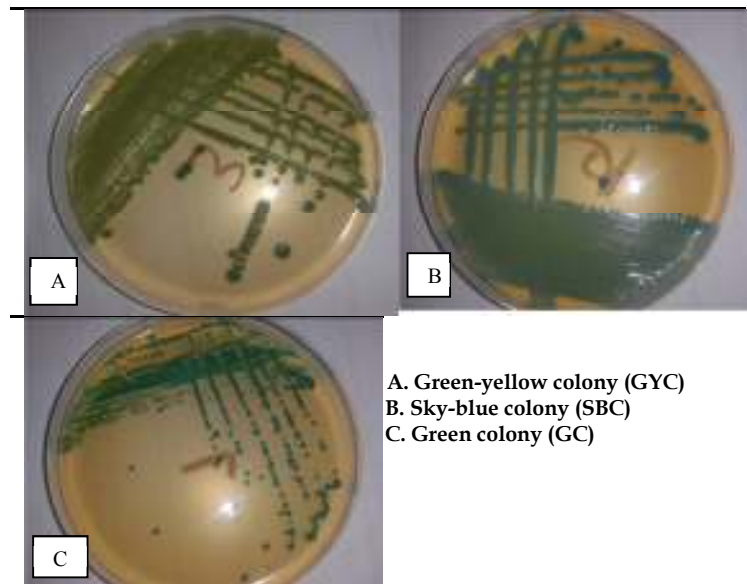


PLATE 1: APPEARANCE OF *LISTERIA* SPP. ON BRILLIANCE *LISTERIA* AGAR

TABLE 1: BIOCHEMICAL CHARACTERISTICS OF GC ISOLATES OF *LISTERIA* SPECIES

Biochemical tests	GC	n = 27(%)
Gram reaction	+	27 (100)
DALAase production	-	27 (100)
Catalase production	+	27 (100)
Oxidase production	-	27 (100)
Xylose fermentation	-	27 (100)
Mannitol fermentation	-	27 (100)
Lactose fermentation	+	21 (77.78)
Galactose fermentation	+	22 (81.48)
Motility at 25°C	+	27 (100)

Key: GC - green colony; +: positive; -: negative; V: variable; DALAase: D-alanyl aminopeptidase

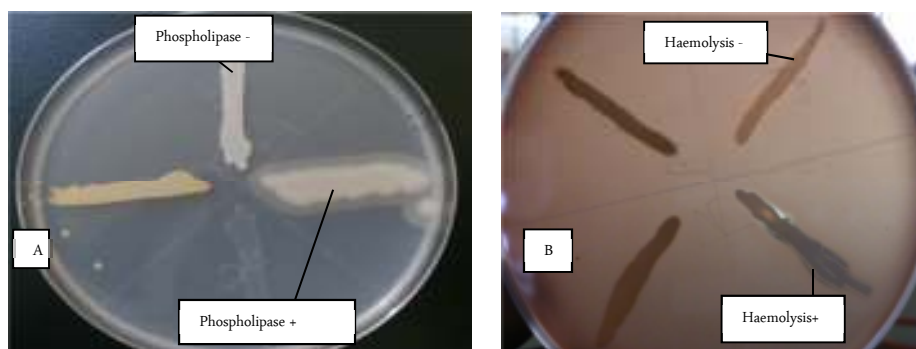


PLATE 2: PRODUCTION OF SOME VIRULENCE FACTORS BY *L. MONOCYTOGENES*
A. showing the production of phospholipase (white halo around colony); B. β -haemolysis (clear area around the edge of colony)

TABLE 2: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON TOTAL PROTEIN (MG/DL) IN SELECTED ORGANS OF RATS

Treatment	Organs		
	Spleen	Kidney	Liver
Control	2.96 ± 0.228 ^a	2.28 ± 0.234 ^a	2.21 ± 0.154 ^a
Oral	2.30 ± 0.595 ^a	2.28 ± 0.312 ^a	1.94 ± 0.334 ^a
Intraperitoneal	2.65 ± 0.420 ^a	2.36 ± 0.228 ^a	3.47 ± 0.453 ^b
Subcutaneous	3.06 ± 0.591 ^a	2.36 ± 0.133 ^a	2.53 ± 0.147 ^{a, b}

Key: Values are Mean ± SEM in mg/dL, values of the same alphabet superscripts have no significant difference ($P < .05$), while values with different alphabet superscripts have significant differences ($P < .05$) by column.

TABLE 3: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON LIPID PROFILE (MG/DL) IN SERUM OF RATS

Treatments	TC	TGC	HDL	LDL
Control	229.30 ± 26.08 ^{a, b}	188.25 ± 14.20 ^b	19.55 ± 1.90 ^a	172.10 ± 24.92 ^a
Oral	354.63 ± 29.41 ^b	115.76 ± 19.05 ^a	17.93 ± 0.57 ^a	313.55 ± 36.98 ^b
Intraperitoneal	211.38 ± 43.16 ^a	126.57 ± 7.74 ^a	20.52 ± 0.90 ^a	165.55 ± 45.27 ^a
Subcutaneous	252.46 ± 28.71 ^{a, b}	183.05 ± 17.16 ^b	19.14 ± 1.86 ^a	196.71 ± 24.69 ^a

Key: Values are Mean ± SEM, values of the same alphabet superscripts have no significant difference, while different superscripts represent values with significant differences ($P < .05$) by column. TC-total cholesterol, TGC-triglyceride, HDL-high density lipoprotein, LDL- intermediate density lipoprotein, LDL-low density lipoprotein

TABLE 4: EFFECTS OF *L. MONOCYTOGENES* INFECTION ON SOME SELECTED ANTIOXIDANT ENZYMES IN ORGANS OF RATS

Organ	Enzyme	Treatment			
		Control	Oral	Intraperitoneal	Subcutaneous
Liver	AST	9.17 ± 4.15 ^a	7.57 ± 2.43 ^b	6.69 ± 1.60 ^b	4.37 ± 0.87 ^c
	ALT	4.11 ± 0.97 ^a	5.82 ± 1.56 ^a	4.07 ± 1.45 ^a	6.98 ± 1.04 ^a
	CAT	283.90 ± 1.06 ^a	338.72 ± 4.46 ^b	328.04 ± 1.37 ^b	316.97 ± 1.93 ^b
	SOD	20.00 ± 3.79 ^a	30.00 ± 1.85 ^b	35.00 ± 2.25 ^b	55.00 ± 0.95 ^c
Spleen	AST	22.12 ± 14.62 ^a	14.27 ± 10.25 ^b	5.67 ± 2.05 ^c	8.00 ± 2.64 ^c
	ALT	4.51 ± 1.43 ^a	4.95 ± 0.61 ^a	4.22 ± 1.37 ^a	5.53 ± 1.55 ^b
	CAT	216.45 ± 1.66 ^a	242.76 ± 1.08 ^b	233.74 ± 0.69 ^a	257.31 ± 0.76 ^b
	SOD	50.00 ± 12.91 ^a	50.00 ± 12.91 ^a	50.00 ± 10.00 ^a	49.98 ± 11.06 ^a

Key: Values are Mean ± SEM in mg/dL, values of the same alphabet superscripts have no significant difference ($P < .05$), while values with different alphabet superscripts have significant differences across row. ALT - alanine aminotransferase, AST - aspartate aminotransferase, CAT - Catalase, SOD - superoxide dismutase

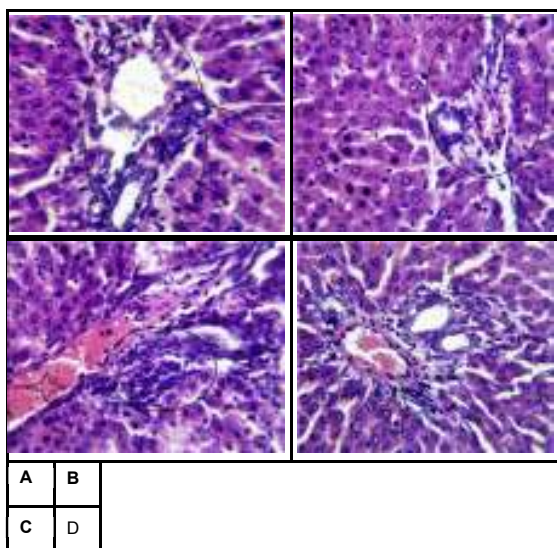


PLATE 3: HAEMATOXYLIN AND EOSIN STAINED SECTION OF LIVER (MAG. X 400)

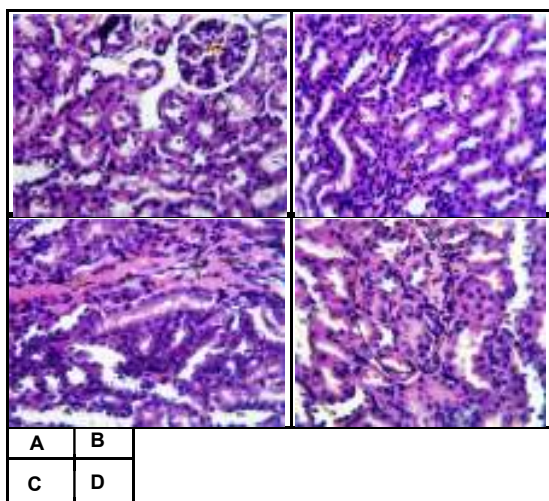
Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous
 A - section of the hepatic tissue composed of hepatocytes (H) disposed in sheet and separated by the sinusoids (S). The portal triads (Circle) composed of the bile duct and vessels. Section is free from collections and inflammatory cells.
 B - Section of the hepatic tissue composed of hepatocytes (H) disposed in sheet and separated by the sinusoids (S). The portal triads (Circle) composed of the bile duct and vessels. Section with mild collections and inflammatory cells.
 C - Section of the hepatic tissue with marked periportal infiltration with inflammatory cell (Circle), there is marked congestion of vessels (Star). The hepatocytes appear unremarkable.
 D - Photomicrograph shows mild periportal infiltration and vascular congestion (Arrow). The sinusoidal spaces appeared mildly dilated (DS). The hepatocytes appear unremarkable.

Significant increases ($p < .05$) were observed in the levels of superoxide dismutase (SOD) and catalase (CAT) among all experimental groups (Table 4). Significant biochemical changes among the tested rats revealed that infection signs were more consistent in Intraperitoneal and Subcutaneous groups than in Oral group.

The haematoxylin and eosin (H and E) stained sections of liver, kidney and spleen showed signs of infection among the experimental groups. Bacteria cells were however not seen in the stained sections. Stained sections of the liver revealed mild collections and inflammatory cells in the portal triad of both oral and subcutaneous group. Section showed infiltration of inflammatory cells in the intraperitoneal group, and hepatocytes appear abnormal (Plate 3). While the renal tubules appear

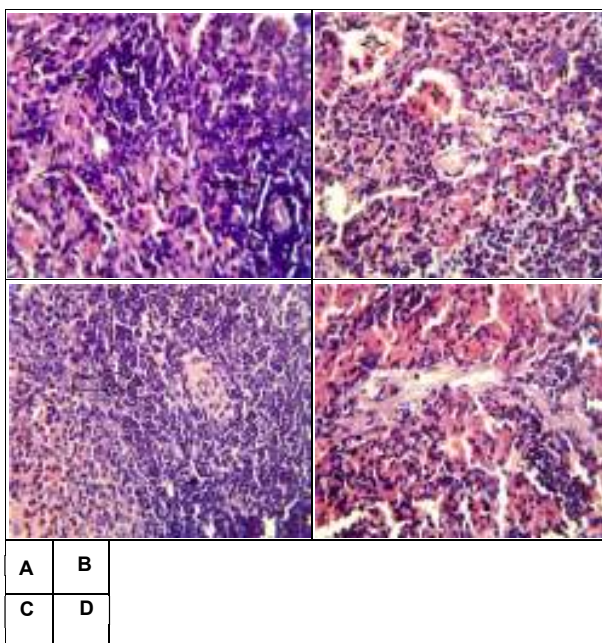
normal with the oral group, mild to heavy tubular necrosis was revealed with stained sections from subcutaneous to Intraperitoneal group respectively (Plate 4). The stained section of the spleen also showed pockets of congestion and most especially marked proliferation of the white pulp which contains the lymphocytes, and the proliferation of lymphocytes among subcutaneous group all indicated infection (Plate 5). In all stained sections, marked signs of infection were more prominent in intraperitoneal group than others.

Both the biochemical and histopathological parameters indicated that an infection had occurred among the rats but none died during the experimental period.



Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous
 A - Photomicrograph shows section of the renal tissue composed of the renal corpuscles (Arrow) composed of the glomerulus (G), the bowman's space (BS) is well defined. The renal tubules (RT) are lined by regular epithelium. Section is free from collection and inflammatory cells.
 B - Photomicrograph shows marked interstitial collection (Star). The renal tubules appear normal.
 C - Photomicrograph shows marked interstitial collection (Star). The renal tubules showed degenerative changes (tubular necrosis).
 D - Section shows marked interstitial congestion and mild inflammatory infiltrates. The renal tubules show mild degenerative changes (TN).

PLATE 4: HAEMATOXYLIN AND EOSIN STAINED SECTION OF KIDNEY (MAG. X 400)



Key: A- Control; B- Oral; C- Intraperitoneal; D- Subcutaneous
A - White pulp (Arrow) and red pulp (Star). The blood vessels (Arrow head).
B - Section shows pockets of congestion (Star)
C - Section shows marked proliferation of the white pulp. (Arrow)
D - Arrow shows cluster of lymphocytes.

PLATE 5: HAEMATOXYLIN AND EOSIN STAINED SECTION OF SPLEEN (MAG. X 400)

DISCUSSION

In the isolation and identification of *L. monocytogenes*, time and accuracy are important factors to consider as the rate of mortality due the organism is relatively high (31, 32, 33, 34). This study employed the use of a chromogenic medium, Brilliance *Listeria* Agar Base (formerly Oxoid Chromogenic *Listeria* Agar, OCLA), which have been reported reliable in the differentiation of *L. monocytogenes* from other species. The sensitivity and specificity of Brilliance *Listeria* agar in this study was at 100 % and 100 % respectively. This report agrees with the study of Park *et al*, (35) at a sensitivity and specificity rate of 92.0 % and 96.5 % respectively. The medium ranked second highest at 98.7 % (second to lecithin and levofloxacin medium (LLM) at 98.8 %) overall for its confirmation rate of *Listeria* species. Other chromogenic media reported alike are BBL CHROMagar *Listeria*, Agar *Listeria* according to Ottaviani and Agosti (ALOA), Rapid'L-mono agar, and CHROMagar *Listeria*. Chromogenic media have been proven efficient in the isolation and differentiation of *Listeria* species (33).

The cycle of relationship among faeces, water and soil in relations to humans makes the samples possible sources of *L. monocytogenes* infection.

Locally, cattle dung is employed in land fertilization for the cultivation of crops meant for human consumption. *Listeria monocytogenes* was isolated from 90 % of cattle faeces in this study, and occurred at 9.7 % in the total population of *L. monocytogenes* from all samples. This result agrees with report of Umeh and Okpokwasili (36) that screened faecal droppings of domesticated animals and found the highest occurrence of *L. monocytogenes* in cattle faeces. Animal droppings have been widely implicated around the world in the occurrence of the organism (37, 38, 39, 40, 41, 42). Most animals have been identified as healthy carriers of *L. monocytogenes* as signs of infection are not noticed on them. These animals shed the organisms in faeces and thus contaminate their products and the environment (39, 43). The report of this study thus discouraged the use of animal dung in farm land fertilization as this can lead to contamination in animals during grazing, water bodies by surface run-offs, and in man through consumption of farm products from such farms.

The pathogenicity of *L. monocytogenes* was studied by using the mouse virulence assay method (44). The relative virulence was at 0 %, with no dead rat at the end of the experiment, and *L. monocytogenes* was not recovered from the stool and the blood. However, biochemical markers and histopathological results showed signs of infection. Isolates used in the pathogenicity tests expressed virulence factors such as listeriolysin, phospholipase and biofilm production (45). Elevated levels of total protein and alanine aminotransferase (ALT) are suggestive of acute hepatocellular damage, while low alkaline phosphatase (ALP) could be due to low protein deficiency among the orally-challenged group. Significant increased levels of superoxide dismutase (SOD) are suggestive of the presence of phagocytes which are known to produce superoxide in great quantities as a weapon against foreign particles such as bacteria. This increase is directly proportional to the significant increase of catalase, as both enzymes are important in neutralizing the damaging effects of superoxide and hydrogen peroxide to water and oxygen (46, 47, 48). *Listeria monocytogenes* strains have been classified as virulent and avirulent. Strains of lineage III have been identified to be avirulent or weakly virulent due to production of lower levels of virulence factor, thus, these strains do not cause death of infected animals (44, 49). The putative gene, *lmo2821* (*inlJ*), has been identified as a means of differentiating virulent from avirulent strains of *L. monocytogenes*. Thus, despite the production of listeriolysin, phospholipase and biofilm, strains may still be avirulent in mouse virulence assay (49).

Conclusion: The rats used in the experiment were immuno-competent, thus, may have successfully eradicated the bacteria as *L. monocytogenes* is an opportunistic pathogen causing diseases in immuno-compromised hosts. Thus, the isolates used in this study can be said to be virulent considering its effects in biochemical and histopathological changes in immuno-competent rats.

Conflict of Interest: Authors declare that there is no conflict of interest in this study.

Funding Statement: This study was self-sponsored by the authors.

Acknowledgements: The authors will like to acknowledge Mr Amos Onikanni, Oladiran Rotimi and Kpolugbo Edirin for their contributions towards the histopathological study. The authors also appreciate the pathology department staff members of Afe Babalola University for their roles in the tissue staining and study section.

Authorship: FO designed and supervised the study, OMA carried out the study and wrote the manuscript, while DOM proofread the manuscript and carried out the data analysis.

REFERENCES

1. Schlech, W.F., Lavigne, P. M., Bortolussi, R. A., Allen, A. C., Haldane, E. V., Wort, A. J., Hightower, A. W., Johnson, S. E., King, S. H., Nicholls, E. S., Broome, C. V. Epidemic listeriosis-evidence for transmission by food. *New England Journal of Medicine*, 1983, 308: 203-206.
2. Flemming, D. W., Coelin, L. S., Macdonald, K. K., Brondun, J., Hayes, S. P., Plikaytis, D. B., Holmes, B. M., Audurier, B. A., Broome, V. C. and Reigold, L. R. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New England Journal of Medicine*, 1985, 312: 404 - 407.
3. McLauchlin, J., Audurier, A. and Taylor, A. G. Aspects of the epidemiology of human *Listeria monocytogenes* infections in Britain 1967-1984; the use of serotyping and phage typing. *Journal of Medical Microbiology*, 1986, 22: 367-377.
4. Ho, J. L., Shands, K.N., Freidland, G., Eckind, P. and Fraser, D. W. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Archives of Internal Medicine*, 1986, 146: 520-524.
5. Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., Hird, D. W., Yonekura, M. L., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B. D., Fannin, S. L., Kleks, A. and Broome, C. V. Epidemic listeriosis associated with Mexican-style cheese. *The New England Journal of Medicine*, 1988, 319: 823-828.
6. Centers for Disease Control and Prevention. Final report: multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado. CDC, Atlanta, GA. 2011
7. Centers for Disease Control and Prevention. Multistate Outbreak of Listeriosis Linked to Imported Frescolina Marte Brand Ricotta Salata Cheese Available at: <http://www.cdc.gov/listeria/outbreaks/cheese-09-12/index.html> 2012 (Accessed 16/1/2015)
8. Centers for Disease Control and Prevention. Multistate Outbreak of Listeriosis Linked to Crave Brothers Farmstead Cheeses Available at: <http://www.cdc.gov/listeria/outbreaks/cheese-07-13/index.html> 2013 (Accessed 16/1/2015) 9. Centers for Disease Control and Prevention. *Listeria* (Listeriosis): Treatment and Outcomes. www.cdc.gov/Listeria/treatment.html. 2014 (Accessed on 23/3/2015)
10. Centers for Disease Control and Prevention. *Listeria* Outbreaks: Multistate Outbreak of Listeriosis Linked to Raw Milk. <https://www.cdc.gov/listeria/outbreaks/>. 2016 (Accessed on 6/4/2016)
11. Sleator, R. D., Gahan, C. G. M. and Hill, C. A postgenomic appraisal of osmo-tolerance in *Listeria monocytogenes*. *Applied Environmental Microbiology*, 2003, 69: 1-9.
12. Gaillard, J. L., Berche, P., Frehel, C., Gouin, E. and Cossart, P. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell*, 1991, 65: 1127-1141.
13. Portnoy D. A., Chakraborty T., Goebel W., Cossart P. Molecular determinants of *Listeria monocytogenes* pathogenesis. *Infect. Immun.* 1992, 60, 1263.
14. Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Gonzalez-Zorn, B., Wehlan, J. and Kreft, J. *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Review*, 2001, 14: 584-640.
15. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe RV. Food-related illness and death in the United States (Reprinted from Emerging Infectious Diseases). *Journal of Environmental Health*, 2000, 62: 9-18.
16. Rocourt, J. and Bille, J. Foodborne listeriosis. *World Health Statistics Quarterly*, 1997, 50: 67-73.
17. Slutsker, L. and Schuchat, A. Listeriosis in Humans. In: *Listeria, Listeriosis, and Food Safety*, E. T. Ryser and E. H. Marth (eds). Marcel Dekker, Inc.: New York, 1999.
18. Ramaswamy, V., Cresence, V. M., Rejitha, J. S., Lekshmi, M. U., Dharsana, K. S., Prasad, S. P. and Vijila, H. M. *Listeria*-review of epidemiology and pathogenesis. *Journal of Microbiology, Immunology and Infection*, 2007, 40:4-13
19. Njoku-Obi, A. N. and Njoku-Obi, J. C. Serological evidence for the prevalence of listeriosis in Nigeria. *Journal of Tropical Medicine and Hygiene*, 1965, 68: 121- 124.
20. Eyo, E. E., Senbanjo, A. O. and Babatunde, E. O. *Listeria monocytogenes* meningitis in an adult Nigerian female. *The West African Medical Journal and Nigerian Practitioner*, 1969, 18: 121-124.

21. Onyemelukwe, G. C. and Lawande, R. V. Listeriosis in a neonate and the mother. *Tropical and Geographical Medicine*, 1982, 34: 87-89.
22. Onyemelukwe, G. C., Lawande, R. V, Egler, L. J. and Mohammed, I. *Listeria monocytogenes* in Northern Nigeria. *Journal of Infection*, 1983, 6: 141-145.
23. Emele, F. E. Etiologic spectrum and pattern of antimicrobial drug susceptibility in bacterial meningitis in Sokoto, Nigeria. *Acta Paediatrica*, 2000, 89: 942-946.
24. Adejuyigbe, E. A., Adeodu, O. O., Ako-Nai, K. A., Taiwo. O. and Owa, J. A. Septicaemia in high risk neonates at a teaching hospital in Ile-Ife, Nigeria. *East African Medical Journal*, 2001, 78: 540-543.
25. Ojukwu, J. U., Abonyi, L. E., Ugwu, J., Orji, I. K. Neonatal septicaemia in high risk babies in South- Eastern Nigeria. *Journal of Perinatal Medicine*, 2006, 34: 166-172.
26. Bolarinwa, R. A., Aboderin, O. A., Odetoyin, B. W. and Adegunloye, A. B. Bacterial contamination of blood and blood components in a tertiary hospital setting in Nigeria. *International Journal of Infection Control*, 2011, 7: 1 -11.
27. Nwadioha, S. I., Nwokedi, E. P., Onwuezube, I., Egesie, J. O. and Kashibu, E. Bacterial isolates from cerebrospinal fluid of children with suspected acute meningitis in a Nigerian tertiary hospital. *Nigerian Postgraduate Medical Journal*, 2013, 20: 9-13.
28. Nwaiwu, O. Mini review: An overview of *Listeria* species in Nigeria. *International Food Research Journal*, 2015, 22(2): 455-464.
29. Hitchins, A. D. Detection and enumeration of *Listeria monocytogenes* in foods. US Food and Drug Administration's Bacteriological Analytical Manual. Chapter 10. Available: <http://www.cfsan.fda.gov/~ebam/bam-10.html>. 2003 (Accessed 10 October 2014)
30. Stelma, G. N. Jr, Reyes, A. L., Peeler, J. T., Francis, D. W., Hunt, J. M., Spaulding, P. L., Johnson, C. H. and Lovett, J. Pathogenicity test for *Listeria monocytogenes* using immunocompromised mice. *Journal of Clinical Microbiology*, 1987, 25: 2085-2089.
31. Painter, J. and Slutsker, L. Listeriosis in humans. In *Listeria, Listeriosis and Food Safety*. 3 edition. Edited by: Ryser ET, Marth EH. Boca Raton: CRC Press; 2007, 85-109.
32. Swaminathan, B. and Gerner-Smidt, P. The epidemiology of human listeriosis. *Microbes and Infection*, 2007, 9: 1236-1243.
33. Pradhan, A. K., Ivanek, R., Gröhn, Y. T., Bukowski, R., Geornaras, I., Sofos, J. N. and Wiedmann, M. Quantitative risk assessment of listeriosis-associated deaths due to *Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. *Journal of Food Protection*, 2010, 73: 620-630.
34. Todd, E. and Notermans, S. Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control*, 2011, 22: 1484-1490.
35. Park, S. H., Chang, P. S., Ryu, S. and Kang, D. H. Development of a novel selective and differential medium for the isolation of *Listeria monocytogenes*. *Applied Environmental Microbiology*, 2014, 80: 1020-1025.
36. Umeh, S. I. and Okpokwasili, G. S. C. Occurrence and antimicrobial profile of *Listeria monocytogenes* in fresh faeces of livestock animals. *Nigerian Journal of Microbiology*, 2009, 23(1): 1869-1875.
37. Kalender, H. Detection of *Listeria monocytogenes* in Faeces from Chickens, Sheep and Cattle in Elazığ Province *Turkish Journal of Veterinary and Animal Science*, 2003, 27: 449 - 451.
38. David, O. M. and Odeyemi, A. T. Antibiotic resistant pattern of environmental isolates of *Listeria monocytogenes* from Ado-Ekiti, Nigeria. *African Journal of Biotechnology*, 2007, 6: 2135-2139.
39. Esteban, J.I., Oporto, B., Aduriz, G., Juste, R. A. and Hurtado, A. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Veterinary Research*, 2009, 5:2.
40. Nwachukwu, N. C. and Orji, F. A. Studies on the isolation of *Listeria monocytogenes* from food, water, and animal droppings: environmental health perspective. In: Oosthuizen J. (Ed) *Environmental health - emerging issues and practice*. 2012. <http://www.intechopen.com/profiles/85404/nkechi-chuks-nwachukwu> on August 2016.
41. Safarpour, D. F., Barati, S., Momtaz, H., Hosseini, A. S. N., and Nejat, D. S. Comparison of shedding and antibiotics resistance properties of *Listeria monocytogenes* isolated from milk, feces, urine and vaginal secretion of bovine, ovine, caprine, buffalo and camel species in Iran. *Jundishapur Journal of Microbiology*, 2013, 6(3): 284 - 294.
42. Weindl, L., Frank, E., Ullrich, U., Heurich, M., Kleta, S., Ellerbroek, L. and Gareis, M. *Listeria monocytogenes* in different specimens from healthy red deer and wild boars. *Food borne pathogens and Disease*, 2016, 13(7): 391 - 397.
43. Szymczak, B., Szymczak, M. and Sawicki, W. Anthropogenic impact on the presence of *L. monocytogenes* in soil, fruits, and vegetables. *Folia Microbiologica*, 2014, 59: 23.
44. Liu, D. *Listeria monocytogenes*: comparative interpretation of mouse virulence assay. *FEMS Microbiology Letter*, 2004, 233: 159-164.
45. Enurah, L. U., Aboaba, O. O., Nwachukwu, S. C. U. and Nwosuh, C. I. Histopathological changes in the liver of mice challenged with *Listeria monocytogenes* in six zones of Nigeria. *Journal of Experimental Biology and Agricultural Sciences*, 2013, 1(5): 392 -397.
46. Pamukcu, T., Yarim, G. F., Kabakci, N., Yarim, M. and Duru, Ö. Experimental listeriosis in Rabbits: Biochemical Changes in Serum and Cerebrospinal Fluid. *Revue de Médecine Vétérinaire*, 2004, 156(5): 253-258.
47. Pandove, G., Sahota, P., Verma, S. K., Kirandeep, S. N., Kaur, B. and Vikal, Y. Epidemiological surveillance, virulence and public health significance of *Listeria* spp. from drinking water. *African Journal of Biotechnology*, 2013, 12(1): 74-83.
48. Kholoud, S. R., Iman, M. K. A., Rasha, E. H. and Soha, S.S. A. The effect of experimental streptococcus infection in myocarditis on some biochemical and inflammatory markers in albino rats. *African Health Sciences*, 2013, 13(4): 1062 - 1070.
49. Liu, D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important food borne pathogen. *Journal of Medicinal Microbiology*, 2006, 55: 645- 659.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2018
AJCEM/1816
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.6>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 113-120

ISBN 1595-689X VOL19 No.2
<http://www.ajol.info/journals/ajcem>

AEROBIC BACTERIA AND FUNGAL ISOLATES IN MAXILLARY SINUSITIS OF ADULTS IN A RESOURCE POOR ENVIRONMENT

Fasunla, A. J., Fayemiwo** S. A., Bakare** R. A., Ijaduola, G. T. A.

Department of Otorhinolaryngology and **Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria.

Correspondence: Dr Ayotunde James FASUNLA, Department of Otorhinolaryngology, College of Medicine, University of Ibadan & University College Hospital, Ibadan, Oyo State. Email address: ayofasunla@yahoo.com
Phone number: +2348033740220

ABSTRACT

Background: Infective rhinosinusitis is a common clinical condition which if left unattended to could result in various degrees of both morbidity and mortality. We aimed to identify aerobic and fungal organisms implicated in acute and chronic maxillary sinusitis and determine their antibiotic sensitivity patterns among adults in South Western Nigeria.

Materials and methods: This was a cross sectional study of adults with clinical and radiological diagnosis of maxillary sinusitis treated at the University College Hospital, Ibadan over a period of one-year. Semi- structured questionnaire was administered to each consented adult to obtain relevant demographic and clinical data. Maxillary antral proof puncture was done to obtain specimen for microscopy, culture and sensitivity for aerobic bacterial and fungal isolates. Descriptive statistics was used in the data analysis.

Results: Seventy-nine patients (49.4% males and 50.6% females) with acute maxillary sinusitis (17.7%), and chronic maxillary sinusitis (82.3%) were recruited into the study. The mean age of the patients was 32.9 years (SD=12.78; Range: 19-59). All patients presented with rhinorrhea while 92.8% had nasal obstruction. Fifty eight (73.4%) patients had history of antibiotic usage before presentation. Eight (57.1%) of the specimens from acute maxillary sinusitis cases and 40 (61.5%) of the specimens from chronic maxillary sinusitis yielded significant growth of bacteria and fungi respectively while 2 (3.5%) yielded mixed bacterial growth. Organisms commonly isolated from these specimens were *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Aspergillus* spp. The bacteria isolates were sensitive to Amoxicillin, Ciprofloxacin, Perfloxacin, Sparfloxacin and Ceftriaxone.

Conclusion: The leading aerobic bacterial isolates from acute and chronic maxillary sinusitis were *Streptococcus pneumoniae* and *Staphylococcus aureus* respectively. Fungal infections are seen only in chronic cases. It is recommended that where there are no microbiologic laboratory facilities, any of Ciprofloxacin, Perfloxacin, Sparfloxacin, and Amoxicillin can be administered empirically to treat infective maxillary sinusitis.

Key words: Aerobic bacteria, Fungus, Maxillary sinus, Rhinosinusitis

LES BACTÉRIES AÉROBIES ET DES ISOLATS FONGIQUES DANS LA SINUSITE MAXILLAIRE D'ADULTES DANS UN ENVIRONNEMENT À FAIBLES RESSOURCES

Fasunla, A. J., Fayemiwo**, S. A., Bakare** R. A., Ijaduola G. T., A.

Ministère de l'oto-rhino-laryngologie et **Département de microbiologie médicale et de parasitologie, Faculté de médecine de l'Université d'Ibadan et de l'University College Hospital, Ibadan, Nigeria.

Correspondance: Dr Ayotunde James FASUNLA, Ministère de l'oto-rhino-laryngologie, Collège de médecine, Université d'Ibadan et Hôpital du Collège universitaire, Ibadan, Oyo State. Adresse e-mail :
Numéro de Téléphone : 2348033740220 ayofasunla@yahoo.com

RÉSUMÉ

Contexte : rhinosinusite infectieuse est une condition clinique commun qui si laissé sans surveillance à pourrait donner lieu à des degrés divers de la morbidité et de la mortalité. Nous avons pour but d'identifier les organismes aérobie et fongiques impliqués dans la sinusite maxillaire aiguë et chronique et de déterminer leur sensibilité aux antibiotiques chez les adultes dans le sud-ouest du Nigeria.

Matériels et méthodes : Il s'agissait d'une étude transversale des adultes avec le diagnostic clinique et radiologique de la sinusite maxillaire traités à l'Hôpital du Collège universitaire, Ibadan sur une période d'un an. Semi- questionnaire structuré a été administré à chaque consenti des profils pour obtenir les données démographiques et cliniques.

Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

La preuve de l'antrite maxillaire crevaison a été fait pour obtenir l'échantillon pour la microscopie, la culture et la sensibilité pour les isolats fongiques et bactériennes aérobies. La statistique descriptive a été utilisé dans l'analyse des données.

Résultats : Soixante-neuf patients (49,4 % d'hommes et 50,6 % de femmes) avec une sinusite maxillaire aiguë (17,7 %), et la sinusite maxillaire chronique (82,3 %) ont été recrutés dans l'étude. L'âge moyen des patients était de 32,94 ans (ET = 12,78 ; Plage : 19-59). Tous les patients présentaient une rhinorrhée tandis que 92,8 % avaient l'obstruction nasale. Cinquante huit (73,4 %) patients avaient histoire de l'utilisation des antibiotiques avant la présentation. Huit (57,1 %) des échantillons de la sinusite maxillaire aiguë et 40 cas (61,5 %) des échantillons de la sinusite maxillaire chronique ont produit une croissance importante de bactéries et de champignons respectivement, tandis que 2 (3,5 %) a donné la croissance bactérienne mixte. Souvent les organismes isolés de ces spécimens ont été *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, et de *Aspergillus* spp. Les bactéries étaient sensibles à l'Amoxicilline, la ciprofloxacine, Perfloxacine, la sparfloxacine et la ceftriaxone.

Conclusion : Le leader des isolats bactériens aérobies de sinusite maxillaire aiguë et chronique ont été *Staphylococcus aureus* *Streptococcus pneumoniae* et respectivement. Organismes fongiques ne sont observées que dans les cas chroniques. Il est recommandé que, lorsqu'il n'y a pas d'installations de laboratoire microbiologique, l'autre de la Ciprofloxacine, Perfloxacine, la sparfloxacine, et de l'amoxicilline peut être administré de manière empirique pour traiter la sinusite maxillaire infectieux.

Mots clés : aérobie des bactéries, champignons, du sinus maxillaire rhinosinusite,

INTRODUCTION

Infective rhinosinusitis is a common clinical condition which if left untreated could result in various degrees of both morbidity and mortality (1, 2). It may start as non-infective rhinosinusitis and later become infected with bacteria. Often, there is involvement of more than one paranasal sinus but maxillary sinus is the most commonly affected. Treatment of infective rhinosinusitis with appropriate antibiotics can prevent complications and result in a satisfactory management outcome (3).

In Nigeria, antibiotics are readily available for procurement in the open market for usage because there is no enforcement of the laid down policies that restrict individuals or patients from having direct access to it without doctor's prescription. This injudicious antibiotics usage might induce growth of resistant bacterial strain with microbial dynamism in infective maxillary sinusitis (4, 5). Furthermore, abuse of antibiotics can lead to replacement of microbial organisms by fungal organisms (5).

There is paucity of literature on the current infective agents implicated in maxillary sinusitis and no agreed antibiotic regimen for the empirical treatment of infective rhinosinusitis in Nigeria. These have led to the injudicious antibiotic usage by the patients with resultant microbial dynamism. In addition, the relative lack of anti-bacteriological sensitivity pattern for infective maxillary sinusitis has caused uncertainty on the part of the clinicians in the choice of the most appropriate antibiotics to be administered as first line therapy where medical laboratory facility is unavailable. The increasing rates of antimicrobial resistance following abuse and misuse of antibiotics hamper logical treatment strategies. This makes it impossible to know which cases of maxillary sinusitis will spontaneously resolve or not hence, trial and error antimicrobial prescription on the part of clinicians is routinely practiced.

When patients have signs suggestive of infective rhinosinusitis, most of the time, they would have

used antibiotics indiscriminately and only when there is no improvement in their clinical condition that they present to the Clinicians, especially Otolaryngologists where available (6). At this stage, it is only by performing microscopy, culture and sensitivity test on the aspirate obtained directly from the maxillary antrum or middle meatus of such patients that the exact organisms responsible for the infection can be isolated and the antimicrobial sensitivity pattern known (7, 8).

Infective rhinosinusitis could result in serious morbidity and complications if neglected or inappropriately treated. The aims of this study were to isolate the pathogenic aerobic bacterial and fungal organisms that are implicated in maxillary sinusitis among adults in South Western Nigeria as well as to determine their antibiotic sensitivity pattern. This would be borne in mind in the selection of empirical treatment pending the result of microscopy, culture and sensitivity for aerobic bacteria and in environment where there is no easy access to microbiologic laboratory facility.

METHODOLOGY

Study design: This was a prospective hospital-based, cross sectional study of adults with maxillary sinusitis managed at the Department of Otorhinolaryngology, University College Hospital, Ibadan. Ethical approval was obtained from UI/UCH Ethical Review Board to conduct the study. An understood, written and verbal informed consent was obtained from all the participants and sample collection was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1996. Participants were adults, 18 years old and above, with clinical and radiological diagnosis of maxillary sinusitis (9).

Data Collection procedures

Questionnaire: A structured interview assisted questionnaire was administered to collect the participants' demographic and relevant clinical data of acute and chronic rhinosinusitis (10). Clinical diagnosis of rhinosinusitis was made if there were 2

or more major factors or 1 major factor and 2 minor factors (9).

Radiologic evaluation: Plain radiography of the paranasal sinuses (Occipitontental, occipitofrontal and lateral views) was performed on all the participants. The radiological features of maxillary sinusitis included haziness or opacification of maxillary antrum, gross mucosal thickening and/or presence of fluid level in the maxillary antrum (11).

Bacteriology and mycology: The maxillary antral specimen was obtained by aspiration through an inferior meatal antrostome created under good illumination using standard sterile and anesthetic procedures. Whenever there was a negative aspiration, 3-5mls of normal saline at body temperature was injected into the maxillary antrum and subsequently aspirated again (12). The maxillary sinus aspirates from the more affected antrum in a participant was sent immediately for microscopy, culture and sensitivity for aerobic bacterial and fungal studies in the diagnostic laboratory of the Department of Medical Microbiology, University College Hospital, Ibadan.

The antral aspirate of each patient was inoculated on Sheep blood and MacConkey agar for the culture of the aerobic bacterial organisms. Inoculated plates were then incubated aerobically at 37°C for 18-24 hrs. Bacterial isolates from these specimens were identified using standard bacteriological methods (13), and were then subjected to antibiotics susceptibility testing following the Clinical and Laboratory Standard Institute (CLSI) for the disc diffusion test (14). Pathogenicity of the isolated organism was determined using established pathogenic properties such as production of toxins and/or other virulence factors, and dominance of the organism in the infecting flora (10^3 cfu/ml). Part of the same specimen was also cultured for fungal organisms on Sabouraud Dextrose Agar at room temperature for 3 weeks and thereafter stained with lactophenol cotton blue for fungi identification if present. We performed disc susceptibility testings on cefuroxime (30ug), augmentin (10ug), amoxicillin (10ug), cloxacillin (10ug) erythromycin (30ug), ceftriaxone (30ug), ceftazidime (30ug), ciprofloxacin (5ug), ofloxacin (5ug), gentamycin (10ug) and Sparfloxacin (5ug). These antibiotics are readily available for use in our environment. The susceptibility patterns of the drugs were interpreted according to standard methods (14). Part of the antral aspirate of each patient was inoculated on Sabouraud Dextrose Agar and incubated at 37°C for 48hrs and room temperature for up to 3 weeks. The diagnosis of fungal infection was made on the basis of the recognisable and characteristic appearance of fungal hyphae fruiting bodies after staining with lacto phenol cotton blue under microscopy.

Data analysis: Data collected were collated and analyzed using Statistical Package for the Social Sciences (SPSS) version 18. The results were then presented in descriptive format, tables, diagrams

and graphs where appropriate. P value of < 0.05 was considered as statistically significant.

RESULTS

Seventy-nine patients (49.4% males and 50.6% females) with acute maxillary sinusitis and chronic maxillary sinusitis were recruited into the study. The mean age of the patients was 32.94 years (SD=12.78; Range: 19-59). Fourteen (17.70%) patients had acute rhinosinusitis and 65 (82.30%) had chronic rhinosinusitis. Fifty-eight (73.40%) patients had history of antibiotic usage before presentation. The clinical presentation of patients with acute rhinosinusitis and chronic rhinosinusitis are shown in tables 1 and 2 respectively.

TABLE 1: CLINICAL PRESENTATIONS OF THE PATIENTS WITH ACUTE RHINOSINUSITIS

Symptoms	Frequency (N)	Percentage (%)
Nasal discharge	14	100.00
Alternating nasal blockage	13	92.86
Facial pressure/ headache	12	85.71
Fatigue	8	57.14
Stuffy nose	8	57.14
Fever	7	50.00
Hyposmia/anosmia	2	14.29
Ear pain	2	14.29
Halitosis	1	7.14

Note: All patients had more than one symptom

TABLE 2: CLINICAL PRESENTATIONS OF PATIENTS WITH CHRONIC RHINOSINUSITIS

Symptoms	Frequency (N)	Percentage (%)
Nasal discharge	57	100.00
Alternating nasal blockage	49	85.96
Itching of eye or ear or nose or throat	44	89.80
Frequent throat hawking & clearing	43	75.44
Excessive sneezing	37	64.91
Hyposmia/anosmia	9	15.79
Facial pain/pressure or headache	7	12.28
Fatigue	5	8.77
Ear pain	5	8.77
Tooth ache	4	7.02
Cheek pain	4	7.02
Hoarseness	4	7.02
Halitosis	3	5.26

Note: All patients had more than one symptom

The diagnosis of maxillary sinusitis was further confirmed with plain radiographs of the paranasal sinuses. Mucosal thickening was found in 47 (59.49%) patients, opacification of the maxillary antrum in 29 (36.71%) patients and fluid level in 3 (3.80%) patients. Fluid level was found only on the Water's view of patients with acute maxillary sinusitis. Only one patient with acute maxillary sinusitis has radiologic evidence of bilateral maxillary antral opacity while four patients with chronic maxillary sinusitis had bilateral maxillary antral opacity.

Out of the 14 specimens from the maxillary antrum of the patients with acute maxillary sinusitis, 8 (57.14%) yielded bacterial growth (Table 3). None of the specimen yielded fungal growth.

However, of the 65 specimens from the maxillary antrum of patients with chronic maxillary sinusitis, only 40 (61.54%) yielded significant aerobic bacterial isolates (Table 4) while 7 (14.58%) yielded fungal isolates (Table 5). Two (3.51%) specimens yielded mixed bacteria growth. Twenty five (38.46%) specimens did not grow any bacteria.

TABLE 3: BACTERIA ISOLATES FROM ANTRAL SPECIMENS OF PATIENTS WITH ACUTE MAXILLARY SINUSITIS

	Bacteria isolated	Frequency(N)	Percentage (%)	Total
Gram Positive	<i>Streptococcus pneumonia</i>	4	50.00	N = 5 (62.50%)
	<i>Staphylococcus aureus</i>	1	12.50	
Gram Negative	<i>Haemophilus influenza</i>	3	37.50	N = 3 (37.50%)
Total		8	100.00	

TABLE 4: BACTERIA ISOLATES FROM ANTRAL SPECIMENS OF PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

	Bacteria isolated	Frequency (N)	Percentage (%)	Total
Gram Positive	<i>Staphylococcus aureus</i>	9	21.43	N = 19 (45.24%)
	<i>Streptococcus pneumoniae</i>	6	14.29	
	<i>Streptococcus pyogenes</i>	2	4.76	
	<i>Staphylococcus epidermidis</i>	1	2.38	
	<i>α-Hemolytic streptococcus</i>	1	2.38	
Gram Negative	<i>Pseudomonas aeruginosa</i>	8	19.05	N = 23 (54.76%)
	<i>Haemophilus influenza</i>	7	16.66	
	<i>Klebsiella spp</i>	6	14.29	
	<i>Escherichia coli</i>	2	4.76	
Total		42	100.00	41(100%)

TABLE 5: FUNGI ISOLATES FROM ANTRAL SPECIMENS OF PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

Fungal isolates	Frequency	Percentage (%)
<i>Aspergillus flavus</i>	3	42.86
<i>Aspergillus fumigatus</i>	2	28.57
<i>Candida albicans</i>	2	28.57
Total	7	100.00

The antibiotic sensitivity patterns of the cultured aerobic bacterial organisms are presented in Tables 6 and 7.

TABLE 6: ANTIBIOTIC SENSITIVITY PATTERN OF AEROBIC BACTERIA CULTURED FROM THE ANTRAL ASPIRATES OF PATIENTS WITH ACUTE MAXILLARY SINUSITIS

Bacteria isolates from acute maxillary antral specimen	In vitro antibiotic sensitivity [n (%)]											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Streptococcus pneumoniae</i>	4 (100)	4 (100)	3 (75)		4 (100)	4 (100)	4 (100)	3 (75)	3 (75)	2 (50)		4 (100)
<i>Staphylococcus aureus</i>		1 (100)			1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)		1 (100)
<i>Haemophilus influenza</i>	3 (100)	3 (100)		3 (100)	2 (66.7)	3 (100)		3 (100)	3 (100)		1 (33.7)	

1 = Pefloxacin, 2 = Ciprofloxacin, 3 = Ofloxacin, 4 = Gentamycin, 5 = Augmentin, 6 = Amoxicillin, 7 = Cloxacillin, 8 = Ceftazidime, 9 = Ceftriaxone, 10 = Cefuroxime, 11 = Erythromycin, 12 = Sparfloxacin

TABLE 7: ANTIBIOTIC SENSITIVITY PATTERN OF AEROBIC BACTERIA CULTURED FROM THE ANTRAL ASPIRATES OF PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

Bacteria isolates from chronic maxillary antral specimen	In vitro antibiotic sensitivity [n (%)]										
	1	2	3	4	5	6	7	8	9	10	12
<i>Staphylococcus aureus</i>	9 (100)	8 (88.9)	7 (77.8)	3 (33.3)	8 (88.9)	9 (100)	9 (100)	8 (88.9)	6 (66.9)		9 (100)
<i>Streptococcus pneumoniae</i>	6 (100)	6 (100)		6 (100)	6 (100)	6 (100)	6 (100)	5 (83.3)	5 (83.3)		6 (100)
<i>Staphylococcus epidermidis</i>	1 (100)	1 (100)			1 (100)	1 (100)		1 (100)	1 (100)	1 (100)	
<i>Streptococcus pyogenes</i>	2 (100)	1 (50)		1 (50)	2 (100)	2 (100)	2 (100)		1 (50)	2 (100)	1 (50)
<i>Haemophilus influenza</i>	7 (100)			7 (100)		7 (100)	6 (100)	7 (100)	7 (100)	4 (66.7)	6 (100)
<i>Pseudomonas aeruginosa</i>	6 (75)	6 (75)	8 (100)	8 (100)	8 (100)	4 (50)		8 (100)	8 (100)	7 (87.5)	8 (100)
<i>Klebsiella spp</i>	6 (100)		5 (83.6)	6 (100)	6 (100)	4 (66.7)			6 (100)		
<i>Escherichia coli</i>		2 (100)		2 (100)		1 (50)		2 (100)	2 (100)	1 (50)	
<i>α-Haemolytic streptococcus</i>	1 (100)		1 (100)	1 (100)	1 (100)	1 (100)	1 (100)				1 (100)

Erythromycin was excluded from Table 7 above as it was *staphylococcus epidermidis* and *streptococcus pneumoniae* from two specimens that were sensitive to it.

1 = Pefloxacin, 2 = Ciprofloxacin, 3 = Ofloxacin, 4 = Gentamycin, 5 = Augmentin, 6 = Amoxicillin, 7 = Cloxacillin, 8 = Ceftazidime, 9 = Ceftriaxone, 10 = Cefuroxime, 11 = Erythromycin, 12 = Sparfloxacin

DISCUSSION

Rhinosinusitis is the fifth most common diagnosis for which an antibiotic is prescribed and accounted for 21% of all adult antibiotic prescriptions (15). However, the sensitivity and/or the resistance patterns of the predominant pathogens vary considerably from region to region (16, 17). Therefore, the initial antimicrobial treatment of acute rhinosinusitis should be with the most narrow-spectrum agent that is active against the likely pathogens. In selecting an appropriate antibiotic for patients with rhinosinusitis, physicians must bear in mind the incidence of antibiotic-resistant bacteria in their community and consider the patient's overall health status. Special attention should be given to diseases that could impede normal recovery from infection and/or predispose to complications such as diabetes mellitus, chronic pulmonary disease, asthma, cystic fibrosis and immune deficiencies.

In this study, acute maxillary sinusitis constituted about 18% of the cases. None of these patients have co-morbid medical conditions. Studies have shown that most cases of maxillary sinusitis will present during the chronic phase and only few cases will present at the acute phase to physicians (8, 18). The relatively low percentage of those with acute maxillary sinusitis may be because of the practice of self-medication with antibiotics in our environment which could have resulted in a complete resolution of most of these patients' symptoms or cure without a need for presentation to the hospital.

This is supported by the evidence that 73.4% participants had history of antibiotic usage before presentation. In addition, some of the patients may not have severe disease hence would have been successfully managed by the General Practitioners or Family Physicians who are usually the first contact for these patients in hospitals. The persistence of the disease beyond 12 weeks will result in its chronicity (9, 19-22) as observed in about 82% of the participants who presented with features of chronic maxillary sinusitis. These may be the products of failed initial self-medication with a resultant severe form of the disease that has a negative impact on the quality of life and performance status of these patients. It could also be that the disease started initially as non-infectious rhinosinusitis which later became infected. The chronic infective maxillary sinusitis may either be caused by bacteria or fungal organisms.

Only 57.14% of the cultured maxillary antral aspirates from patients with acute maxillary sinusitis in this study yielded pathogenic organisms. It is possible that anaerobic organism, which was not included in this study because of non-availability of funds, was responsible for infection in the remaining 42.86% of maxillary antral aspirates which did not grow any organism. Anaerobic organisms have been reported as causes of acute maxillary sinusitis. (19, 21, 23) Similar studies have also reported that bacterial cultures were negative in certain proportion of suspected

cases of acute community acquired sinusitis (24, 25). This is similar to the finding in this study.

The spectrum of aerobic bacterial isolates from antral specimens of patients with acute maxillary sinusitis in this study is similar to what had been previously reported in the literature (12, 22, 25-30). However, variability exists in the frequencies at which these pathogenic organisms occurred. The frequency of *Streptococcus pneumoniae* or *Haemophilus influenza* as a leading cause of acute maxillary sinusitis varies from study to study (12, 22, 25-30). *Streptococcus pneumoniae* accounted for 50% of the isolated pathogen in this study. This was followed by *Haemophilus influenza* in 37.50% and the least was *Staphylococcus aureus* in 12.50%. The low frequency of *Staphylococcus aureus* in this study is also similar to what had been reported from a similar previous study (31). *Staphylococcus aureus* could be a normal nasal flora in 28 – 35% of healthy individuals (32) and if precaution was not taking during samples collection, it could contaminate maxillary antral aspirates. *Staphylococcus aureus* isolated in this study is likely to be a pathogen rather than a contaminant as the specimen was aspirated with a canula directly from the maxillary antrum into a sterile syringe before being cultured.

Out of the 57 specimens from the maxillary antrum of patients with chronic maxillary sinusitis, only 39 (68.42%) yielded pathogenic aerobic bacterial organisms. No growth was found in the culture from 18 (31.58%) specimens. Similar studies have also reported varied proportion of maxillary antral specimens which yielded pathogenic growth as observed by Aneke et al (57.41%), and Mantovani et al (53.2%) (8, 33). Anaerobic bacteria appear to play an important role in patients with chronic paranasal sinusitis (12). It is a possibility that anaerobic bacteria and other higher organisms, which were not included in this study for lack of funds, could have been responsible for the infection in these patients (42.86%) whose maxillary antral aspirates did not grow any aerobic organism. Hence, a similar study that will include isolation of anaerobic organisms from maxillary antral aspirate is desired. Some of these patients' maxillary antra had an initial negative tap or aspirate as at the time of the study but this was washed and the aspirate from it cultured. The antibiotic abuse among other medications by most of our patients could have rendered the antrum sterile as at the time of the study. Pathogenic anaerobic organisms have been isolated with varied frequencies from similar studies that included anaerobic isolation from maxillary antral specimens of patients with chronic maxillary sinusitis (19, 33-35).

The commonest aerobic bacterial isolate from chronic maxillary sinusitis in this study was *Staphylococcus aureus*, which constituted 21.43% of all isolated bacteria. This has been described as a

pathogenic cause of chronic infection of the paranasal sinuses. (35) Hence, its isolation as a pathogen in this study should not be regarded as a mere contaminant from a normal nasal flora (8). In a similar study by Aneke et al, *Staphylococcus aureus* topped the list of their total isolated pathogenic organisms with a frequency of 32.3% respectively (8).

Gram negative bacteria constituted the majority (54.76%) of the aerobic organisms isolated from the maxillary antral specimens of patients with chronic maxillary sinusitis in this study and also, *Pseudomonas aeruginosa* constituted 19.05% of all the aerobic isolates and 34.78% of the gram negative organisms. The predominance of gram negative organisms among the aerobic pathogenic agents in chronic maxillary sinusitis was also reported in similar studies (8, 20, 24, 25). This has been attributed to nonhygienic care and misuse of antibiotics, a problem in developing countries, which generally leads to persistence of resistance strains and chronicity. The *Haemophilus influenza* isolated from the maxillary antral aspirates in this study had also been reported by other workers (8, 23). This may be the cause of an acute exacerbation of chronic maxillary sinusitis because it has been more frequently implicated in acute maxillary sinusitis. *Streptococcus pyogenes* accounted for 4.76% of the isolates from this study. No single isolates of this organism was demonstrated from the similar study in this environment. (8, 25) *Staphylococcus epidermidis* accounted for 2.38% of isolates in this study. This has been demonstrated as one of the most frequent isolates (normal flora) from the nasal cavities of healthy individuals. (36) *Alpha-Hemolytic Streptococcus* was also cultured in one (2.38%) of our specimen.

Normal individuals have on the average more than two different fungal forms that are present in their nasal cavities (37). These could be normal body flora or contaminants even though could be pathogenic in some immunocompetent patients. Thus, over-colonization of the sinuses by fungi can also occur in immunocompromised individual with resultant opportunistic infections even though it was not sought for in this study. The low pH and decreased mucociliary clearance will trap the fungal spores and mycelia and lead to their growth and spread (38). Many authors have reported varied prevalence of fungal infection in chronic rhinosinusitis (39-42). In this study, only seven maxillary antral specimens yielded fungal growth (Table 5). The main fungal organisms isolated were *Aspergillus* and *Candida* species. *Aspergillus* spp have been isolated more frequently than other fungal agents in the maxillary aspirates (41, 42). The abuse of antibiotic usage seen in some of our patients might have contributed to the opportunistic fungal infection in them. None of the maxillary antral

specimen that grew fungus was positive for bacteria growth.

Amoxicillin and trimethoprim-sulfamethoxazole [Bactrim, Septrim]) have been recommended for use in the management of uncomplicated, acute bacterial rhinosinusitis (43, 44). Trimethoprim-sulfamethoxazole was not included in our antibiotic sensitivity pattern as it was observed from our institution that most organisms have developed resistance to it. This might be because of the abuse of the drug which can be easily procured from the counter in our environment. All the aerobic organisms cultured from the maxillary antral aspirate from acute maxillary sinusitis showed 100% in vitro sensitivity to Amoxicillin and Ciprofloxacin (Table 6).

Chronic maxillary sinusitis usually results primarily from a non-infective etiology but may have secondary superimpose bacteria infections.¹² Both Gram positive and Gram negative aerobic and anaerobic organisms have been reportedly cultured from the maxillary antral aspirates of some of these patients (25-27). The types of aerobic bacterial organisms isolated from this study is similar to what had been reported (5,8,12,25). The in vitro sensitivity activities of antibiotics against the isolated organisms from the maxillary antral specimen of patients with chronic maxillary sinusitis are shown in Table 7.

Quinolones (Ciprofloxacin, Pefloxacin and Sparfloxacin) and Penicillin based drugs (Amoxillin, Augumentin, Ceftriaxone) appear to have good sensitivity pattern against most of the

aerobic bacterial isolates. Gentamycin has 100% sensitivity pattern against most of the cultured organisms especially gram negative organism. However, *Staphylococcus aureus* and *Streptococcus pyogenes* displayed poor sensitivity to gentamycin. Nevertheless, Gentamycin should be used with caution as it has some ototoxic and nephrotoxic side effects.

Conclusion and Recommendation

The aerobic bacteria isolated from the acute and chronic maxillary sinusitis did not differ much from what had been known. However, *Streptococcus pneumoniae* and *Staphylococcus aureus* were the commonest bacterial isolates from the maxillary antrum of patients with acute maxillary sinusitis and chronic maxillary sinusitis respectively. *Aspergillus flavus* is the commonest fungal pathogen. The in vitro antibiotic sensitivity pattern varies hence the need for microscopy, culture and sensitivity of the antral aspirate in selecting appropriate antibiotics for the patients with rhinosinusitis. Nevertheless, where microbiologic laboratory facilities are unavailable, empirical Amoxicillin or Ciprofloxacin can be administered as a first line antibiotic therapy in the management of bacterial rhinosinusitis.

Acknowledgement

The authors appreciate all the patients who participated in the study. We also thank all ENT surgeons at the Department of Otorhinolaryngology, University College Hospital, Ibadan who gave permission to recruit their patients into the study.

REFERENCES

1. Fasunla, J.A., Adeleye, A.O., Onakoya, P.A., Okolo, C.A., and Nwaorgu, G.O. Recurrent nasal polyp and pansinus mucopyocele associated with bilateral blindness: a case report. Ghana Med. J. 2010;44(4):165-168
2. Fasunla, A.J., Nwaorgu, O.G.B. Adult Chronic Rhinosinusitis: Spectrum of Clinical Features in a Tertiary Health Institution and Literature Review. East Cent. Afr. J. Surg. 2011;16(1):12-18.
3. Goodyear, P.W.A., Firth, A.L., Strachan, D.R., and Dudley, M. Periorbital swelling: the important distinction between allergy and infection. Emerg. Med. J. 2004;21:240-242.
4. Mounghthong, G., Suwas, A., Jaruchida, S., Chantaratchada, S., Phonphok, Y., and Rangsin, R. Prevalence of etiologic bacteria and beta-lactamase-producing bacteria in acute and chronic maxillary sinusitis at Phramongkutklao Hospital. J. Med. Assoc. Thai. 2005; 88: 478 - 483.
5. Brook, I., Frazier, E.H., and Foote, P.A. Microbiology of the transition from acute to chronic maxillary sinusitis. J. Med. Microbiol. 1996; 45: 372 - 375
6. Poole, M.D., and Portugal, L.G. Treatment of rhinosinusitis in the outpatient setting. Am. J. Med. 2005; 118 Suppl 7A:45S-50S.
7. Manes, R.P., and Batra, P.S. Bacteriology and antibiotic resistance in chronic rhinosinusitis. Facial Plast. Surg. Clin. North Am. 2012;20(1):87-91.
8. Aneke, E.C., and Ezeanolue, B.C. Profile of aerobic bacteria isolated in chronic maxillary sinusitis patients. Niger. Postgrad. Med. J. 2004; 11: 116 - 120.
9. Lanza, D.C., Dhong, H.J., Tantilipikorn, P., Tanabodee, J., Nadel, D.M., and Kennedy, D.W. Fungus and chronic rhinosinusitis: from bench to clinical understanding. Ann. Otol. Rhinol. Laryngol. Suppl. 2006;196:27-34.
10. Benninger, M.S., Ferguson, B.J., and Hadley, J.A. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. Otolaryngol Head Neck Surg. 2003; 129: 1-32
11. Ezeanolue, B.C., Aneke, E.C., and Nwagbo, D.F. Correlation of plain radiological diagnostic features with antral lavage results in chronic maxillary sinusitis. West Afr. J. Med. 2000; 19: 16 - 18.
12. Adachi, M., Furuta, S., Suzuki, S., and Maeda, T. Bacterial examination of sinusitis using antral puncture and irrigation. Nihon Jibiinkoka Gakkai Kaiho. 2002;105(9):925-930.
13. Cowan, S.T., Barrow, G., Steel, K.J., and Feltham, R. Cowan and Steel's manual for the identification of medical bacteria: Cambridge university press; 2004.

14. Ginocchio, C.C. Role of NCCLS in antimicrobial susceptibility testing and monitoring. American journal of health-system pharmacy: AJHP: official journal of the American Society of Health-System Pharmacists. 2002; 59(8 Suppl 3):S7-11.
15. Brook, I., Foote, P.A., and Frazier, E.H. Microbiology of acute exacerbation of chronic sinusitis. Ann. Otol. Rhinol. Laryngol. 2005;114(7):573-576.
16. Kaplan, A. Canadian guidelines for acute bacterial rhinosinusitis: clinical summary. Can. Fam Physician. 2014;60(3):227-234.
17. Felmingham, D., Feldman, C., Hryniewicz, W., Klugman, K., Kohno, S., Low, D.E., Mendes, C., and Rodloff, A.C. Surveillance of resistance in bacteria causing community-acquired respiratory tract infections. Clin. Microbiol. Infect. 2002; 8 Suppl 2:12-42.
18. Uri, N., Cohen-kerem, R., Elmalah, I., Doweck, I., and Greenberg, E. Classification of fungal sinusitis in immunocompetent patients. Otolaryngol. Head Neck Surg. 2003; 129(4):372-378.
19. Brook, I. The role of anaerobic bacteria in sinusitis. Anaerobe. 2006;12(1):5-12.
20. Brook, I. Microbiology of chronic rhinosinusitis. Eur. J. Clin. Microbiol. Infect. Dis. 2016;35(7):1059-1068.
21. Poole, M.D. Acute bacterial rhinosinusitis: clinical impact of resistance and susceptibility. Am. J. Med. 2004; 117 Suppl 3A:29S-38S.
22. Christmas, D.A., Mirante, J.P., and Yanagisawa, E. Maxillary sinusitis caused by nasoseptal obstruction. Ear Nose Throat J. 2006;85(3):144 - 146.
23. Hoban, D., and Felmingham, D. The PROTEKT surveillance study: antimicrobial susceptibility of *Haemophilus influenza* and *Moraxella catarrhalis* from community-acquired respiratory tract infections. J. Antimicrob. Chemother. 2002;50 Suppl S1: 49 - 59.
24. Marghani, O.A., Alherabi, A.Z., Herzallah, I.R., Saati, F.A., Tantawy, E.A., Alandjani, T.A., Faidah, H.S., Bawazeer, N.A., Marghalani, A.A., and Madani, T.A. Acute rhinosinusitis during Hajj season 2014: Prevalence of bacterial infection and patterns of antimicrobial susceptibility. Travel Med. Infect. Dis. 2016;14(6):583-587.
25. Ologe, F.E., and Nwabuisi, C. Bacteriology of chronic sinusitis in Ilorin, Nigeria. Afr. J. Clin. Exp. Microbiol. 2003;4:91-97.
26. Dubin, M.G., Ebert, C.S., Coffey, C.S., Melroy, C.T., Sonnenburg, R.E., and Senior, B.A. Concordance of middle meatal swab and maxillary sinus aspirate in acute and chronic sinusitis: a meta-analysis. Am. J. Rhinol. 2005;19(5):462-470.
27. Olwoch, I.P. Microbiology of acute complicated bacterial sinusitis at the University of the Witwatersrand. S. Afr. Med. J. 2010;100(8):529-533.
28. Brook, I., and Hausfeld, J.N. Microbiology of acute and chronic maxillary sinusitis in smokers and nonsmokers. Ann. Otol. Rhinol. Laryngol. 2011;120(11):707-712.
29. Wald, E.R. Staphylococcus aureus: is it a pathogen of acute bacterial sinusitis in children and adults? Clin. Infect. Dis. 2012;54(6):826-831.
30. Brook, I. Bacteriology of Chronic Sinusitis and Acute Exacerbation of Chronic Sinusitis. Arch. Otolaryngol. Head Neck Surg. 2006; 132(10): 1099 - 1101.
31. Payne, S.C., and Benninger, M.S. Staphylococcus aureus is a major pathogen in acute bacterial rhinosinusitis: a meta-analysis. Clin. Infect. Dis. 2007;45(10):e121-127.
32. Bergogne-Bérézin, E. Controlling acute bacterial maxillary sinusitis. Expert opin. Pharmacother. 2002; 3(10):1471-1479.
33. Mantovani, K., Bisanha, A.A., Demarco, R.C., Tamashiro, E., Martinez, R., and Anselmo-Lima, W.T. Maxillary sinuses microbiology from patients with chronic rhinosinusitis. Braz. J. Otorhinolaryngol. 2010;76(5):548-551.
34. Ikeda, K., Ono, N., Lizuka, T., Kase, K., Minekawa, A., Inoshita, A., and Kusunoki, T. Bacteriologic evaluation of sinus aspirates taken by balloon catheter devices in chronic rhinosinusitis: preliminary study. ORL. J. Otorhinolaryngol. Relat. Spec. 2011;73(5):271-274.
35. Ivanchenko, O.A., Karpishchenko, S.A., Kozlov, R.S., Krechikova, O.I., Otvagin, I.V., Sopko, O.N., Piskunov, G.Z., and Lopatin, A.S. The microbiome of the maxillary sinus and middle nasal meatus in chronic rhinosinusitis. Rhinology. 2016;54(1):68-74.
36. Finegold, S.M., Flynn, M.J., Rose, F.V., Jousimies-somer, H., Jakielaszek, C., McTeague, M., Wexler, H.M., Berkowitz, E., and Wynne, B. Bacteriologic Findings Associated with Chronic Bacterial Maxillary Sinusitis in Adults. Clin. Infect. Dis. 2002; 35(4):428-433.
37. Lackner, A., Stammberger, H., Buzina, W., Freudenschuss, K., Panzitt, T., Schosteritsch, S., and Braun, H. Fungi: a normal content of human nasal mucus. Am. J. Rhinol. 2005;19(2):125-129.
38. Correll, D.P., Luzi, S.A., and Nelson, B.L. Allergic fungal sinusitis. Head Neck Pathol. 2015;9(4):488-491.
39. Gelber, J.T., Cope, E.K., Goldberg, A.N., and Pletcher, S.D. Evaluation of Malassezia and common fungal pathogens in subtypes of chronic rhinosinusitis. Int. Forum. Allergy. Rhinol. 2016;6(9):950-955.
40. Nazeri, M., Hashemi, S.J., Ardehali, M., Rezaei, S., Seyedmousavi, S., Zareei, M., and Hosseini, E. Fungal rhino sinusitis in tehran, Iran. Iran J. Public Health. 2015;44(3):374-379.
41. Murr, A.H., Goldberg, A.N., Pletcher, S.D., Dillehay, K., Wymer, L.J., and Vesper, S.J. Some chronic rhinosinusitis patients have elevated populations of fungi in their sinuses. Laryngoscope. 2012;122(7):1438-1445.
42. Marple, B.F. Allergic fungal rhinosinusitis: current theories and management strategies. Laryngoscope. 2001;111(6):1006-1019.
43. Sabino, H.A., Valera, F.C., Aragon, D.C., Fantucci, M.Z., Titoneli, C.C., Martinez, R., Anselmo-Lima WT, and Tamashiro, E. Amoxicillin-clavulanate for patients with acute exacerbation of chronic rhinosinusitis: a prospective, double-blinded, placebo-controlled trial. Int. Forum Allergy Rhinol. 2017;7(2):135-142.
44. Won, Y.S., Brichta, A., Baroody, F., Boonlayangoor, S., and Naclerio, R. Bactrim reduces the inflammatory response in a murine model of acute rhinosinusitis. Rhinology. 2000;38(2):68-71.

CASE REPORT

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2018 ISBN 1595-689X VOL19 No.2
AJCEM/1817 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.7>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2):121-124

ATYPICAL PRESENTATION OF GENITAL HERPES IN A RETROVIRAL DISEASE PATIENT ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY

Nwadike¹, V. U., Anaedobe², C. G., Azeez¹, R. A., Jinadu¹, S.S., & Chigozie¹, J. O.

¹Medical Microbiology Unit, Department of Pathology, Federal Medical Center, Abeokuta;

² Department of Medical Microbiology and Parasitology, College of Health Sciences, University of Abuja

Correspondence: Dr. Anaedobe Chinenye Gloria, Department of Medical Microbiology and Parasitology, University of Abuja, FCT, Nigeria. Phone: +234 806 784 4403 Email: chimedico@yahoo.com

ABSTRACT

Herpes Simplex Virus type 2 (HSV-2) is the leading cause of Genital Ulcer Disease (GUD) worldwide. In HIV infected persons, it typically presents with increased number of recurrent genital lesions which often have severe and prolonged presentations. There are reports that patients receiving highly active antiretroviral therapy (HAART) may be more prone to chronic genital ulcers and a higher risk of acyclovir-resistant herpes infection than is seen in immune competent patients. We present a case of recurrent genital herpes infection in a sexually active 15 year old known HIV patient.

Keywords: Recurrent genital ulcer, HSV-2, HIV, HAART

PRÉSENTATION ATYPIQUE DE L'HERPÈS GÉNITAL CHEZ UN PATIENT DE LA MALADIE RÉTROVIRALE HAUTEMENT ACTIVE SUR LA THÉRAPIE ANTIRÉTROVIRALE

Nwadike¹, V. U., Anaedobe², C. G., Azeez¹, R. A., Jinadu¹, S.S., & Chigozie¹, J. O.

¹Unité de microbiologie médicale, Département de Pathologie, centre médical fédéral, Abeokuta;

²Département de microbiologie médicale et parasitologie, Collège des Sciences de la santé, l'Université d'Abuja

Correspondance: Dr. Anaedobe Chinenye Gloria, Département de microbiologie médicale et de parasitologie, Université d'Abuja, Nigéria, FCT. Téléphone : +234 806 784 4403 Email : chimedico@yahoo.com

RÉSUMÉ

Herpès simplex type 2 (HSV-2) est la principale cause d'ulcérations génitales (UG) dans le monde entier. Les personnes infectées par le VIH, en règle générale, elle présente à l'augmentation du nombre de lésions génitales récurrentes qui ont souvent des présentations grave et prolongée. Il y a des rapports que les patients recevant un traitement antirétroviral hautement actif (HAART) peuvent être plus sujets à des ulcérations génitales chroniques et un risque plus élevé de l'infection d'herpès résistant à l'acyclovir qu'est vu dans l'abri les patients compétents. Nous présentons un cas d'herpès génital récurrent dans une infection sexuellement actif 15 ans VIH connues du patient.

Mots-clés: ulcère génital récurrent, HSV-2, le VIH, la multithérapie

INTRODUCTION

Genital herpes is typically caused by HSV-2 but can also be caused by HSV-1 (1, 2). In HIV patients with genital herpetic ulcers, particularly those receiving HAART, the presentation is often more severe and chronic with increasing episodes of recurrent genital ulcers. Several reasons have been postulated including immune reconstitution inflammatory syndrome (3, 4). It is believed that more severe clinical symptoms occurs following HAART due to

the improved immune status and more effective ability to mount an immune response against a pathogen (3).

Case Presentation

A fifteen year old boy from an urban lower class setting presented to the General Out-Patient Clinic (GOPD) of Federal Medical Center, Abeokuta, with a seven (7) month history of non-healing ulcerative lesion affecting the penile organ.

The ulcer was said to have been painful, pruritic and discharging serous fluid several months earlier. However, there was neither history of trauma to affected region, nor use of drugs besides prescribed ones. He had unprotected sexual intercourse on multiple occasions, two weeks before onset of the penile ulcer. Patient is known with vertically transmitted HIV infection and had been commenced on highly active anti-retroviral therapy (HAART) 22 months prior to presentation. He was compliant on his medications up to the time of presentation. His past medical history was otherwise notable for recurrent left neck sore of two years duration.

Patient presented afebrile and vital signs were within normal limits. Examination of the genitals revealed a non-tender, non-pruritic shallow ulcer extending from the lower border of his glans penis anteriorly to the anterolateral border of the shaft of his penis. This can be seen in Figure 1. All other systemic examinations were non-contributory.

VDRL done at presentation was non-reactive and wound swab culture yielded no growth after 48 hours of incubation, however, serology for HSV-2 done via ELISA was positive in the patient and absolute CD4+ count was 480 cells/mm³. He was commenced on a 10 day course of oral Acyclovir 200mg four times daily; he was also prescribed acyclovir cream, erythromycin and his routine anti-retroviral medications. He was booked for regular weekly STI clinic visits. Follow up clinic visits showed marked improvement of ulcer and resolution of the wound.



FIGURE 1: PENILE ULCER AT FIRST PRESENTATION

Six months later, the patient presented to the STI clinic with a recurrent penile ulcer that first appeared as a discharging blister with associated itching and pain on contact three weeks earlier. The lesion was large, measuring about 4cm by 3cm, had no clearly defined edges, and was tender to touch. There was no inguinal lymphadenopathy.

A diagnosis of reactivation of latent herpes simplex genital ulcer was subsequently made and he was placed on oral and topical acyclovir. He was also advised on good wound care and scheduled for follow up clinic visits. Subsequent clinic visits revealed increasing size of penile ulcer despite antiviral therapy. Patient however gave a history of poor drug compliance due to severe financial constraint. Efforts were made to ensure availability of drugs and proper drug compliance while patient was placed on acyclovir 400 mg PO daily as suppressive therapy. Subsequent follow up clinic visits several weeks later confirmed complete resolution of lesion.

DISCUSSION

Genital herpes, a well-recognized and globally endemic sexually transmitted infection (1, 2) is mostly as a result of HSV-2 infection (1). However, both HSV-1 and HSV-2 are capable of establishing latent infection in the sensory root ganglion of the lumbosacral region with capability for subsequent reactivation of the disease in later time. The statistics for the current prevalence and incidence of HSV-2 in Nigeria is not well documented. However, as at 2012, global estimated figures revealed that 417 million people aged 15–49 years were living with HSV-2 infection, of which 267 million were women (5). Also, 19.2 million individuals aged 15–49 years were newly-infected (5). The highest burden was in Africa (5).

Genital Herpes typically presents as a cluster of painful vesicular or ulcerative muco-cutaneous lesions on an erythematous base. These lesions often crust, re-epithelialize and heal without scarring. Transmission typically occurs when the virus enters into the body through broken skin or mucous membranes by direct sexual contact with the secretions or mucosal surfaces of an infected person (1). The virus subsequently multiplies at the point of entry before ascending along the sensory nerve roots to the dorsal root ganglion where it establishes latency. Reactivation triggers the transfer of the virus from the dorsal root ganglion back down to the nerve root on the muco-cutaneous surface where it usually causes symptoms to occur. However, subclinical viral shedding has been documented in more than 80 percent of HSV-2 seropositive persons with no reported lesions (6).

Index case is a case of a 15 year old boy known with vertically transmitted HIV infection who presented to our facility on account of a seven months history of recurrent penile ulcer which he noticed two weeks after series of unprotected sexual intercourse. He was subsequently diagnosed serologically positive for HSV-2 IgG. With onset of sexual activity at age of 15 years, this patient typifies those infected with their

first genital herpes infection which coincides with the onset of sexual activities. The history of immunosuppression further increases the risk of contracting the disease. There is a well-known relationship between human immunodeficiency virus and genital herpes infection as it has been observed and documented that the interaction between HSV-2 and HIV may result in more efficient transmission of HIV and an increased rate of HIV replication during HSV-2 reactivation (6-8).

Genital herpes in HIV infected persons typically presents with increased number and size of lesions both in primary and recurrent infections, atypical disease presentations and increased risk of genital shedding, more than is seen in immune competent patients (3, 4, 8). Also, the lesions are usually chronic, more severe and may or may not be associated with pain (3, 4). Studies have shown that as immunosuppression worsens with a drop in CD4 cell count, recurrent genital herpes increase in frequency, chronicity and severity unless interventions are made (3-6). Although HAART has been shown to reduce the duration of genital ulcers; there are reported cases of Immune Reconstitution Inflammatory Syndrome (IRIS) in HIV patients with genital herpes (3, 4, 7, 8). This is a paradoxical clinical worsening of an existing condition or the appearance of a new condition after initiating antiretroviral therapy in HIV-infected patients.

HSV-2 is one of the most common causes of IRIS and HSV-2 IRIS is associated with increased severity of ulcerative disease and increased frequency of recurrences (8). Some patients present with worsening genital ulcers despite receiving therapy, this is perhaps due to excessive immune reaction to the infectious agent. The IRIS can occur within few months following the initiation of HAART. There should be a high index of suspicion for it in the presence of atypical disease presentation, treatment failure with standard regimen amidst improving CD4

cell count and decreasing viral load. In this case, the patient's medical history of recurrent genital ulcers, the background of retroviral disease on HAART, atypical presentation of the ulcer and failure to respond to standard treatment regimen helped to confirm the diagnosis.

Recurrent genital herpes in HIV patients often poses a significant problem as patient often do not respond to standard treatment regimen. Acyclovir-resistant HSV infections are often seen and successful treatment with parenteral Valacyclovir, Famcyclovir and in some case topical corticosteroids, Imiquimod cream, have been reported (3, 4, 7). Suppressive Acyclovir therapy at the initiation of HAART in HIV/HSV-2 co-infected patients has been shown to blunt the anticipated increase in GUD incidence (8). It is worthy of note that an extended period of treatment with oral acyclovir was employed in the index case following failure of significant relief of symptoms after the standard 10 days of oral acyclovir therapy as per protocol. This suggests that in HIV/HSV-2 co-infected patients and particularly those on HAART, acyclovir-resistance should be looked out for and alternative treatment initiated early.

Conclusion: Genital herpes is frequent in HIV positive populations particularly those receiving HAART and may present with more severe, chronic and widespread lesions. They are more likely to be recurrent and be resistant to standard treatment regimen with oral acyclovir. Clinicians managing patients with HIV/HSV-2 co-infection should anticipate these atypical presentations and consider the use of suppressive acyclovir therapy or effective alternatives in order to reduce the severity of genital herpes in these patients.

Competing interests: The authors declare no competing interest.

Funding: The case report received no financial support.

REFERENCES

1. Looker KJ, Garnett GP, Schmid GP. An Estimate of the Global Prevalence and Incidence of Herpes Simplex virus type 2 infection. *Bull World Health Organ* 2008; 86(10): 805-12
2. Catriona Ooi and Vijay Zawar, "Hyperaesthesia following Genital Herpes: A Case Report," *Dermatology Research and Practice*, vol.2011, Article ID 903595, 3 pages, 2011.

3. Shelburne SA 3rd, Hamill RJ, Rodriguez-Barradas MC, Greenberg SB, Atmar RL, et al. Immune reconstitution inflammatory syndrome: Emergence of a unique syndrome during highly active antiretroviral therapy. *Medicine (Baltimore)* 2002; 81: 213-227
4. Couppié P, Sarazin F, Clyti E, El Guedj M, Vaz T, et al. Increased Incidence of Genital Herpes after HAART Initiation: A Frequent Presentation of Immune Reconstitution Inflammatory Syndrome (IRIS) in HIV-

- Infected Patients. *AIDS patient care STDs* 2006; **20**: (3) 143-145
5. Katharine J. Looker, Amalia S. Magaret, Katherine M. E. Turner, Peter Vickerman, Sami L. Gottlieb *et al.* Global Estimates of Prevalent and Incident Herpes Simplex Virus Type 2 Infections in 2012. *PLoS one* 2015; **10**(5): e0114989. <https://doi.org/10.1371/journal.pone.0114989>
6. Wald A, Zeh J, Selke S, Warren T, Ryncarz AJ, Ashley R, et al. Reactivation of genital herpes simplex virus 2 infection in asymptomatic seropositive persons. *N Engl J Med.* 2000; **342**:844-50
7. McKendry A, Narayana S, Browne R Atypical presentations of genital herpes simplex virus in HIV-1 and HIV-2 effectively treated by imiquimod. *Int J STD AIDS* 2014; **9**: 98-99.
8. Kenneth H. Fife, Kenneth Mugwanya, Katherine K Thomas, Jared M Baeten, Connie Celum, *et al.* Transient Increase in Herpes Simplex Virus Type 2 (HSV-2)-Associated Genital Ulcers Following Initiation of Antiretroviral Therapy in HIV/HSV-2-Co-infected Individuals. *The Journal of Infectious Diseases* 2016; **213** (10): 1573-1578,

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY
AJCEM/1818
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.8>
AFR. J. CLN. EXPER. MICROBIOL 19 (2): 125 -132

MAY 2018 ISBN 1595-689X VOL19 No.2
<http://www.ajol.info/journals/ajcem>

CO-INFECTION OF HEPATITIS B AND C VIRUSES AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED CHILDREN IN LAGOS, NIGERIA

RUNNING TITLE: CO-INFECTION OF HBV AND HCV IN HIV POSITIVE CHILDREN

Salu, O. B.^{1,2}, Oyefolu, A. O. B.³, Gbadegesin, A.⁴, James, A. B.^{2,5}, Oke, B. O.¹, Ashaka, O. S.⁶, Anjorin, A. A.³, Akinyemi, K. O.³ and Omilabu^{1,2*}, S. A.

¹Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos, Nigeria; ²Virology Research Laboratory, Central Research Laboratory, College of Medicine, University of Lagos, Lagos, Nigeria; ³Department of Microbiology, Lagos State University, Ojo P. M. B. 0001, LASU, Ojo, Lagos, Nigeria; ⁴Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria; ⁵Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria; ⁶Department of Medical Microbiology and Parasitology, College of Health Sciences, University of Ilorin, Ilorin, Kwara, Nigeria. *Professor, Team Lead and the Director, Central Research Laboratory, College of Medicine, University of Lagos, Lagos State, Nigeria.

Corresponding Author: Salu, Olumuyiwa B.; E-mail: obsalu@yahoo.com; Tel: +2348023055077

ABSTRACT

Introduction: The co-infection of Human immunodeficiency virus (HIV), Hepatitis B and C viruses remains a public health problem particularly in resource limited setting like Nigeria. Studies on these co-infections have been done principally among adult and pregnant women with limited information on the pediatric population. The study aims at documenting the burden and the patterns of HIV/HBV, HIV/HCV and HIV/HBV/HCV co-infections in children in Lagos, Nigeria.

Methods: A cross-sectional study carried out at the Virology Research Laboratory, College of Medicine of the University of Lagos between December 2008 and January 2014. A total of 393 confirmed HIV infected children aged between <1 to 15 years were screened from two tertiary health facilities; Lagos State University Teaching Hospital (LASUTH, n=272) and Lagos University Teaching Hospital (LUTH, n=121), Lagos. Plasma samples were screened for markers for HBV (HBsAg, HBeAg, HBcAb, HBcIgM) and HCV (anti-HCV) using a fourth generation enzyme-linked immunosorbent assay (DIA. PRO. Diagnostic Bioprobes Srl., Italy).

Results: Out of the 393 samples analyzed, 40 (10.2%) were sero-positive for dual HIV/HBV co-infection, comprising 21 (52.5%) females and 19 (47.5%) males, while 15 (3.8%) had detectable antibodies to HCV consisting of 7 (46.7%) females and 8 (53.3%) males without any statistical significance. On the overall, two (0.5%) of the participants were seropositive for triple HIV, HBV and HCV co-infections. HIV/HBV co-infection was detected among all the age groups, whereas, HIV/HCV co-infection was not seen among children <1 to 5 years.

Conclusion: This analysis confirmed a high prevalence of HBV, low prevalence of HCV and suggests that chronic hepatitis may be prevalent among our HIV-infected children. Thus, routine screening and early detections are therefore necessary for an appropriate treatment plan for children co-infected with HIV/HBV and or with HCV.

Keywords: Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Co-infection and Enzyme-Linked Immunosorbent Assay (ELISA).

. Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

CO-INJECTION DE VIRUS DE L'HÉPATITE B ET C CHEZ LES ENFANTS INFECTÉS PAR LE VIRUS DE L'IMMUNODÉFICIENCE HUMAINE À LAGOS, NIGERIA

TITRE COURANT: LA CO-INFECTION PAR LE VHB ET LE VHC DANS LES ENFANTS SÉROPOSITIFS

Salu, O. B.^{1,2}, Oyefolu, A. O. B.³, Gbadegesin, A.⁴, James, A. B.^{2,5}, Oke, B. O.¹, Ashaka, O. S.⁶, Anjorin, A. A.³, Akinyemi, K. O.³ and Omilabu^{1,2*}, S. A.

1Département de microbiologie médicale et de parasitologie, Faculté de médecine de l'Université de Lagos, l'État de Lagos, Nigeria ; Unité de virologie, Laboratoire 2 Laboratoire de recherche centrale, Collège de médecine, Université de Lagos, l'État de Lagos au Nigeria ; 3Département de microbiologie, Université d'État de Lagos, Ojo P. M. B., 0001, LASU Ojo, l'État de Lagos, Nigeria ; 4Département d'obstétrique et de gynécologie, Hôpital d'enseignement de l'Université d'État de Lagos, Ikeja, Lagos State, Nigeria ; 5Département de biochimie, Collège de La médecine, l'Université de Lagos, l'État de Lagos, Nigeria ; 6Département de microbiologie médicale et parasitologie, Collège des Sciences de la santé, Université d'Ilorin, Ilorin. L'État de Kwara, le Nigeria. *Le professeur, chef d'équipe et le directeur, Laboratoire de recherche du Centre, Collège de médecine, Université de Lagos, l'État de Lagos au Nigeria.

Auteur correspondant : Salu, Olumuyiwa B. ; E-mail : obsalu@yahoo.com ; Tel : 2348023055077

RÉSUMÉ

Introduction: La co-infection du virus de l'immunodéficience humaine (VIH), le virus de l'hépatite B et C reste un problème de santé publique en particulier dans les pays à ressources limitées comme le Nigeria. Des études sur ces co-infections ont été réalisées principalement chez les adultes et les femmes enceintes avec l'information limitée sur la population pédiatrique. L'étude vise à documenter le fardeau et l'évolution du VIH/VHB, VIH/VHC et VIH/VHB/VHC co-infections chez les enfants à Lagos, Nigeria.

Méthodes: Une étude transversale effectuée au Laboratoire de recherche de virologie, Collège de médecine de l'Université de Lagos, entre décembre 2008 et janvier 2014. A confirmé un total de 393 enfants infectés par le VIH âgés de moins de 1 à 15 ans ont été sélectionnés à partir de deux établissements de santé tertiaires ; hôpital d'enseignement de l'Université d'État de Lagos (LASUTH, n =272) et l'Hôpital d'enseignement de l'Université de Lagos (luth, n =121), Lagos. Des échantillons de plasma ont été projetés pour les marqueurs pour le VHB (AGHBS, l'AGHBE, HBeAb, HBcIgM) et du VHC (anti-VHC) à l'aide d'une quatrième génération de dosage immuno-enzymatique (DIA. PRO. Bioprobes Diagnostic Srl., Italie).

Résultats: Sur les 393 échantillons analysés, 40 (10,2 %) étaient séropositifs pour le VIH/VHB co-infection, composée de 21 (52,5 %) et 19 femmes (47,5 %) hommes et 15 (3,8 %) présentaient des anticorps détectables au VHC composé de 7 (46,7 %) et 8 femmes (53,3 %) hommes sans aucune signification statistique. Sur l'ensemble, deux (0,5 %) des participants étaient séropositifs pour le VIH, VHB et VHC co-infections. Le VIH/VHB co-infection n'a été détectée parmi tous les groupes d'âge, tandis que, le VIH/VHC co-infection n'a pas été observé chez les enfants de moins de 1 à 5 ans.

Conclusion: Cette analyse a confirmé une forte prévalence du VHB, une faible prévalence du VHC et suggère que l'hépatite chronique peut être répandue chez nos enfants infectés par le VIH. Ainsi, le dépistage systématique et au début les détections sont donc nécessaires pour un plan de traitement approprié pour les enfants co-infectés par le VIH et le VHB et par le VHC ou.

Mots-clés: virus de l'immunodéficience humaine (VIH), l'hépatite B (VHB), le virus de l'hépatite C (VHC), la co-infection et de dosage immuno-enzymatique (ELISA).

INTRODUCTION

The convergence of Human immunodeficiency virus (HIV), Hepatitis B and C viruses is of great individual clinical significance and a public health challenge at large [1]. While HIV is the causative agent of Acquired Immune Deficiency Syndrome (AIDS), the other two viruses cause Hepatitis, which is a hepatocytic inflammation of the liver [2, 3].

The hallmarks of HIV infection are transmissibility, chronicity and progression to AIDS through gradual

destruction of the immune system, when there is no appropriate intervention [4]. Despite recent declines in HIV/AIDS mortality globally, there is considerable heterogeneity in the levels and patterns of the infection across countries. While many countries have experienced reductions in HIV/AIDS annual new infections and mortality, other countries have had insignificant responses or upsurges in rates of annual new infections [5, 6]. An estimated 60% of new HIV infections in Central and Western Africa was reported from Nigeria in 2015. The country's adult HIV prevalence stands at 3.1% in the report of National

Agency for Control of AIDS (NACA)-2015. The same report gave an estimate of 260,000 children (0 to 14 years) in Nigeria living with HIV as of 2015 [7].

Globally, an estimated 257 million and 71 million people were living with chronic HBV and HCV infection respectively in 2015 [8]. Hence, due to similar or shared routes of transmission, the occurrence of Hepatitis B and C viruses with HIV, albeit as a single or co-infection remains a common public health problem [3, 9]. However perinatal transmission is the most important route of spread to children, due to high rates of infection of these viruses in pregnant women [10, 11]. Although, co-infection with HBV and HCV is a global challenge, the impact is greater on health resource poor settings, particularly Sub-Saharan Africa. This is accentuated by an earlier report of prevalence rates of HBV and HCV of 15% and 7%, respectively among HIV infected people in the region [12].

Varied prevalence rates have been recorded for HBV in Nigeria for children ranging from 4.1% to 44.7% [13], with the country said to have a pooled prevalence of 14% for Hepatitis B virus infection [14]. There is however a dearth of similar data for HCV in the country. Similarly, there is a dearth of combined data for co-infection of HBV and HCV among children with HIV in the country. Studies elsewhere in Africa have shown significant association of HBV and HCV (9.7% and 7% respectively) in HIV infected children [15].

Infections with HBV and or HCV leading to liver hepatitis are leading causes of morbidity and mortality in HIV infected children. As such the WHO recommends that such children should be diagnosed and provided with appropriate and effective treatment for both HIV and the hepatitis [8]. However, this is a challenge in resource limited countries where resources and capacities are inadequate to offer appropriate full laboratory hepatitis viral assays, to evaluate the co-infections of HBV and HCV with HIV. More studies are therefore required to address the burden of the convergence of these viruses in this setting, which informed this study.

MATERIALS AND METHODS

Study Centre: This study was a cross-sectional study carried out at the Virology Research Laboratory (Central Research Laboratory), College of Medicine of the University of Lagos.

Study Population: The study participants were 393 HIV infected children aged between <1 to 15 years confirmed to be HIV positive with Polymerase Chain Reaction (PCR) for children <18 months or Western blot for children ≥18 months born to HIV-positive

mothers attending either the Paediatrics/ Obstetrics and Gynecology Centers of two tertiary health facilities; Lagos State University Teaching Hospital (LASUTH, n=272) and Lagos University Teaching Hospital (LUTH, n=121), Lagos, Nigeria between December 2008 and January 2014. Ethical approval was obtained from both institutional Health Research and Ethics Committee and from the Lagos State Hospital Management Board. Only individuals who consented that their data and samples can be used for research were recruited for the study and they were then identified with unique codes to protect their confidentiality.

The minimum sample size (N) calculated was 162, determined using the equation as described by Naing et al., 2006[16]:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n= sample size, Z=statistics for a level of 95% confidence interval=1.96, P=prevalence rate. Assumed to be= 12% according to Owolabi et al., 2014 [17] and d= precision (allowable error) =5%=0.05.

Data/Sample Collection and analyses: Socio-demographic data were documented using structured and pretested interviewer administered questionnaire and possible risk factors for HBV/HCV transmission. About 3ml of whole blood samples were collected aseptically into sterile ethylene diamine tetraacetic acid (EDTA) vacutainer bottle, centrifuged within 2 hours of collection to obtain plasma and stored at -70°C for serological assays. All samples were screened for markers for HBV (HBsAg, HBeAg, HBeAb, HBcIgM) and HCV (anti-HCV) using a fourth generation enzyme-linked immunosorbent assay (DIA. PRO. Diagnostic Bioprobes Srl., Italy). All assay protocols and interpretation of results using cut-off values were done according to the manufacturer's instructions.

All quantitative data were entered in the computer Microsoft Excel sheet and analyzed using SPSS version 17 for Windows. Descriptive statistics was computed for all relevant data. Associations between HBV/HCV/HIV infections and the socio-demographic and major risk factors were tested using Chi-square. All significance was accepted at $P < 0.05$.

RESULTS

Three hundred and ninety-three (393) HIV infected children aged <1 to 15 years with mean age of 4.79 ± 3.17 years were enrolled for this study. There were 186 (47.3%) males and 207 (52.7%) females. Out of the 393 samples analyzed, 40 (10.2%) were sero-positive for dual HIV/HBsAg co-infection, comprising 21 (52.5%) females and 19 (47.5%) males, while 15 (3.8%) had detectable antibodies to HCV consisting of 7 (46.7%) females and 8 (53.3%) males without any

statistical significance. On the overall, two (0.5%) of the participants were seropositive for triple HIV, HBsAg and anti-HCV co-infections (Figure 1).

Majority, 185 (47.1%) of the participants were aged between 1 – 5 years, while 107 (27.2%) and 69 (17.6%) were within the age group 6 – 10 and <1 years respectively (Table 1). Based on age group, HIV/HBV co-infection was highest (43.8%) amongst age group 11 – 15 years as compared with 15.9%, 4.3%

and 3.2% amongst of those age groups 6 – 10, <1 and 1 – 5 years respectively. The differences were not statistically significant ($P=0.59$). Co-infection of HIV/HCV was only and predominantly seen in age groups 6 – 10 and 11 – 15 years with prevalence of 46.7% and 53.3% respectively (Table 1). However, 50% each among age groups 6 –10 and 11 – 15 years had triple infection of HIV/HBV/HCV (Table 1).

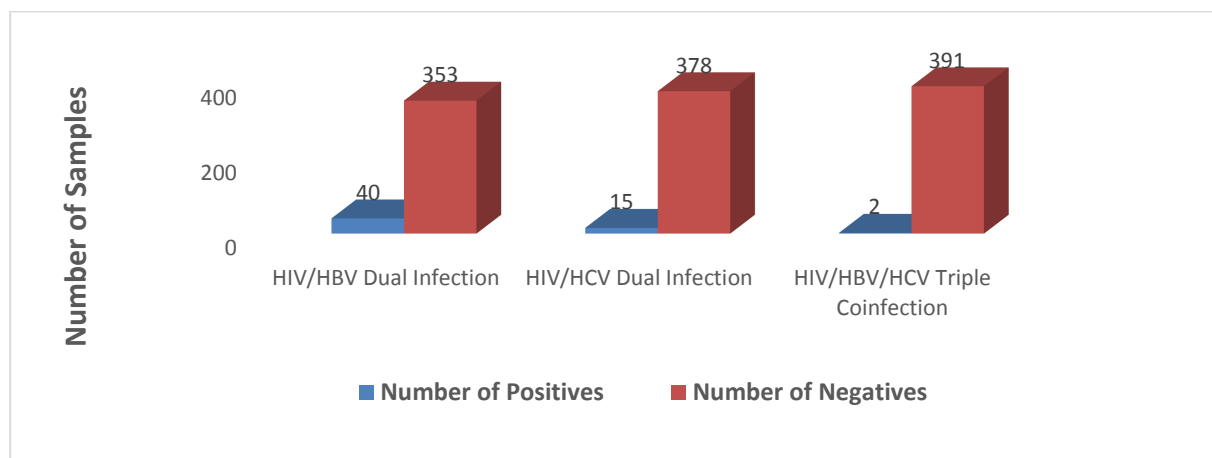


FIGURE 1: CO-INFECTION PATTERN OF HIV WITH HBV AND HCV AMONG PATIENTS IN LAGOS, NIGERIA

TABLE 1: AGE DISTRIBUTION OF HIV CO-INFECTION WITH HBV AND HCV AMONG PATIENTS IN LAGOS, NIGERIA

Age Group	Total (%)	HIV/HBV (%)	HIV/HCV	HIV/HBV/HCV
< 1yr	69 (17.6)	3 (4.3)	0 (0)	0 (0)
1-5yrs	185 (47.1)	6 (3.2)	0 (0)	0(0)
6-10yrs	107 (27.2)	17 (15.9)	7 (46.7)	1 (50)
11-15yrs	32 (8.1)	14 (43.8)	8 (53.3)	1 (50)
Total	393	40	15	2

Analysis of other markers of Hepatitis B virus among the 40 participants that were positive for HBsAg revealed that, 13 (32.5%) and 25 (62.5%) had acute and chronic infections respectively that were not active

based on the combination of markers as shown in table 2. However, 2 (5%) of this participants had chronic infection that is still active based on the combination of markers (Table 2).

TABLE 2: SERO-POSITIVITY OF HBV MARKERS IN PATIENTS AND IMPLICATIONS

Serologic Patterns Observed	Frequency of Occurrence (%)	Interpretation
HBsAg+, HBsAb+, HBeAg-, HBeAb+, HBcAb+	13 (32.5)	Acute Infection (Low or No replicative state)
HBsAg+, HBsAb-, HBeAg-, HBeAb+, HBcAb+	25 (62.5)	Chronic Infection (Low or No replicative state)
HBsAg+, HbsAb-, HbeAg+, HbeAb+, HbcAb+	2 (5.0)	Chronic Infection (ongoing high level replication)
Total	40 (100)	

DISCUSSION

The co-infections of Human immunodeficiency virus (HIV), Hepatitis B and C viruses remain a global

public health challenge on health resource poor settings particularly in Sub-Saharan Africa where the impact is greater. The prevalence rate of dual

HIV/HBV, HIV/HCV and triple HIV/HBV/HCV co-infection in this study was 10.2%, 3.8% and 0.5% respectively. These findings adds to the available data that Hepatitis B virus (HBV) and Hepatitis C virus (HCV) co-infections are important co-morbidities to consider when making management decisions particularly in HIV infected children. Moreso, that the prevalence of HBV or HCV infection and its impact on HIV-infected children has been poorly characterized and studied in our environment.

Varying prevalences had been documented in literature for HBV and HCV co-infections in similar cohort studies on HIV positive children, the prevalence of 10.2% for Hepatitis B surface antigen in this study was higher than the respective 7.7%, 8.4%, 1.2%, 2.6%, 4%, and 4.9% prevalences reported by Sadoh et al. [18] in Benin, Rawizza et al. [19] in Nigeria, Telatela et al. [20] in Tanzania, Toussi et al. [21] in New York, USA, Chakraborty et al. [22] in Kenya, and Zhou et al. [23] in China. Furthermore, the prevalence of 10.2% from our study was lower than the respective 19%, and 12.1% prevalence reported by Ashir et al. [24] in Maiduguri, Nigeria, and Rouet et al. [25] in Ivory Coast also in cohorts of pediatric HIV-infected children. The differences in the prevalence rate of HIV/HBV co-infection in this study and that of others may reflect the differences in the geographical distribution, assay type and sample population.

Although, the prevalence of 3.8% observed for HCV in this study, was higher than the 1.5%, 2.7%, 3.1% prevalences reported by Schuval et al [26] in USA, Rawizza et al [19] in Nigeria and Toussi et al [21] in USA. It is however, lower than the respective 5.2%, 9.6% and 13.8% published by Sadoh et al [18] in Nigeria, Zhou et al [23] in China and by Telatela et al [20] in Tanzania. In the contrary, Rouet et al [25] reported no co-infection of HIV with hepatitis C among a cohort of HIV-infected children in Cote d'Ivoire.

The mean ages of children with HIV/HBV, HIV/HCV co-infections and HIV/HBV/HCV triple infections were not different statistically. Co-infection with hepatitis B was documented in all age groups of children, with those greater than 10 years been more affected. However, no hepatitis C co-infection was detected among children under 5 years of age in this study. This findings was in contrast to a report from Tanzania where children under 5 years of age was found to have a higher incidence of HIV/HCV co-infection [20]. Our findings suggests that there were no vertical transmission of HCV from mother to child as compared to that of HBV since children under 5

years of age were infected with the virus and thus, may reflect differing modes of transmission of HCV which may be peculiar to our environment.

The pattern of the occurrence of the HBV markers from this study revealed that majority (32.5%) and (62.5%) had acute and chronic HBV infections respectively with low or no ongoing replication of the virus. The observed 5% of these children had chronic infection with presently ongoing high level replication of the virus and therefore very important in the transmission cycle of HBV particularly among children population in our environment. It will therefore be appropriate for these small group of children to be provided with suitable and effective treatment for both HIV and hepatitis infections.

CONCLUSION: This study revealed that co-infections of HBV and HCV with HIV among children may further increase the undesirable chances of chronic liver diseases which will consequently reduce the life expectancy of children in our environment. The observed different patterns of co-infection in this study may therefore necessitate the need for full and better understanding of the clinical outcomes and molecular epidemiology of these viruses with a view to alleviating the public health impetus associated with these diseases.

ACKNOWLEDGMENT: The authors would like to acknowledge and thank all patients, students and staffs who contributed to the collection and analysis of samples in the laboratory, especially the Medical Laboratory Scientists of the Virology Research Laboratory, College of Medicine of the University of Lagos.

COMPETING INTERESTS: The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

AUTHORS' CONTRIBUTIONS: S.O.B., O.A.O.B and G.A conceptualized the study and were responsible for the experimental and project design, analysis of data and writing the manuscript. J.A.B, O.B.O, A.O.S and A.A.A made conceptual contributions, performed some of the experimental analysis and assisted in preparing the manuscript. A.K.O made conceptual contributions and assisted in preparing the manuscript, while O.S.A was the laboratory director, team lead of the Virology Research Group and was responsible for the experimental and project design, analysis of data and writing the manuscript. All authors read and approved the manuscript.

REFERENCES

- Otegbayo JA, Taiwo BO, Akingbola TS, Odaibo GN, Adedapo KS, Penugonda S, Adewole IF, Olaleye DO, Murphy R, Kanki P. Prevalence of hepatitis B and C seropositivity in a Nigerian cohort of HIV-infected patients. *Annals of hepatology* 2008; 7: 152-6.
- Sepkowitz KA. AIDS – The First 20 Years. *New England Journal of Medicine* 2001; 344: 1764-72.
- Zenebe Y, Mulu W, Yimer M, Abera B. Sero-prevalence and risk factors of hepatitis B virus and human immunodeficiency virus infection among pregnant women in Bahir Dar city, Northwest Ethiopia: a cross sectional study. *BMC infectious diseases* 2014; 14: 118.
- Cohen MS, Hellmann N, Levy JA, Decock K, Lange J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *The Journal of clinical investigation* 2008; 118: 1244-54.
- Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, Gakidou E, Hay SI, Mills EJ, Trickey A, Msemburi W, Coates MM, Mooney MD, Fraser MS, Sligar A, Salomon J, Larson HJ, Friedman J, Abajobir AA, Abate KH, Abbas KM, Razek MM, Abd-Allah F, Abdulle AM, Abera SF, Abubakar I, Abu-Raddad LJ, Abu-Rmeileh NM, Abyu GY, Adebisi AO, Adediji IA, Adelekan AL, Adofu K, Adou AK, Ajala ON, Akinyemiju TF, Akseer N, Lami FH, Al-Aly Z, Alam K, Alam NK, Alasfoor D, Aldhahri SF, Aldridge RW, Alegretti MA, Aleman AV, Alemu ZA, Alfonso-Cristancho R, Ali R, Alkerwi A, Alla F, Mohammad R, Al-Raddadi S, Alsharif U, Alvarez E, Alvis-Guzman N, Amare AT, Amberbir A, Amegah AK, Ammar W, Amrock SM, Antonio CA, Anwar P, Arnlov J, Artaman A, Asayesh H, Asghar RJ, Assadi R, Atique S, Atkins LS, Avokpaho EF, Awasthi A, Quintanilla BP, Bacha U, Badawi A, Barac A, Barnighausen T, Basu A, Bayou TA, Bayou YT, Bazargan-Hejazi S, Beardsley J, Bedi N, Bennett DA, Bensenor IM, Betsu BD, Beyene AS, Bhatia E, Bhutta ZA, Biadgilign S, Bikbov B, Birlik SM, Bisanzio D, Brainin M, Brazinova A, Breitborde NJ, Brown A, Burch M, Butt ZA, Campuzano JC, Cardenas R, Carrero JJ, Castaneda-Orjuela CA, Rivas JC, Catala-Lopez F, Chang HY, Chang JC, Chavan L, Chen W, Chiang PP, Chibalabala M, Chisumpa VH, Choi JY, Christopher DJ, Ciobanu LG, Cooper C, Dahiru T, Damtew SA, Dandona L, Dandona R, Das Neves J, De Jager P, De Leo D, Degenhardt L, Dellavalle RP, Deribe K, Deribew A, Des Jarlais DC, Dharmaratne SD, Ding EL, Doshi PP, Driscoll TR, Dubey M, Elshrek YM, Elyazar I, Endries AY, Ermakov SP, Eshрати B, Esteghamati A, Faghmous ID, Farinha CS, Faro A, Farvid MS, Farzadfar F, Fereshtehnejad SM, Fernandes JC, Fischer F, Fitchett JR, Foigt N, Fullman N, Furst T, Gankpe FG, Gebre T, Gebremedhin AT, Gebru AA, Geleijnse JM, Gessner BD, Gething PW, Ghiwot TT, Giroud M, Gishu MD, Glaser E, Goenka S, Goodridge A, Gopalani SV, Goto A, Gughani HC, Guimaraes MD, Gupta R, Gupta R, Gupta V, Haagsma J, Hafezi-Nejad N, Hagan H, Hailu GB, Hamadeh RR, Hamidi S, Hammami M, Hankey GJ, Hao Y, Harb HL, Harikrishnan S, Haro JM, Harun KM, Havmoeller R, Hedayati MT, Heredia-Pi IB, Hoek HW, Horino M, Horita N, Hosgood HD, Hoy DG, Hsairi M, Hu G, Huang H, Huang JJ, Iburg KM, Idrisov BT, Innos K, Iyer VJ, Jacobsen KH, Jahanmehr N, Jakovljevic MB, Javanbakht M, Jayatilake AU, Jeemon P, Jha V, Jiang G, Jiang Y, Jibat T, Jonas JB, Kabir Z, Kamal R, Kan H, Karch A, Karema CK, Karletsos D, Kasaeian A, Kaul A, Kawakami N, Kayibanda JF, Keiyoro PN, Kemp AH, Kengne AP, Kesavachandran CN, Khader YS, Khalil I, Khan AR, Khan EA, Khang YH, Khubchandani J, Kim YJ, Kinfu Y, Kivipelto M, Kokubo Y, Kosen S, Koul PA, Koyanagi A, Defo BK, Bicer BK, Kulkarni VS, Kumar GA, Lal DK, Lam H, Lam JO, Langan SM, Lansingh VC, Larsson A, Leigh J, Leung R, Li Y, Lim SS, Lipshultz SE, Liu S, Lloyd BK, Logroscino G, Lotufo PA, Lunevicius R, Razek HM, Mahdavi M, Majdan M, Majeed A, Makhlof C, Malekzadeh R, Mapoma CC, Marcenes W, Martinez-Raga J, Marzan MB, Masiye F, Mason-Jones AJ, Mayosi BM, Mckee M, Meaney PA, Mehndiratta MM, Mekonnen AB, Melaku YA, Memiah P, Memish ZA, Mendoza W, Meretoja A, Meretoja TJ, Mhimbira FA, Miller TR, Mikesell J, Mirarefin M, Mohammad KA, Mohammed S, Mokdad AH, Monasta L, Moradi-Lakeh M, Mori R, Mueller UO, Murimira B, Murthy GV, Naheed A, Naldi L, Nangia V, Nash D, Nawaz H, Nejjari C, Ngalesoni FN, De Dieu Ngirabega J, Nguyen QL, Nisar MI, Norheim OF, Norman RE, Nyakarahuka L, Ogbo FA, Oh IH, Ojelabi

- FA, Olusanya BO, Olusanya JO, Opio JN, Oren E, Ota E, Padukudru MA, Park HY, Park JH, Patil ST, Patten SB, Paul VK, Pearson K, Peprah EK, Pereira CC, Perico N, Pesudovs K, Petzold M, Phillips MR, Pillay JD, Plass D, Polinder S, Pourmalek F, Prokop DM, Qorbani M, Rafay A, Rahimi K, Rahimi-Movaghar V, Rahman M, Rahman MH, Rahman SU, Rai RK, Rajsic S, Ram U, Rana SM, Rao PV, Remuzzi G, Rojas-Rueda D, Ronfani L, Roshamdel G, Roy A, Ruhago GM, Saeedi MY, Sagar R, Saleh MM, Sanabria JR, Santos IS, Sarmiento-Suarez R, Sartorius B, Sawhney M, Schutte AE, Schwebel DC, Seedat S, Sepanlou SG, Servan-Mori EE, Shaikh MA, Sharma R, She J, Sheikhbahaei S, Shen J, Shibuya K, Shin HH, Sigfusdottir ID, Silpakit N, Silva DA, Silveira DG, Simard EP, Sindi S, Singh JA, Singh OP, Singh PK, Skirbekk V, Sliwa K, Soneji S, Sorensen RJ, Soriano JB, Soti DO, Sreeramareddy CT, Stathopoulou V, Steel N, Sunguya BF, Swaminathan S, Sykes BL, Tabares-Seisdedos R, Talongwa RT, Tavakkoli M, Taye B, Tedla BA, Tekle T, Shifa GT, Temesgen AM, Terkawi AS, Tesfay FH, Tessema GA, Thapa K, Thomson AJ, Thorne-Lyman AL, Tobe-Gai R, Topor-Madry R, Towbin JA, Tran BX, Dimbuene ZT, Tsilimparis N, Tura AK, Ukwaja KN, Uneke CJ, Uthman OA, Venketasubramanian N, Vladimirov SK, Vlassov VV, Vollset SE, Wang L, Weiderpass E, Weintraub RG, Werdecker A, Westerman R, Wijeratne T, Wilkinson JD, Wiysonge CS, Wolfe CD, Won S, Wong JQ, Xu G, Yadav AK, Yakob B, Yalew AZ, Yano Y, Yaseri M, Yebo HG, Yip P, Yonemoto N, Yoon SJ, Younis MZ, Yu C, Yu S, Zaidi Z, Zaki Mel S, Zeeb H, Zhang H, Zhao Y, Zodpey S, Zoeckler L, Zuhlke LJ, Lopez AD, Murray CJ. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. *The lancet HIV* 2016; 3: e361-87.
6. WHO. Executive Summary of the Global Update on the Health Sector Response to HIV. *World Health Organisation Report* 2014. Assessed 12 July 2017; Available from <http://www.who.int/hiv/pub/progressreports/update2014/en/>
 7. UNAIDS. Prevention Gap Report. 2016. Assessed 15 July 2017; Available from http://www.unaids.org/sites/default/files/media_asset/2016-prevention-gap-report_en.pdf
 8. WHO. Global Hepatitis Report. Assessed 12 July 2017. Available from: <http://who.int/hepatitis/publications/global-hepatitis-report2017/en/>
 9. Okeke TC, Obi SN, Okezie OA, Ugwu EO, Akogu SP, Ocheni S, Ezenyeaku CC. Coinfection with hepatitis B and C viruses among HIV positive pregnant women in Enugu south east, Nigeria. *Nigerian journal of medicine : Journal of the National Association of Resident Doctors of Nigeria* 2012; 21: 57-60.
 10. Jenkins CN, Mcphee SJ, Wong C, Nguyen T, Euler GL. Hepatitis B immunization coverage among Vietnamese-American children 3 to 18 years old. *Pediatrics* 2000; 106: E78.
 11. Ezechi OC, Kalejaiye OO, Gab-Okafor CV, Oladele DA, Oke BO, Musa ZA, Ekama SO, Ohwodo H, Agahowa E, Gbajabiamilla T, Ezeobi PM, Okwurawe A, Audu R RA, Okoye RN, David AN, Odunukwe NN, Onwujekwe DI, Ujah IA. Sero-prevalence and factors associated with Hepatitis B and C co-infection in pregnant Nigerian women living with HIV Infection. *The Pan African Medical Journal* 2014; 17: 197.
 12. Barth RE, Huijgen Q, Taljaard J, Hoepelman AI. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *International journal of infectious diseases : IJID : Official publication of the International Society for Infectious Diseases* 2010; 14: e1024-31.
 13. Ikobah J, Okpara H, Elemi I, Ogarepe Y, Udoh E, Ekanem E. The prevalence of hepatitis B virus infection in Nigerian children prior to vaccine introduction into the National Programme on Immunization schedule. *The Pan African Medical Journal* 2016; 23: 128.
 14. Musa B, Bussell S, Borodo M, Samaila A, Femi O. Prevalence of hepatitis B virus infection in Nigeria, 2000-2013: A systematic review and meta-analysis. *Nigerian Journal of Clinical Practice* 2015; 18: 163-72.
 15. Muro FJ, Fiorillo SP, Sakasaka P, Odhiambo C, Reddy EA, Cunningham CK, Buchanan AM. Seroprevalence of Hepatitis B and C Viruses Among Children in Kilimanjaro

- Region, Tanzania. *Journal of the Pediatric Infectious Diseases Society* 2013; 2: 320-6.
16. Naing L, Winn T, Rusli BN. Practical Issues in Calculating the Sample Size for Prevalence Studies. In: *Archives of Orofacial Sciences* 2006; 1: 9-14.
17. Owolabi LF, Ibrahim A, Musa BM, Gwaram BA, Dutse AI, Hamza M, Yakasai AM, Habib AG, Borodo MM. Prevalence and Burden of Human Immunodeficiency Virus and Hepatitis B Virus Co-infection in Nigeria: A Systematic Review and Meta-Analysis. *J AIDS Clin Res* 2014; 5: 308. doi:10.4172/2155-6113.1000308
18. Sadoh AE, Sadoh WE, Iduoriyekemwen NJ. HIV co-infection with hepatitis B and C viruses among Nigerian children in an antiretroviral treatment programme. *South African Journal of Child Health* 2011; 5(1): 7-10.
19. Rawizza H, Ochigbo S, Chang C, Meloni M, Oguche S, Osinusil K, Ekong E, Kanki P, for the APIN PEPFAR Team. Prevalence of Hepatitis Coinfection Among HIV-Infected Nigerian Children in the Harvard PEPFAR ART Program. Conference on Retroviruses and Opportunistic Infections; February 16th -19th; San Francisco CA; 2010. Poster abstract S-181, <http://retroconference.org/2010/Abstracts/39148.htm>.
20. Telatela SP, Matee MI, Munubhi EK. Seroprevalence of hepatitis B and C viral co-infections among children infected with human immunodeficiency virus attending the paediatric HIV care and treatment center at Muhimbili National Hospital in Dar-es-Salaam, Tanzania. *BMC Public Health* 2007; 7: 338.
21. Toussi SS, Abadi J, Rosenberg M, Levanon D. Prevalence of hepatitis B and C virus infections in children infected with HIV. *Clinical Infectious Diseases* 2007; 45(6): 795-798.
22. Chakraborty R, Pulver A, Pulver LS, Musoke R, Palakudy T, D'Agostino A, Rana F. Viral coinfections among African children infected with human immunodeficiency virus type 1. *Clinical Infectious Diseases* 2003; 36(7): 922-924.
23. Zhou S, Zhao Y, He Y, Li H, Bulterys M, Sun X, Dou Z, Robinson M, Zhang F. Hepatitis B and hepatitis C seroprevalence in children receiving antiretroviral therapy for human immunodeficiency virus-1 infection in China, 2005-2009. *Journal of Acquired Immune Deficiency Syndromes* 2010; 54(2): 191-196.
24. Ashir GM, Rabasa AI, Gofama MM, Bukbuk D, Abubakar H, Farouk GA. Study of hepatic functions and prevalence of hepatitis B surface antigenaemia in Nigerian children with human immunodeficiency virus infection. *Nigerian Journal of Medicine* 2009; 18(30): 260-262.
25. Rouet F, Chaix ML, Inwoley A, Anaky MF, Fassinou P, Kpozehouen A, Rouzioux C, Blanche S, Msellati P, for the Programme Enfant Yopougon (Agence Nationale de Recherches sur le SIDA et les Hépatites Virales B et C 1244/1278). Frequent occurrence of chronic hepatitis B virus infection among West African HIV type-1 infected children. *Clinical Infectious Diseases* 2008; 46(3): 361-366.
26. Schuval S, Van Dyke RB, Lindsey JC, Palumbo P, Mofenson LM, Oleske JM, Cervia J, Kovacs A, Dankner WN, Smith E, Nowak B, Ciupak G, Webb N, Eagle M, Smith D, Hennessey R, Goodman-Kerkau M, Levin MJ; Pediatric AIDS Clinical Trials Group Protocol 1028S Team. Hepatitis C Prevalence in Children with Perinatal Human Immunodeficiency Virus Infection Enrolled in a Long-term Follow-up Protocol. *Arch Pediatr Adolesc Med* 2004; 158:1007-1013.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2018 ISBN 1595-689X VOL19 No.2
AJCEM/1819 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.9>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 133-140

FACTORS AFFECTING THE PARASITIC CONTAMINATION OF EDIBLE LOCALLY PRODUCED DRY SEASON LEAFY VEGETABLES CULTIVATED IN SOUTH EAST ENUGU, NIGERIA

Chijioke¹, U. O., Onyemelukwe², N., Ogboi³, S. J.

^{1,2,3}Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus

Correspondence: Ogboi Sonny Johnbull, Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus Nigeria.
Email address: ogboijb@yahoo.com

ABSTRACT

Background/Aim: Outbreaks of human infections associated with consumption of fresh fruits and vegetables have occurred with increased frequency during the past decade. This study evaluated parasitic contamination of locally produced dry season vegetables cultivated in Enugu metropolis, Enugu State, Nigeria.

Materials/Method: Vegetables, water and soil samples selected from vegetable farms were investigated. A total of 160 vegetable samples, 15 soil samples and 15 water samples were investigated. The samples were washed separately in saline and the resulting solution was subjected to standard sedimentation and flotation techniques. Physiochemical analysis of soil and water samples was done using the standard DREM technique. Furthermore, the water samples were investigated for *C. parvum* using the modified Ziehl-Neelsen staining technique.

Results: Parasitic contamination of the different vegetables were *Teifeairia occidentalis* 242 (18.1%), *Amaranthus hybridus* 193 (15.1%), *Cucurbita maxima* 192 (15.0%), *Solanum* species 191 (14.9%), *Ocimum grattissimum* 136 (10.6%), *Solanum nigrum* 114 (8.9%), *Gongronema latifolium* 101 (7.9%), *Talinum triangulare* 73 (5.7%) and *Murraya koenigii* 39 (3.0%). The parasites identified included; *Ascaris lumbricoides*, *Taenia* spp, *Fasciola hepatica*, *Schistosoma* spp, *Trichuris* spp, *Enterobius vermicularis*, *Toxocara* spp, *Giardia lamblia*, *Entamoeba* spp and *Cryptosporidium parvum*. *Entamoeba* species was the highest contaminating parasite 529 (41.3%) while *Schistosoma haematobium* was the least, 14 (1.1%). Physiochemical analysis revealed a strong positive correlation between parasite load in water and total suspended solids (TSS) ($r = 0.59$, $p = 0.021$) and turbidity ($r = 0.55$, $p = 0.032$) and also a strong positive correlation between parasite load in the soil and organic matter (OM), ($r = 0.71$, $p = 0.003$) and silt ($r = 0.63$, $p = 0.011$).

Conclusion: These vegetables should be considered as potential sources of parasitic infections, especially when they are not properly cooked (the type called 'Half Done') or consumed raw like the local salad (abacha) because of the high risk of parasitic infections such as giardiasis, amoebiasis, enterobiasis, cryptosporidiosis, ascariasis, etc. Public enlightenment is necessary to enhance the adoption of effective food safety approaches by agronomic practices of vegetable farmers which will in turn reduce foodborne illnesses.

Keywords: Parasite, contamination, vegetables, Enugu.

FACTEURS AFFECTANT LA CONTAMINATION PARASITAIRE DE SAISON SÈCHE PRODUITE LOCALEMENT COMESTIBLES LÉGUMES FEUILLUS CULTIVÉS DANS LE SUD-EST DE ENUGU AU NIGÉRIA

Chijioke¹, U. O., Onyemelukwe², N., Ogboi³, S. J.

^{1,2,3} Département des sciences de laboratoire médical, Faculté des Sciences de la santé et de la technologie, Faculté de médecine de l'Université du Nigéria, Enugu Campus

Correspondance: Ogboi Johnbull Sonny, Département des sciences de laboratoire médical, Faculté des Sciences de la santé et de la technologie, Faculté de médecine de l'Université du Nigéria, Campus Nigeria d'Enugu. E-mail: ogboijb@yahoo.com

RÉSUMÉ

Fond/Aim: foyers d'infections humaines associées à la consommation de fruits et légumes se sont produits avec l'augmentation de la fréquence au cours de la dernière décennie. Cette étude a évalué la contamination parasitaire de saison sèche légumes cultivés en métropole, l'état d'Enugu Enugu, Nigéria. **Matériaux/Méthode:** légumes, des échantillons d'eau et de sol sélectionné dans les fermes maraîchères ont été étudiés. Un total de 160 échantillons de légumes,

Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

15 échantillons de sol et 15 échantillons d'eau ont été étudiés. Les échantillons ont été lavés séparément dans une solution saline et la solution résultante a été soumise à des techniques de flottation et de sédimentation standard. L'analyse physico-chimiques des échantillons de sol et d'eau a été réalisée en utilisant la technique standard de DREM. En outre, les échantillons d'eau ont été étudiés du *C. parvum* en utilisant la technique de coloration de Ziehl-Neelsen modifiée.

Résultats: La contamination parasitaire des légumes différents ont été *Teifeairia occidentalis* 242 (18,1 %), *Amaranthus hybridus* 193 (15,1 %), *Cucurbita maxima*, 192 (15,0 %), 191 espèces de *Solanum* (14,9 %), *Ocimum gratissimum* 136 (10,6 %), *Solanum nigrum* 114 (8,9 %), *Gongronema latifolium* 101 (7,9 %), *Talinum triangulare* 73 (5,7 %) et de *Murraya koenigii* 39 (3,0 %). Les parasites identifiés inclus ; *Ascaris lumbricoides*, *Taenia* spp, *Fasciola hepatica*, *Schistosoma* spp., *Trichuris* spp., *Enterobius vermicularis*, *Toxocara* spp, *Giardia lamblia*, *Entamoeba* spp et *Cryptosporidium parvum*. Espèce *Entamoeba* a été la plus forte contamination parasite 529 (41,3 %) tandis que *Schistosoma haematobium* était le moins, 14 (1,1 %). L'analyse physico-chimiques a révélé une forte corrélation positive entre la charge parasitaire dans l'eau et de solutés en suspension (MES) ($r = 0,59$, $p = 0,021$) et la turbidité ($r = 0,55$, $p = 0,032$) et aussi une forte corrélation positive entre la charge parasitaire dans le sol et la matière organique (MO), ($r = 0,71$, $p = 0,003$) et de limon ($r = 0,63$, $p = 0,011$).

Conclusion: Ces légumes doivent être considérées comme des sources potentielles d'infections parasitaires, surtout quand ils ne sont pas bien cuits (le type appelé "Demi-Fait") ou consommés crus comme la salade locale (abacha) en raison du risque élevé d'infections parasitaires telles que l'amibiase, giardiase, entérobiase, cryptosporidiosis, l'ascaridiase, etc. L'information du public est nécessaire pour améliorer l'adoption d'approches efficaces de sécurité sanitaire des aliments par les pratiques agronomiques de maraîchers qui, à son tour, réduire les toxi-infections alimentaires.

Mots-clés: Parasite, contamination, légumes, Enugu.

INTRODUCTION

One of the most important needs of man is to be healthy. Over half of the world's population are affected by parasites, especially in underdeveloped countries like Nigeria. There are so many food-borne illnesses caused by intestinal parasites that remain a public health problem in the developing countries [1]. Intestinal parasites are among the major public health problems around the globe, particularly in tropical and subtropical countries [2]. Several enteric parasites cause severe morbidity and death rate in both man and animals worldwide. In developed settings, enteric parasites (helminths and protozoa) are frequently dismissed as a case of diarrheal illness due to apparently better hygienic conditions, and as such, very little effort is produced toward the laboratory diagnosis of these beings. Although these parasites contribute to the high burden of infectious diseases, estimates of their true prevalence are sometimes struck by the lack of sensitive diagnostic techniques to observe them in clinical and environmental specimens and proper documentation. Despite recent improvements in the epidemiology, molecular biology, and treatment of parasitic infections, health problems involving foodborne infections of parasitic origin still soars.

Intestinal parasites such as *Cryptosporidium* spp., *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis*, *Trichuris trichiura*, *Toxocara* spp., *Hymenolepis* spp., *Taenia* spp., *Fasciola* spp., etc., can infect humans as a consequence of consumption of contaminated, uncooked (raw), or improperly cooked vegetables [3]. The outbreak of human infections associated with ingestion of fresh fruits and vegetables has

occurred with increased frequency during the past ten years. Factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immunocompromised consumers. A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in character, to their inaccessibility to locations within structures and tissues that may harbor pathogens [4]. Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors which are depended upon by all levels of humans as food supplements or nutrients and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers [5].

Epidemiological studies have also indicated that, in areas of the world where parasitic diseases are endemic and where wastewater is used to irrigate vegetables, which are eaten raw, the consumption of wastewater irrigated vegetables without proper washing may lead to parasitic infection [5]. Different parasitic stages can contaminate vegetables. Most contamination occurs in vegetables before harvest, either by contaminated manure in soil, sewage, irrigation water, and wastewater from livestock operations or directly from wild and domestic animals [1]. Other contaminations occur during harvesting, transport, processing, distribution, and marketing or even at home [6]. Therefore, the main aim of the study was to investigate the parasitic contamination of locally produced dry season leafy vegetables cultivated in Enugu metropolis with specific objectives of

determining the parasite load on such vegetables, soil and water samples where the vegetables are cultivated.

MATERIALS AND METHODS

Study area

This study was undertaken in the expanding and residential areas of Enugu metropolis and its peri-urban zones in south east, Nigeria, during the dry season, when vegetables are grown. Enugu metropolis was grouped into five zones, and each zone consists of three prominent dry season leafy vegetable farming locations. The areas were as follows; Zone one (Maryland, Ugwuaji and New Artisan), Zone two (Trans-Ekulu, Abakpa (1) Iji Nike and Abakpa (11) Ugebe Nike), Zone three (Thinkers' Corner, Emene and Independence Layout) Zone four (Uwani, Achara Layout and Garikki) and Zone five (GRA, Artisan, and New Haven). Samples for the control study were obtained from the University of Nigeria, Enugu Campus (UNEC)'s gardens.

Sample collection

This study was carried out in Enugu metropolis and its peri-urban zones (October 2014 to February 2015) after an initial visitation to the different vegetable farming areas. In addition to the sample collection, interactions with the gardeners on their agronomic practices with regards to the water and manure sources they used for the vegetable cultivation was obtained. The leafy vegetables collected for the study included *Amaranthus hybridus* commonly known as green, *Teffairia occidentalis* (fluted pumpkin or ugu in Igbo), *Ocimum gratissimum* (scent leaves or nchuanwu in Igbo), *Talinum triangulare* (waterleaf or mgbolodi in Igbo), *Cucurbita maxima* (morning glory or Ugbogulu in Igbo), *Solanum* specie (anara in Igbo) *Solanum nigrum* (Awa in Igbo), *Gongronema latifolium* (Utazi in Igbo) and *Murraya koenigii* (curry). These vegetables were picked at random from the gardens. Three (3) samples of a particular vegetable constituted a sample from a farm. Nine different types of vegetable samples were collected from each of the vegetable farm locations listed above. A total of 160 samples of vegetables, fifteen (15) soil samples on which the vegetables were grown and fifteen (15) samples of the water used for irrigating the vegetables were collected and taken to the laboratory in sterile polythene bags, jars and plastic bottles respectively.

Sample processing

Macroscopic and Microscopic examination of samples were conducted. Macroscopically, the vegetables were carefully examined for the presence of adult forms of parasites according to a standard technique by [7]. Microscopic examinations were done following the standard sedimentation and floatation techniques that

involve washing the vegetable and soil samples. Physiochemical analyses of the soil and water samples from the various gardens were also done.

Microscopic Examination of the Samples

A Simple Sedimentation Method which involves the concentration of parasite eggs or ova and cysts as sediments and used to get operculated helminth eggs.

The vegetables, soil and water samples

The vegetable samples were washed using the standard washing technique [8, 9]. The filtrate was used to carry out both sedimentation and floatation techniques on each of the soil samples. The preparation was examined under the light microscope for various stages of intestinal parasites such as helminth eggs, larvae, cysts and protozoan using x10 and x40 objectives [8, 10]. The processed samples from vegetables, soil and water that were not centrifuged, were used to carry out the floatation technique according to standard method [11], especially for the sand samples. Apart from the sedimentation and floatation techniques for investigating the samples, the water samples from some of the farms situated near piggeries were also stained with the modified Ziehl Neelsen staining technique for *Cryptosporidium* organisms.

Source of funding: There was no funding from external source or government but the source of funding was personal contributions from the authors.

Conflict of Interest: I hereby state that there was no conflict of interest among the authors that participated in this research study

Data analysis: The mean parasite load was calculated according to vegetable types, soil and water samples of the different farms. The data obtained were subjected to descriptive statistics involving frequencies and percentages. Analysis of variance (ANOVA), Chi-square tests of the SPSS software version 17, Pearson's Product Moment Correlation and Fishers LSD (Least Significant Difference) tests. Probability less than 0.05 was considered significant, P -values < 0.05 [12].

RESULTS

All the 15 samples of each vegetable were parasitized except one sample of *Murraya coenigii*; hence, only *Murraya coenigii* had prevalence less than 100%. The results also show the mean intensity and parasitic loads of the vegetables, soil and water samples from various zones (Table 1). When comparing the parasitic load in the vegetables, a Chi-Square Test revealed a significant difference between them, $p < .001$; with *Teffairia occidentalis* having the highest parasitic load. The parasitic load for the different vegetables in the descending order was as follows: *Teffairia occidentalis* (242),

Amaranthus hybridus (193), *Cucurbita maxima* (192),
Solanum species (191), *Ocimum grattissimum* (136),
Solanum nigrum (114), *Gongronema latifolium* (101),

Talinum triangulare (73) and *Murraya coenigii* (39)
 Figure 1.

TABLE 1: PREVALENCE AND MEAN PARASITIC LOAD IN VEGETABLES

Vegetables	No. of Sample	No. parasitized sample	Prevalence (%)	No. of Parasites	Mean Parasitic Load	Range (Min-Max)
<i>Teifeairia occidentalis</i>	15	15	100.00	242	16.13 ±8.43	5-37
<i>Amaranthus hybridus</i>	15	15	100.00	193	12.87±7.35	4-31
<i>Ocimum grattissimum</i>	15	15	100.00	136	9.07±4.88	2-20
<i>Cucurbita maxima</i>	15	15	100.00	192	12.80±7.10	3-25
<i>Talinum triangulare</i>	15	15	100.00	73	4.87±4.09	1-15
* <i>Murraya coenigii</i>	15	14	93.33	39	2.6±1.76	0-6
<i>Gongronema latifolium</i>	15	15	100.0	101	6.73±3.97	1-16
<i>Solanum species</i>	15	15	100.00	191	12.73±4.88	4-21
<i>Solanum nigrum</i>	15	15	100.00	114	7.60±5.64	2-23

All the 15 samples of each vegetable were parasitized except one sample of **Murraya coenigii*; hence, only *Murraya coenigii* had prevalence less than 100%.

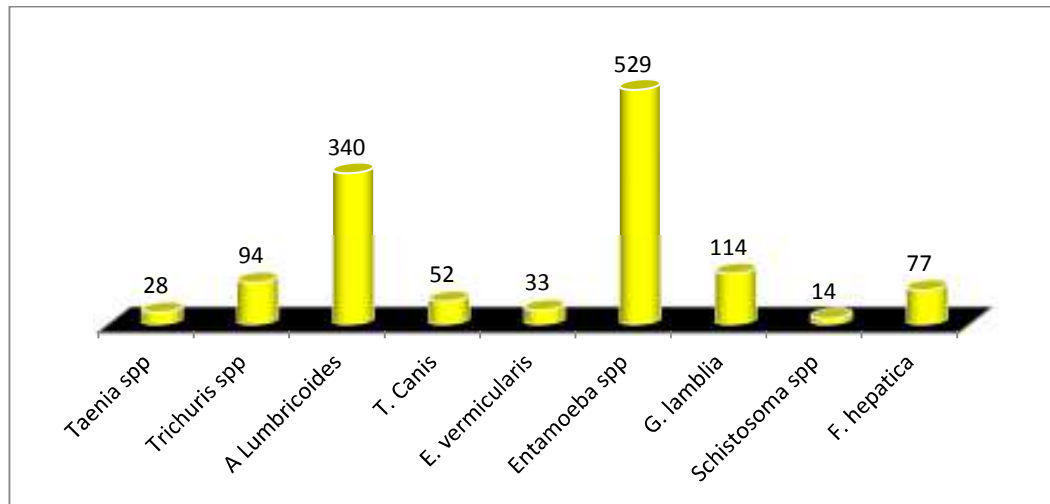


FIGURE 1: PARASITIC LOAD IN VARIOUS VEGETABLES

Four (4) vegetables, *Teifeairia occidentalis*, *Amaranthus hybridus*, *Cucurbita maxima* and *Solanum species* were contaminated by all the parasites. In nine vegetables examined, *Entamoeba spp.* was the highest, followed by *Ascaris lumbricoides* while *Talinum triangulare* was the least contaminated with 4 parasites. In 7 vegetables examined, *Schistosoma*

spp load was least in 7 vegetables (*Ocimum grattissimum*, *Cucurbita maxima*, *Talinum triangulare*, *Murraya coenigii*, *Solanum species*, *Solanum nigrum* and *Gongronema latifolium*), followed by *Taenia*, which was least in 4 vegetables (*Teifeairia occidentalis*, *Talinum triangulare*, *Murraya coenigii* and *Solanum nigrum*)(Table 2).

TABLE 2: PARASITE DISTRIBUTION ACCORDING TO VEGETABLES

Vegetables	Parasites									
	Taenia	Trich	Ascaris	Toxo	Entero	Entam	Gia	Sch	Fas	Total
<i>*Teifeairia occidentalis</i>	4	19	54	11	7	102	21	5	19	242
<i>*Amaranthus hybridus</i>	4	11	46	6	3	83	19	6	15	193
<i>Ocimum grattisimum</i>	3	12	30	5	4	56	12	0	14	136
<i>*Cucurbita maxima</i>	8	16	48	12	7	75	15	2	9	192
<i>Talinum triangulare</i>	0	0	25	0	0	41	4	0	3	73
<i>Murraya coenigii</i>	0	3	16	0	1	17	2	0	0	39
<i>*Solanum species</i>	5	17	54	6	5	76	16	1	11	191
<i>Solanum nigrum</i>	0	3	37	1	3	47	17	0	6	114
<i>Gongronema latifolium</i>	4	13	30	11	3	32	8	0	0	101
Total	28	94	340	52	33	529	114	14	77	1281

Four vegetables **Teifeairia occidentalis*, **Amaranthus hybridus*, **Cucurbita maxima* and **Solanum species* were contaminated by all the parasites

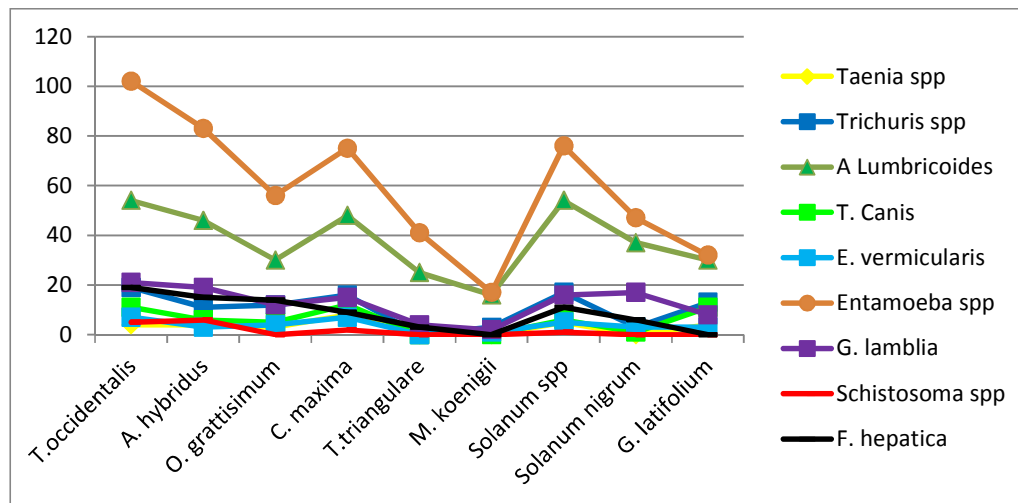


FIG 2: PARASITE-DISTRIBUTION ACCORDING TO VEGETABLES

Ascaris lumbricoides, *Entamoeba* spp and *Giardia lamblia* contaminated all the vegetables, while *Schistosoma* spp was the least contaminated parasites followed by *Taenia* spp

The diversity level of the vegetables is as follows: *Gongronema latifolium* (0.782), *Cucurbita maxima* (0.767), *Ocimum grattisimum* (0.758), *Teifeairia occidentalis* (0.752), *Solanum species* (0.745), *Amaranthus hybridus* (0.741), *Solanum nigrum* (0.704), *Murraya coenigii* (0.649) and *Talinum triangulare* (0.570). This implies that the probability of randomly getting two parasites that were different from a vegetable was highest in *Gongronema latifolium* than the other vegetables (figure 2).



FIGURE 3, WASTE PIPE CONNECTED TO CHANNEL WASTE INTO VEGETABLE FARMS

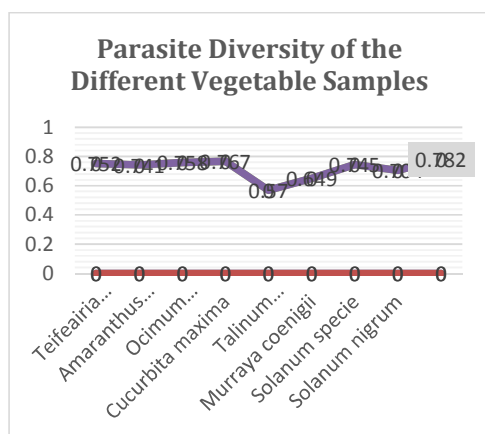


FIGURE 4. PARASITIC DIVERSITY OF DIFFERENT VEGETABLES

Pearson Correlation revealed a strong positive relationship between the parasite load in the vegetables in a given location and parasite load in the water source in that location, which was statistically significant, $r = 0.532$, $n = 15$, $p = 0.041$. A Pearson Correlation revealed a strong positive relationship between the parasite load in the vegetables in a given location and parasite load in the soil in that location, which was statistically significant, $r = 0.718$, $n = 15$, $p = 0.003$. In the physiochemical analysis of both soil and water samples, the result revealed a moderate positive correlation between the level of parasite load and water parameters, Pearson revealed a strong positive relationship between parasite load in the soil and the soil organic matter (OM) with $r = 0.712$, $p = 0.003$. A strong positive relationship also existed between parasite load and silt with $r = 0.632$, $p = 0.011$.

DISCUSSION

Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors which are depended upon by all levels of humans as food supplements or nutrients and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers [5]. Parasitic infections, caused by intestinal helminths and protozoan parasites, are among the most prevalent infections in humans in developing countries. It was observed that the rate of contamination also varied probably according to whether the vegetable leaf surfaces were very fine (smooth) e.g. the leaves of *Murraya koenigii*, *Gongronema latifolium*, *Talinum triangulare*, etc or very coarse (rough) e.g. the leaves of *Cucurbita maxima*, *Teifeairia occidentalis*, *Amaranthus hybridus*, etc. Vegetable samples with coarse or rough leaves had higher parasitic load when compared with the vegetable samples with fine or smooth leaves. Going by this assumption, our study revealed that

Teifeairia occidentalis (fluted pumpkin) had the highest level of parasitic contamination while *Murraya koenigii* had the least level of contamination. This finding agrees with other research works [13], on the detection and enumeration of parasitic eggs. There was high incidence of intestinal parasites found in communities that consume raw vegetables, especially where those vegetables are cultivated on farms fertilized with untreated human and animal wastes as fertilizers (figure 4). This only confirmed in our study the different types of parasitic contaminants (helminth and protozoan) on the vegetables from the different zones which were examined and the parasites identified based on the morphological characteristics of their eggs and cysts respectively.

Among the helminth eggs and protozoan cysts identified in the analyzed leafy vegetable samples were *Taenia* spp, *Ascaris*, *Toxocara* spp, *Trichuris* spp, *Schistosoma* spp, *Enterobius vermicularis*, and *Fasciola hepatica* eggs (helminths) and *Entamoeba* sp and *Giardia lamblia* (protozoa).

It was remarkable to note in our study that among the nine different intestinal parasites identified in the study, *Entamoeba* species recorded the highest prevalence 529 (41.3 %) while *Schistosoma* spp were the least 14 (1.1%). This high prevalence of *Entamoeba* species especially *E. histolytica* agrees with a previous study in South West of Saudi Arabia which demonstrated that eggs of *Ancylostoma* and *Ascaris* together with cysts of *Entamoeba* species were the most common parasites found in the 5 leafy vegetable plants investigated [14]. However in contrast to this finding, Robertson and Gjerde [15] did not encounter *Ascaris* in their investigations. The contrasting reports can be explained by different environmental factors including some physiochemical parameters of the soil on which the vegetables were grown or in the water used in irrigating the vegetables. .

In this study, *Cryptosporidium parvum* was the only parasite whose investigation involved a staining technique and yielded a level of contamination which was as high as 91% in the water samples. This finding is in close agreement with a study done in Alexandria in Egypt [14], on the detection of *Cryptosporidium* oocysts in irrigation water from El Mahmoudeya canal. Here, a high rate of irrigation water contamination with this parasite was (100%). Another study in USA demonstrated that *Cryptosporidium* oocysts were capable of strongly adhering to spinach plants after contact with contaminated water and also internalized within the leaves [16]. *Cryptosporidium* is a common cause of acute diarrhea in the immunocompetent but severe and prolonged diarrhea in the immunocompromised hosts, particularly patients with HIV/AIDS [14].

Findings from this study show that sources of contamination on these vegetables were the soil on which the vegetables were grown and the water from where the vegetables were irrigated. The organic fertilizers used by these vegetable farmers often consist of both animal and human waste even though some of the farmers, during a little interaction with them claimed that the name of the organic fertilizer they applied is called 'black soil'. To identify vegetables as a source of parasitic infection, it has been proposed that it is necessary to provide the following three conditions: firstly, they must be highly contaminated, secondly they must be cultivated in a warm season when the parasite eggs can develop, and thirdly they must be eaten uncooked [17].

According to Abougraina et al [10], the most likely hypothesis of contamination is that it must have occurred before harvest, either by contaminated manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations or directly from wild and domestic animals. These potential contaminants are all plausible and consistent with the assumption that the level of contamination must have been high. In contrast, Ettehad et al, [18] reported slightly lower level of contamination of consumed native garden vegetables with intestinal parasites (29%) in Ardabil city, Iran [19]. It was also reported that in Abha, most of the vegetables in the market sampled, were irrigated with well water which revealed high contamination levels of *Entameba* species. However, in this study, our findings is not consistent with any of the above reports because 169 parasites were recorded from the soil samples while 139 parasites were recorded from the water samples. The analysis showed that there was no statistically significant difference in the parasitic load of the soil and water samples to have had any of them tagged as the only or major source of parasitic contamination of the vegetables here in Enugu metropolis.

During field visits in this study, evidence of indiscriminate human defecation and piggery waste pipes channeled to flow directly into these vegetable farms were observed (figure 3). Physiochemical analysis of both soil and water samples revealed a moderate positive correlation between the parasite load and water parameters such as total dissolved solutes (TDS) and turbidity. Also there was a strong positive correlation between parasite load and soil parameters like organic matter (OM) and silt. The results of this study show that the higher the levels of these

parameters in a vegetable farm the higher the parasitic contamination of the vegetables from the farm.

The diversity level of the vegetables was high in our study and this implies that the probability of randomly getting two parasites that were different from a vegetable was highest in *Gongronema latifolium* than the other vegetables (figure 4).

The physiochemical analysis revealed a high positive correlation between the parasitic load in the water samples and the organic matter such that the more the organic matter content of the soil the more the parasitic load will be. The correlation was as high as $r = 0.71$, $p < 0.05$. This shows that soil organic matter affects the chemical and physical properties of the soil (soil physiochemical parameters), soil structure and porosity, the water infiltration rate and moisture holding capacity of soils for the diversity and biological activities of soil organisms, and plant nutrient availability. As they break down the organic matter, any excess nutrients (N, P and S) are released into the soil in forms that plants can use. This release process is called mineralization. The waste products produced by micro-organisms are also soil organic matter. This waste materials are less decomposable than the original plant and animal material, but it can be used by a large number of organisms for their biological activities. From this study, our findings show that the soil of the vegetable farms investigated must be rich in organic matter hence sustaining such level of parasitic load. Each species and group of organisms exists where it can find appropriate food supply, space, nutrients and moisture.

However, the results of this study showed good evidence of parasitic contamination of the dry season leafy vegetables cultivated here in Enugu metropolis and the levels of contamination were significant. These vegetables should be considered as potential sources of parasitic infections especially when they are not properly cooked (the type called 'Half Done') or consumed raw in our foods like the local salad (*Abacha*, etc) because of the high risk of contracting parasitic infections such as giardiasis, amoebiasis, enterobiasis, cryptosporidiosis, ascariasis, etc. Certain physiochemical parameters of soil and water are positively correlated with the level of parasitic contamination on the vegetables. Therefore, increased awareness and overall education of the public is necessary to enhance the adoption of effective food safety approaches which will in turn reduce foodborne illnesses.

REFERENCES

- [1]. Pires S.M, Vieira A.R, Perez E, Lo F, Wong D, Hald T. Attributing Human Foodborne Illness to Food Sources and Water in Latin America and the Caribbean Using Data from Outbreak Investigations. *International Journal of Food Microbiology*, (2012) 52(3):129-38.
- [2]. Wakid M.H. Improvement of Ritchie technique by identifying the food that can

- [3]. be consumed pre-analysis. *Journal of Applied Sciences Research*, (2009) 5(3):293-296.
- [4]. Kozan E, Sevimi F.K, Kose M, Eserm M and Cicek H. Examination of helminth contaminated wastewaters used for agricultural purposes in Afyonkarahisar, *Turkish Journal of Parasitology*, (2007) vol. 31, pp. 197-200.
- [5]. Beuchat L.R (2002) Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infection*;4 (4):413-418.
- [6]. Van Duyn M.A, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of American Diet Association* (2000) 100(12):1511-1521.
- [7]. Damen J.G, Banwat E.B, Egah .DZ, Allanana J.A. Parasitic Contamination of Vegetables in Jos, Nigeria. *Annals of African Medicine*, (2007) 3: 115 - 118.
- [8]. Garcia L.S. Macroscopic and microscopic examination of fecal specimens. In: Garcia LS, editor. *Diagnostic Medical Parasitology*. 5th ed. Washington, DC: American Society for Microbiology; (2007) 782-830.
- [9]. Fallah A.A, Pirali-Kheirabadi K, Fatemeh S, Saei-Dehkordi S. Prevalence of Parasitic Contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Iranian Food Control*; (2012) 25(2): 331-333.
- [10]. Tello R, Terashima A, Marcos L.A, Machicado J, Canales M, Gotuzzo E. Highly effective and inexpensive parasitological technique for diagnosis of intestinal parasites in developing countries: spontaneous sedimentation technique in tube. *International Journal of Infectious Disease*, (2001) 16(6):414- 416.
- [11]. Abougrain A.K, Nahaisi M.H, Madi N.S, Saied M.M, Ghenghesh K.S. Parasitological Contamination in Salad Vegetables in Tripoli-Libya. *Food Control*, (2009) 19(8):3004; 10-15.
- [12]. Ayres R.M, Mara D.D. Analysis of Wastewater for Use in Agriculture: A laboratory Manual of Parasitological and Bacteriological Techniques. WHO, Geneva, (1996) 7-11.
- [13]. Das P.N. Instant Medical Biostatistics. Ane Books Pvt Ltd, New Delhi, (2009) 170-185.
- [14]. Mba O.O. Detection and enumeration of parasite eggs in irrigated vegetable and salad crops in Plateau State, *Nigeria Journal Medical Laboratory Sciences*; (2000) 9:30-36.
- [15]. Uga S, Kimura D, Kimura K, Margono S.S. Intestinal parasitic infections in Bekasi District, west Java, Indonesia and a comparison of the infection rates determined by different techniques for fecal examination. *Southeast Asian Journal of Tropical Medicine in Public Health*; (2002) 33: 462-467.
- [16]. Robertson L.J, Gjerde B. Occurrence of Parasites on Fruits and Vegetables in Norway. *Journal of Food Protection*, (2001) 64(11):1793-1798.
- [17]. Macarasin D, Bauchan G, Fayer R, Spinacia O.L Leaf stomata harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. *Applied Environmental Microbiology*; (2010) 76:555-559.
- [18]. Takemasa K, Kimura K, May SI. Epidemiological survey of intestinal parasitic infections of diarrhoeal patients in Nepal and Lao PDR. *Nepal Medical College Journal*; (2004) 6: 7-12.
- [19]. Ettehad G.H, Sharif M, Ghorbani L, Ziaei H Prevalence of Intestinal parasites in vegetables consumed in Ardabil. *Iran Food Control*; (2008)19:790-794.
- [20]. Mercanoglu-Taban B, Halkman A.K. Do leafy green vegetables and their ready-to-eat RTE salads carry a risk of foodborne pathogens? *American Food Journal* (2011) 17(6):286-297

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2018 ISBN 1595-689X VOL19 No.2
AJCEM/1820 <http://www.ajol.info/journals/ajcem>
COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.10>
AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 141-148

ANTIFUNGAL PROPERTIES OF METHANOLIC EXTRACTS OF SOME MEDICAL PLANTS IN ENUGU, SOUTH EAST NIGERIA

Ndam P¹, Onyemelukwe N², Nwakile C.D³, Ogboi S.J⁴, Maduakor U⁵

^{1,2,4,5}Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus; ³Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State.

Correspondence: Ogboi Sonny Johnbull, Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria. Email address: ogboijb@yahoo.com

ABSTRACT

Objective: The current study evaluated the anti-fungal activities of methanolic extracts of the leaves and fruits of some medicinal plants of health value in Enugu, southeast Nigeria.

Materials and methods: Volumes of the different extracts into molten Sabouraud Dextrose Agar to make up for concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. Concentrations of 125 µg/ml, 250 µg/ml and 500 µg/ml of Miconazole were incorporated to serve as a positive control while 0.5 ml of DMSO served as a negative control. The plants extracts employed were from *Azadirachta indica* (Neem), *Anacardium occidentale* (Cashew), *Euphorbia hirta* (Asthma weed), *Jatropha curcas* (Physic nut), *Acanthus montanus* (Mountain thistle) and *Picralima nitida* (ósúgwe in Igbo). A 2 mm agar disc cut out from the margin of actively growing cultures of each dermatophyte (*Trichophyton soudanense*, *T. mentagrophytes*, *Cladosporium* sp, *T. rubrum* and *Fusarium* sp) were inoculated on the agar plates containing varying concentrations of the different plant extracts, the standard antifungal agent and negative control in triplicates and incubated at 28 °C.

Results: All the extracts exhibited antifungal activities of varying degrees with radial growth inhibitions (RGIs) ranging from 0-0.45 mm radius. Fifty percent (50 %) of the plants extracts comprising of *Picralima nitida*, *Euphorbia hirta* and *Acanthus montanus* exhibited complete inhibition at 100 mg/ml against all the clinical isolates under investigation ($p < 0.05$). *Picralima nitida* seed was the only plant extract with complete inhibition at 25 mg/ml against *T. soudanense* and *T. rubrum*. *Picralima nitida* was the closest in activity to miconazole. *Azadirachta indica* (Neem) did not exhibit complete inhibition against any of the clinical isolates at 100 mg/ml yet exhibited a significantly lower RGI at the lowest concentration of 25 mg/ml better than others.

Conclusion: Antifungal activities showed that leaves extract of fruits plants against the clinical isolates are good source of medicinal applications

Key words: Antifungal agents, medical plants, Enugu, Nigeria.

PROPRIÉTÉS ANTIFONGIQUES D'EXTRAITS MÉTHANOLIQUES DE CERTAINES PLANTES MÉDICALES À ENUGU, AU SUD-EST DE NIGERIA

Ndam P¹, Onyemelukwe N², Nwakile C.D³, Ogboi S.J⁴, Maduakor U⁵

^{1,2,4,5}Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus; ³Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State.

Correspondence: Ogboi Sonny Johnbull, Department of Medical Laboratory Sciences, Faculty of Health Sciences and technology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria. Email address: ogboijb@yahoo.com

RÉSUMÉ

Objectif : La présente étude a évalué les activités anti-fongiques des extraits méthanoliques des feuilles et fruits de certaines plantes médicinales de valeur santé à Enugu, au sud-est du Nigeria. **Matériel et méthodes :** Volumes des différents extraits dans molten Sabouraud Dextrose Agar pour compenser pour les concentrations de 25 mg/ml, 50 mg/ml et 100 mg/ml respectivement. Les concentrations de 125 µg/ml, 250 µg/ml et 500 µg/ml de miconazole ont été intégrés pour servir comme contrôle positif alors que 0,5 ml de DMSO a servi de témoin négatif. Les extraits de plantes utilisés étaient d'*Azadirachta indica* (neem), noix de cajou (*Anacardium occidentale*), *Euphorbia hirta* (asthme), *Jatropha curcas* (pourghère), *Acanthus montanus* (chardon de montagne) et de *Picralima nitida* (ósúgwe en Igbo).

. Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

Un disque de 2 mm d'agar coupé à partir de la marge de croissance active, les cultures de chaque (dermatophytes *Trichophyton soudanense*, *T. mentagrophytes*, *Cladosporium* sp, *T. rubrum* et *Fusarium* sp) ont été inoculés sur les géloses contenant des concentrations variables des différents extraits de plantes, la norme agent antifongique et contrôle négatif en triplicats et incubées à 28 °C. Résultats : Tous les extraits de l'exposé des activités antifongiques avec divers degrés d'inhibitions de la croissance radiale (RGIs) allant de 0-0,45 mm de rayon. Cinquante pour cent (50 %) des extraits de plantes comprenant de *Picralima nitida*, *Euphorbia hirta* et *Acanthus montanus* présentaient une inhibition complète à 100 mg/ml contre tous les isolats cliniques soumis à l'enquête ($p < 0,05$). *Picralima nitida* semence a été le seul extrait de plantes avec une inhibition complète à 25 mg/ml contre *T. soudanense* et *T. rubrum*. *Picralima nitida* était la plus proche de l'activité pour le miconazole. *Azadirachta indica* (neem) n'ont pas d'inhibition complète à l'égard d'aucun des isolats cliniques à 100 mg/ml mais montrent un souci nettement inférieur à la plus faible concentration de 25 mg/ml mieux que d'autres. Conclusion: activités antifongique a montré que l'extrait de feuilles de fruits plantes contre les isolats cliniques sont une bonne source d'applications médicinales

Mots clés: antifongiques, plantes médicinales, Enugu, Nigéria.

INTRODUCTION

The increasing incidence of growing resistance to antifungal agents despite the intensive use of antifungal drugs in the treatment of fungal infection [1], has become a great health challenge. However, there has been some claims by the traditional healers that some medicinal plants are more efficient in the treatment of infectious diseases than synthetic antibiotics. Medicinal plants have been practiced for centuries as remedies for human diseases as they incorporate elements of therapeutic value. At that place are numerous plant natural products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally [2]. Medicinal plants are considered to be an important source of new chemical substances with possible therapeutic effects [3]. The World Health Organization (WHO) estimates that up to 85% of people still rely primarily on traditional remedies such as herbaceous plants for their medicine [4]. Traditional healing plays an integral role in black African culture as it provides health care needs for a large majority of the society [5]. Presently, there is growing awareness of scientific and medicinal plants in the health care system of many developing countries [6]. In Nigeria, there is a rich tradition in the use of herbal plant products for the treatment of several ailments. Plants such as *Euphorbia hirta* (Asthma weed), *Anacardium occidentale* (Cashew), *Picralima nitida* (Ösúigwe), *Jatropha curcas* (Physic nut), *Azadirachta indica* (Neem) and *Acanthus montanus* (Mountain thistle) have been used by the people within the Enugu metropolis for the local treatment of skin diseases because of their effectiveness, availability and cost effectiveness.

Furthermore, nature has bestowed a very rich botanical wealth of vegetation, and a large number of diverse types of plants grow in different parts of the country. They constitute the richest source of drugs for traditional systems of medicine, modern medicines, food supplements [7]. The increasing prevalence of serious mycoses coupled with the frequent use of the available antifungal drugs has resulted in rising resistance of fungal pathogens to

antifungal agents. However, these topical drugs are generally ineffective against fungal infections of the nails due to their inability to penetrate the entire nail unit and eradicate the infection. The increasing prevalence of serious mycoses coupled with the frequent use of antifungal drugs currently available has resulted in rising resistance of fungal pathogens to antifungal agents. There is, therefore, a need for new broad-spectrum antifungal agents that can be used empirically in immune compromised patients, organ transplant patient, and other challenging situations. Use of herbal medicine in the treatment of infection with microorganisms predates the introduction of antibiotics [8]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented too, as well as representing a rich source of antimicrobial agents [9], with many of them readily available in rural areas at a relatively cheaper price. Medicinal plants extracts are promising as alternative or complementary control means because of their antimicrobial activity, non-phytotoxicity, as well as biodegradability properties [10].

Considering the vast potential of plants materials in the health care and the challenges in the management of fungal infections, this current study was designed to evaluate the antifungal properties of methanolic extracts of some traditional medicinal plants in Enugu, South East, Nigeria and their phytochemistry properties in order to reformulate the existing antifungals that are essential for improving patient management.

MATERIALS AND METHODS

Preparation of Plant Materials

Fresh leaves of the plants, *Azadirachta indica* (Neem), *Anacardium occidentale* (cashew), *Euphorbia hirta* (Asthma weed), *Jatropha curcas* (Physic nut), *Acanthus montanus* (Mountain thistle) and the *Picralima nitida* (Ösúigwe) Seeds & Rind were collected within the Enugu metropolis and authenticated in the Botany Department of the University of Nigeria, Nsukka. They were washed

under running tap water and air dried in room for 5 days (for the leaves) and 12 days (for the seeds and rind). The materials were ground into fine powder and stored in labelled air tight containers.

Preparation of Crude Extracts

100 g of each of the powdered form of the plant materials was exhaustively extracted by Soxhlet extraction method [11], using absolute methanol at 30 °C.

Preparation of fungal isolates: Microscopically identified isolates of *Trichophyton soudanense*, *T. mentagrophytes*, *T. rubrum*, *Cladosporium* sp and *Fusarium* sp, were obtained from the Mycology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. The isolates were subcultured in Sabouraud Dextrose Agar (SDA) to get pure cultures. Pure cultures were prepared in slanted cultures, stored in McCartney bottles and kept at 2-8 °C for further experimental purposes.

Determination of Antifungal Activities

Reconstitution of plant extracts: A stock concentration was reconstituted by weighing 2 g of the individual plant residue by adding 1 ml of DMSO to it in a test tube to make a concentration of 2 g/ml (2000 mg/ml).

Preparation of molten SDA plates containing the extract: The agar plate method was adopted as described [12].

Culture Process: The Culture medium of the different concentrations was inoculated with 2 mm agar disc cut out from the margin of the actively growing culture of the dermatophyte. This was carried out in triplicates and incubated at 28 °C, with controls run concurrently. The radial growth was measured daily for 4 days and the average reading taken for the test, positive and negative controls.

Percentage radial growth inhibition was calculated by employing the following formula:

Percent inhibition = $C - T \div C \times 100$ [13]. Where, C = Radial growth of negative control; T = Radial growth of the test

Column Chromatography

This was done using n-hexane, chloroform and ethyl acetate based on increasing polarity [14].

Phytochemical Analysis

The phytochemical analysis of fractionated *Picralima nitida* seed extract was carried out using standard methods [15].

Other test analysis for alkaloids, flavonoids, steroids and terpenoids, saponins, tannins carbohydrate, glycosides, resins (Precipitation test), proteins, fats and oil were carried using standard methods.

Data analysis: All generated data were subjected to statistical analysis using a one-way analysis of variance (ANOVA), followed by Dunnetts test (multiple comparison post-test) at $p < 0.05$.

RESULTS

Antifungal activities of the methanolic extracts of six medicinal plants were determined against five clinical isolates of fungi species. Miconazole nitrate was used as the standard for comparing the plant's extract. In Table 1, Miconazole nitrate at 500 µg/ml had the highest antifungal effect on the growth of *Cladosporium* sp by inhibiting it completely with a percentage radial growth inhibition (PRGI) of 100 %. *Picralima nitida* seed extract exhibited the highest antifungal effect with all the three concentrations (Tables 2 and 3).

TABLE 1: PERCENTAGE RADIAL GROWTH INHIBITION OF POSITIVE CONTROL ANTIFUNGAL AGENT (MICONAZOLE) AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Miconazole nitrate		
	125 µg/ml	250 µg/ml	500 µg/ml
<i>T.soudanense</i>	89.2 %	90 %	93.8 %
<i>T.mentagrophytes</i>	75 %	78 %	85 %
<i>Cladosporium</i> sp	86.7 %	95 %	100 %
<i>T.rubrum</i>	94 %	95 %	98 %
<i>Fusarium</i> sp	82.9 %	75.3 %	95.3 %

TABLE 2: PERCENTAGE RADIAL GROWTH INHIBITION OF *PICRALIMA NITIDA* SEED EXTRACT AGAINST FUNGAL ISOLATES

Fungal Isolates	Concentrations of extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial Growth inhibition		
<i>T.soudanense</i>	100 %	100 %	100 %
<i>T.mentagrophytes</i>	90 %	100 %	100 %
<i>Cladosporium</i> sp	91.7 %	95 %	100 %
<i>T.rubrum</i>	100 %	100 %	100 %
<i>Fusarium</i> sp	94.1 %	100 %	100 %

TABLE 3: PERCENTAGE RADIAL GROWTH INHIBITION OF *PICRALIMA NITIDA* SEED FRACTIONS AGAINST FUNGAL ISOLATES

Fungal Isolates	Hexane fraction (100 mg/ml)	Chloroform fraction (100 mg/ml)	Ethylether fraction (100 mg/ml)
	Radial growth	Inhibition	
<i>T.soudanense</i>	55 %	77.1 %	26.7 %
<i>T.mentagrophytes</i>	-33.3 %	25 %	-66.7 %
<i>Cladosporium</i> sp	25 %	50 %	-50 %
<i>T.rubrum</i>	35.7 %	88.6 %	57.1 %
<i>Fusarium</i> sp	-14.3 %	83.3 %	0%

At 100 mg/ml the extract inhibited completely all the investigated isolates with PRGI of 100 %. At 50 mg/ml, there was complete inhibition against all isolates except against *Cladosporium* spp. and at 25 mg/ml it inhibited completely *T.soudanense* and *T.rubrum*. The inhibitions exhibited by *T.mentagrophytes*, *Cladosporium* spp. and *Fusarium* spp. were, however, significant. *T.soudanense* and *T.rubrum* appeared to be the most sensitive of the fungi under investigation. This was followed by *T.mentagrophytes* and *Fusarium* spp. with a PRGI of 90 – 100 %. *Cladosporium* spp. with a PRGI of 91.7 -100 % appeared to be the least sensitive. The inhibition range of *Picralima nitida* seed extract for all the isolates was 90 -100 %. There was no inhibition observed in the negative control. The PRGIs for the antifungal activities of *Picralima nitida* seed extract at 25 mg/ml, 50 mg/ml and 100 mg/ml, respectively were significant ($P < 0.05$) compared with the standard.

The phytochemistry of the chloroform fraction of *Picralima nitida* seed revealed the presence of flavonoids, alkaloids, and terpenoids (Table 3). The highest antifungal effect was exhibited by Chloroform fraction with a PRGI of 88.6 % against *T.rubrum*. All the fungi under investigation appeared sensitive to the chloroform extract. Ethyl ether fraction had a lesser antifungal effect on the experimental isolates. There was a significant difference of ($p < 0.05$) in the treatment of chloroform fraction against control.

From Table 4, *Picralima nitida* rind extract had its highest antifungal effect at 100 mg/ml by inhibiting completely all the investigated isolates with a PRGI of 100 %. RGs of 0.03 ± 0.03 mm to 0.1 ± 0.05 mm at 25 mg/ml and 0.03 ± 0.03 mm to 0.05 ± 0.05 mm at 50 mg/ml were observed for all the isolates. The activities of *Picralima nitida* rind at these concentrations show a significant difference ($p < 0.05$) compared to the activities of the standard antifungal agent. Table 5 shows that *Azadirachta*

indica (Neem) seed extract did not inhibit any of the fungi completely at its highest concentration of 100 mg/ml. The inhibition range of Neem seed extract for all the isolates was 54.2-95.4 %. Neem seed was statistically significant ($p < 0.05$) only at 100mg/ml when compared with Miconazole nitrate.

TABLE 4: PERCENTAGE RADIAL GROWTH INHIBITION OF *PICRALIMA NITIDA* RIND EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
<i>T.soudanense</i>	92.3 %	95.4 %	100 %
<i>T.mentagrophytes</i>	80 %	94 %	100 %
<i>Cladosporium</i> sp	86.7 %	91.7 %	100 %
<i>T.rubrum</i>	94 %	94 %	100 %
<i>Fusarium</i> sp	90.6 %	94.1 %	100 %

Anacardium occidentale (cashew) leaf extract as shown in Table 5 had its highest antifungal effect of 100 % RGI at 100 mg/ml. All investigated isolates were completely inhibited at 100 mg/ml by the extract except for *Cladosporium* sp with a PRGI of 91.7 %. *T.rubrum* had a PRGI of 88-100 % and appeared to be the most sensitive followed by *T.soudanense* with a PRGI of 76.9-100 %. The percentage inhibition range of *A.occidentale* extract for all the isolates was between 23.5-100 % giving a significant difference ($p < 0.05$) on all the concentrations with respect to the standard control (Table 6),

TABLE 5: PERCENTAGE RADIAL GROWTH INHIBITION OF *AZADIRACHTA INDICA* SEED EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
<i>T.soudanense</i>	86.2 %	95.4 %	89.2 %
<i>T.mentagrophytes</i>	64 %	94 %	94 %
<i>Cladosporium</i> sp	54.2 %	66.7 %	91.7 %
<i>T.rubrum</i>	94 %	94 %	95 %
<i>Fusarium</i> sp	88.2 %	79.4 %	89.4 %

In table 7, *Euphorbia hirta* exhibited its highest antifungal effect at 100 mg/ml by completely inhibiting all the fungal isolates. No inhibition was observed in most of the isolates under investigation at 25 mg/ml. *T.rubrum* appeared to be the most sensitive fungi with a PRGI of 60 % at 50 mg/ml and 100 % at 100 mg/ml. However, the concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml had a significant difference of ($p < 0.05$)

when compared with the Standard. In Table 8, *Jatropha curcas* exhibited its highest antifungal effect of 100 % at 100 mg/ml by inhibiting all tested isolates except for *Fusarium* sp. Mild inhibition of 58.3 % was observed at 25 mg/ml and 50 mg/ml for *Cladosporium* sp. Others appeared to be resistant at these concentrations.

TABLE 6: PERCENTAGE RADIAL GROWTH INHIBITION OF ANACARDIUM OCCIDENTALE LEAF (CASHEW) SEED EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
<i>T.soudanense</i>	76.9 %	84.6 %	100 %
<i>T.mentagrophytes</i>	68 %	82 %	100 %
<i>Cladosporium</i> sp	43.3 %	80 %	91.7 %
<i>T.rubrum</i>	88 %	92 %	100 %
<i>Fusarium</i> sp	23.5 %	52.9 %	100 %

TABLE 7: PERCENTAGE RADIAL GROWTH INHIBITION OF EUPHORBIA HIRTA LEAF EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth inhibition		
<i>T.soudanense</i>	30.8 %	46.2 %	100 %
<i>T.mentagrophytes</i>	0 %	40 %	100 %
<i>Cladosporium</i> sp	0 %	33.3 %	100 %
<i>T.rubrum</i>	0 %	60 %	100 %
<i>Fusarium</i> sp	-5.9 %	35.3 %	100 %

TABLE 8: PERCENTAGE RADIAL GROWTH INHIBITION OF JATROPHA CURCAS LEAF EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial Growth Inhibition		
<i>T.soudanense</i>	38.5 %	53.8 %	100 %
<i>T.mentagrophytes</i>	10 %	20 %	100 %
<i>Cladosporium</i> sp	58.3 %	58.3 %	100 %
<i>T.rubrum</i>	30 %	40 %	100 %
<i>Fusarium</i> sp	29.4 %	47.1 %	97.1 %

TABLE 9: PERCENTAGE RADIAL GROWTH INHIBITION OF ACANTUS MONTANUS LEAVES EXTRACT AGAINST FUNGAL ISOLATES

Fungal Isolates	Concentration of extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial Growth inhibition		
<i>T.soudanense</i>	84.6 %	92.3 %	100 %
<i>T.mentagrophytes</i>	52 %	64 %	100 %
<i>Cladosporium</i> sp	50 %	66.7 %	100 %
<i>T.rubrum</i>	-40 %	0 %	100 %
<i>Fusarium</i> sp	41.2 %	52.9 %	100 %

Cladosporium sp appeared to be the most sensitive fungi with a PRGI of 58.3-100 %. The extract was significantly higher ($p < 0.05$) at the three concentrations with respect to the standard. In Table 9 shows that *Acanthus montanus* at 100 mg/ml exhibited its highest antifungal effect by completely inhibiting all the tested isolates at a 100 % RGI. *T.rubrum* and *Fusarium* sp were not inhibited at 25 mg/ml and 50 mg/ml of extract. *T.soudanense* appeared to be the most sensitive with a PRGI of 84.6-100 % followed by *T.mentagrophytes* with PRGI of 52-100 %. However, there was a significant difference ($p < 0.05$) in activities of the plant extract to the standard. The phytochemical studies of these plants revealed the presence of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Table 10).

DISCUSSION AND CONCLUSION

The methanolic extracts of six different plant species were assayed for antifungal activities and compared with Miconazole nitrate using the agar plate method. Miconazole belongs to the azole group of antifungal agents used clinically against fungal infections. They owe their antifungal activity by inhibiting the enzyme lanosterol 1,4- α -demethylase; the enzyme necessary to convert lanosterol to ergosterol, which is the predominant sterol in fungal cell membranes responsible for maintaining cell integrity, viability, function and normal growth [16]. Results from this study showed that the methanolic extract of *Picralima nitida* (seed and rind), *Azadirachta indica* (Neem), *Anacardium occidentale* (Cashew), *Euphorbia hirta*, *Acanthus montanus* and *Jatropha curcas* exhibited antifungal activity against *T. soudanense*, *T.mentagrophyte*, *T.*

rubrum, *Cladosporium* sp and *Fusarium* sp. These plants were considered to possess biochemically related substances with similar inhibitory properties with miconazole with the antifungal activities varying differently at the same concentrations of each of the extracts against the respective fungal isolate. It generally increases with increase in concentration of the extracts. This finding agrees with other study [17], which showed higher concentrations of antimicrobial substances led to appreciable growth inhibition.

TABLE 10: PHYTOCHEMICAL CONSTITUENTS OF CHLOROFORM EXTRACT

S/No	Phytoconstituent	CE
1	Alkaloids	+
2	Carbohydrates	-
3	Flavonoids	+
4	Glycosides	-
5	Fats and oil	-
6	Tannins	-
7	Saponins	-
8	Resins	-
9	Proteins	-
10	Terpenoids	+
11	Steroids	-

Key: + = Present, - = Absent, CE = Chloroform extract

The phytochemical studies of these plants also reveal the presence of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Table 10). The antifungal activities of these plants may probably be due to the presence of these rich secondary metabolites in plants which is tandem with other studies [18, 19, 20]. The patterns of radial growth inhibition of these plant extracts in this study were similar to those of miconazole nitrate. This could suggest the presence of similarly active ingredients present in Miconazole nitrate (the control antifungal agent) which is used predominantly as a drug of choice against superficial fungal infection especially *Trichophyton* spp, *Epidermophyton* spp and *Micosporium* spp. The activities of *Picralima nitida* seed extract appeared to be better than the other plant extracts investigated. This could be based on the concentration of active antifungal ingredients such as the phenolics (Tannins and flavonoids) present in that part of the plant material. The activities of *Picralima nitida* (seed

and rind) in this study justifies its use in the treatment of skin conditions including of *Tinea corporis* (ringworm of the skin), *Tinea capitis* (ringworm of the head) as reported by other works [21, 22]. *Azadirachta indica* (Neem) seed oil has been used in the treatment of various skin infections by alternative system of medicine [23]. Its activities in this study are in agreement with the work done [24], where neem oil was shown to have different inhibitory effects on different fungi including *T. rubrum*, *T. mentagrophytes* and *Fusarium* sp. However, though there was no complete inhibition observed in this study with any of the fungi, which could be due to neem oil from different localities may possess different rates of the antifungal activities with significant effect on all the fungal species tested. This variation could be due to the difference in the quality of the active ingredients in the oil sample. The leaves of *Anacardium occidentale* (cashew) in this study exhibited poor inhibitions at lower concentrations, though Rajesh *et al* [25] reported that, the nuts have proven antifungal properties of more than 94 % inhibition against *Aspergillus fumigatus*, *A. niger*, *Curvalaria* sp and *Fusarium* sp. This is an indication of uneven distribution of the active ingredients of the plant. *Euphorbia hirta* exhibited resistance at lower concentrations on some of the isolates, but complete inhibition at a higher concentration. This finding agrees with the other findings [9], as sensitivity increases with increase in concentration. *Jatropha curcas* in this study showed a poor inhibition of 10 % and 30 % at 25 mg/ml for *T. mentagrophytes* and *T. rubrum* respectively. However, Adejumo *et al.* 2009 who worked on *Fusarium* sp observed it to be the most resistant. The difference in their susceptibilities could be attributed to an inherent resistance factor of the test organisms among other factors, though other studies [26, 27] observed that, the leaves of *Jatropha curcas* had no inhibitory effect against *T. mentagrophytes* rather it was observed in the seed extract. This discrepancy is likely due to the fact that no two plants of the same species may have experienced the same environmental challenges considering the fact that plants phytoanticipins are uniformly distributed within the plants while phytoalexins are restricted to the tissue colonized by the fungus and the cells surrounding the infection site [28]. Some antifungal compounds may be present constitutively in one part of a plant, but induced as phytoalexins in other organs. *Acanthus montanus* had not really been widely used in folk medicine for the treatment of skin diseases, rather for pain, inflammation and other ailments [29, 30]. Its antifungal activities in this study support its usage in folklore treatment. *T. rubrum* and *T. soudanense* appear to be the most sensitive fungi at lower concentrations of the extracts. This could be due to the difference in the concentrations of the bioactive components in the sample or synergistic reactions of the various phytochemicals in the extract.

Euphorbia hirta (Asthma weed), *Anacardium occidentale* L (Cashew), *Picralima nitida* (Akuamma plant), *Jatropha curcas* (Barbados nut), *Azadirachta indica* A (Neem plant), and *Acanthus montanus* (Mountain thistle) have antifungal activities. Their antifungal activities increase with increase in concentrations. Crude extract of *Picralima nitida* seed possesses the highest antifungal activity. The phytochemistry of its chloroform fraction reveals

the presence of flavonoids, alkaloids and terpenoids. *Trichophyton rubrum* was the most sensitive fungal under investigation. Further investigation of the purified components of the seed extracts of *Picralima nitida* to determine the metabolites responsible for their activities will make it serve as a good base for consideration in the pharmaceutical industries for the production and packaging of antifungal products.

REFERENCES

- [1]. Svetaz, L., Zuljan, F. and Derita, M.. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. *Journal of Ethnopharmacology*. (2010)127 (1):137-158.
- [2]. Demetrio LV, Jeanie IA, Juliana Janet MP, Esperanza CC, Windell L. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria *Asian Pacific Journal of Tropical Biomedicine*, (2015), Volume 5, Issue 7.
- [3]. Farnsworth, N.R. Screening plants for new medicine In: Wilson, E.O. (Ed), *Biodiversity*. Part 11. National Academy Press, Washington. (1989) 88-97.
- [4]. Leena, T. and Jaindranath, T.. *Tropical Journal of Pharmaceutical Research*. (2003) 2(2): 243-253.
- [5]. World Health Organization, (2002). Traditional medicine strategy. 2002-2005.
- [6]. Ame, I.O, and Salah, E.I. Therapeutic utility constituents and toxicity of some medicinal plants. *Veterinary and Human Toxicology*. (1995) 37(3):46.
- [7]. Hammer, K.A., Carson, C.F., Riley, T.V. Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*. (1999) 86(6): 985.
- [8]. Owoyale, J.A., Olatunji, G.A., Oguntonye, S.O. Antifungal and antibacterial activities of an Alcoholic Extract of *Senna alata* leaves. *Journal of Applied Sciences and Environmental Management*, (2005) 9(3): 105-107.
- [9]. Momoh, A.R.M., Idonije, O.B., Okhai, O., Iribhogbe, O.I., Momoh, A.A., Otamere, H.O., Ekhatior, C.N., Okolo, P.O., Oseghale, D.A. The Antifungal Effects of Extract of *Euphorbia hirta* on selected Pathogenic fungi. *New York Science Journal*, (2011). 4(80).
- [10]. Timothy, S.Y., Goji, S.Y., Abdusallam, B., Mava, Y. and Galadima, I.H. Antibacterial and Phytochemical screening of the ethanolic leaf extract of *Azadirachta indica* (Neem). *International Journal of Applied Biology and Pharmaceutical Technology*. (2011) 2:194.
- [11]. Tejas, G., Mayank, Patel and Bharat, k. D. Scholars Research Library. *Journal of National production and plant resource*. (2012) 2(1): 135-142.
- [12]. Oseni, L., Adebayo, Alphonse, Prince Kofi. Comparism of antibacterial properties of solvent extracts of different parts of *Jatropha curcas* (linn). *International Journal of Pharmaceuticals and Phyto Pharmacological Research*. (2011) 1(3): 117 - 123.
- [13]. Vinit, K. M. In vitro antagonism of *Trichoderma* species against *Pythium Aphanidermatum*. *Journal of Phytology*, (2010) 2(9): 28-35.
- [14]. Geetu, G. Chromatography (A Refresher Course CPDHE) (2008) on Sivaprakashan, K., and Vidhyasekaran, P., (1993). Phenylalanine ammonia lyase gene for crop management. In: Vidhyasekaran, P. ed. *Genetic Engineering, Molecular Biology and Tissue Culture for Crop, Pest and Disease Management*. Delhi, India. pp: 113-122.
- [15]. Trease, G. E and Evans, N. C. *Textbook of Pharmacognosy* 15th edition. W.B. Saunders Company Ltd, London, (1996). 168:249-251.
- [16]. Ghannoum, M.A., and Rice, L.B. Antifungal agents: Mode of action, Mechanisms of Resistance, and Correlation of these mechanisms with bacterial resistance. *Clinical Microbiology Review*. (1999) 12: 501-517.
- [17]. Banso, A., Adeyemo, S.O., Jeremiah, P. Antimicrobial properties of *Vernonia amygdalina* extract. *Journal of Applied Science and Management* (1999) (3):9-11.

- [18].Baba-Moussa, F. and Akpagana, K.B. Antifungal activities of Seven West African Combretaceae used in Traditional Medicine. *Journal of Ethnopharmacology*, (1999) 66(3):335-338.
- [19].Cowan, M.M.A Plants Products as Antimicrobial Agents. *Clinical Microbiology Review*. (1999) 12:564-582.
- [20].Reyes-Chilpa, Garibay,M.T., Jimenez-Estrada and Quiroz-Vasquez. Flavonoids and Isoflavonoids with Antifungal properties from *Platymiscium yucatanum* heartwood Holzforschuna. *International Journal of Biological, Chemical and Physical Technology Wood* (2009) pg 52.
- [21].Wosu,L.O., and Ibe,C.C. Use of extract of *Picralima nitida* bark in the treatment of experimental trypanosomiasis: A preliminary Study. *Journal of Ethnopharmacology* (1989) 25:263-268.
- [22].Ezeamuzieji, C., Ojiamaka, M.C., Uzogara, E.O. and Orji, S.E. Anti-inflammatory, Anti-pyretic and Anti-malarial activities of a West African Medicinal Plant *Picralima nitida*. *African Journal of Medical Sciences* (1994) 23:85-90
- [23].Natarajan, V., Venugopal, P.V., and Menon, T. Effect of *Azadirachta indica* (Neem) on the growth pattern of Dermatophytes. *Indian Journal of Medical Microbiology* (2003) 21(2):98-101.
- [24].Khan, M., and Wassilew, S.W. *Natural Pesticides from the Neem Tree and Other Tropical Plants* (eds Schutterer, H. and Asher, K.R.S.), GT2, Eschborn, Germany, (1987) pp 645-650.
- [25].Rajesh, K., Verma, L.C., and Sadhana, K. Potential antifungal plants for controlling building fungi. *Natural Product Radianc*. (2007) 7(4):374-387.
- [26].Ayanbimpe, G.M and Fagbemi, O.I. Antifungal activity of extracts from a hedges plant *Jatropha curcas* on some pathogenic fungi. *Nigerian Annals of Natural Sciences*. (2005) 6(1):18 – 22.
- [27].Ekpo, M.A. and Etim, P.C. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. *Journal of Medical and Plants Research*. (2009) 3 (9):621-664.
- [28].Dixon, R. Natural products and plant disease resistance. *Nature*. (2001) 411: 843-847.
- [29].Asongalem, E.A., Nana, P., Foyet, H.S., Dimo, T., Kamtchouing, P. Antifertility and fetotoxic activities of *Acanthus montanus* extracts in Wister rats. *Pubmed Clinical Pharmacology*, (2008) 30(7): 521-528.
- [30].Adejumo, T.O and Bamidele, B.S. Control of dermatophyte-causing agent (*Trichophyton mentagrophytes* and *Trichophyton rubrum*) using six medicinal plants. *Journal of Medicinal Plant Research* (2009): 3(11):906-913.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X 2018 VOL19 No.

AJCEM/1811

<http://www.ajol.info/journals/ajcem>

COPYRIGHT 2018 <https://dx.doi.org/10.4314/ajcem.v19i2.11>

AFR. J. CLN. EXPER. MICROBIOL. 19 (1): 148-158

CROSS-SEASONAL ANALYSIS OF BACTERIOLOGICAL PROFILE OF WATER SOURCES AS A DISEASE RISK MEASURE

Odonkor^{1*}, S.T. and Addo², K.K.

¹ Greenhill College, Ghana Institute of Management and Public Administration
Accra, Ghana; ² Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana,
Accra, Ghana

*Correspondence: Stephen T. Odonkor, Greenhill College, Ghana Institute of Management and Public
Administration, P. O. Box AH 50, Achimota-Accra, Ghana. Tel. +(233) 24 21550 +(233) 23 4709702 Fax: +(233) 302 404664
E-mail: stodonkor@gimpa.edu.gh

ABSTRACT

Water is a natural resource and is essential to sustain life. Poor drinking water quality is the cause of several diseases. The aim of this paper was to investigate bacteriological profile of water sources as a measure of disease risk, aimed at providing useful information towards rural water resources management. Five hundred and twenty bacterial isolates (520) were obtained from water samples collected during the period of study. Majority of the Isolates (305) representing 58.65% of the total were obtained during the dry season, as against (205) representing 41.35% in the rainy season. There was a statistical differences ($P > 0.05$) of the microbes isolated seasonally. The highest occurring was *Klebsiella* spp. (9.83 ± 6.99 , $P > 0.05$) in the dry season and the least *Shigella* spp. $P > 0.05$. Furthermore dam water sources was observed to poses a high disease risk among the five water sources investigated, while borehole water sources possess a lower diseases risk. An alarming observation was the presences of bacteria of public health importance in the water sources. These included *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerae* (cholera). In a nutshell, to reduce the level of bacterial contamination of drinking water sources there should be an incessant education on issues such as: environmental awareness, (cultivation sanitation habits and ensure that their surroundings and water sources are not indiscriminately polluted), causes, modes of transmission and prevention of water and sanitation related diseases.

Key words: *E. coli*, water, Public health and disease

L'ANALYSE SAISONNIÈRE DU PROFIL BACTÉRIOLOGIQUE DES SOURCES D'EAU COMME UNE MESURE DU RISQUE DE MALADIES

Odonkor^{1*}, S.T. et Addo², K.K.

Greenhill College, le Ghana Institute of Management and Public Administration
Accra, Ghana ; 2 Département de bactériologie, Noguchi Memorial Institute for Medical Research, Université du
Ghana, Accra, Ghana

*Correspondance : Stephen T. Odonkor, Greenhill College, le Ghana Institute of Management and Public
Administration, P. O. Box AH 50, Achimota-Accra, au Ghana. Tél. (233) 2421550 (233) 23 4709702 Fax : (233) 302404664
E-mail : stodonkor@gimpa.edu.gh

Résumé

L'eau est une ressource naturelle et indispensable à la vie. La mauvaise qualité de l'eau potable est la cause de plusieurs maladies. L'objectif de cet article était d'étudier le profil bactériologique des sources d'eau comme mesure de risque de maladie, visant à fournir des informations utiles à la gestion des ressources en eau en milieu rural. Cinq cent vingt (520) des isolats bactériens ont été obtenues à partir des échantillons des eaux recueillies au cours de la période d'étude. La majorité des isolats (305) représentant 58,65 % du total ont été obtenus pendant la saison sèche, contre (205) représentant 41,35 % dans la saison des pluies. Il y avait une différence statistique ($p > 0,05$) des microbes isolés en saison. Le plus haut lieu de *Klebsiella* spp. a été ($9,83 \pm 6,99$, $P > 0,05$) pendant la saison sèche et la moins *Shigella* spp. $P > 0,05$. De plus les sources d'eau du barrage a été observé à pose un risque de maladie élevé parmi les cinq sources d'eau d'une enquête, les sources d'eau forage whiles possèdent un plus faible risque de maladies.

Copyright ©2017 AJCEM. This work is licensed under the Creative Commons Attribution 4.0 International License CC-BY

Un constat alarmant a été la présence de bactéries d'importance pour la santé publique dans les sources d'eau. Ceci inclus les *Shigella* spp. (La dysenterie), *Salmonella typhi* (la fièvre typhoïde et les infections diarrhéiques aiguës), *Salmonella typhi* (fièvre typhoïde) et *Vibrio cholerae* (choléra). En un mot, pour réduire le niveau de contamination bactérienne des sources d'eau potable il devrait y avoir une éducation incessante sur des questions telles que : sensibilisation à l'environnement, la culture et les habitudes d'assainissement (s'assurer que leur environnement et les sources d'eau ne sont pas pollués sans discernement), causes, modes de transmission et prévention des maladies liées à l'eau et l'assainissement.

Mots clés: E. coli, de l'eau, de la santé publique et de la maladie.

INTRODUCTION

Water is essential for survival. It has been stated that our existence is "intimately connected with the quality of water available to us. [1,2]. An adequate supply of safe drinking water is one of the major prerequisites for a healthy life. Waterborne disease is still a major cause of death in many parts of the world, particularly in children, and it is also a significant economic constraint in many subsistence economies. Waterborne diseases are as a result of contaminations: the presence of elevated concentrations of substances in the environment above the natural background level for the area and for the organism [3-5].

Drinking water is derived from two basic sources: surface water, such as rivers and reservoirs, and groundwater such as wells and boreholes. All water contains natural contaminants, particularly inorganic contaminants that arise from the geological strata through which the water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals. In general, groundwater is less vulnerable to pollution than surface waters [6]. There are a number of possible sources of man-made contaminants, some of which are more important than others. These fall into the categories of point and diffuse sources. For example a badly sited latrines and septic tanks are a significant source of contamination, especially of wells [7].

The most common and deadly pollutants in drinking water in developing countries are of biological origin [8]. WHO [9] states that the "infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread health risk associated with drinking water". The use of such water for drinking, cooking, contact with it during washing, bathing, or even inhalation of its fine droplets as aerosols, may then result in infection. The minimum infectious dose [the smallest number of ingested pathogens necessary to cause disease) for the average healthy adult varies widely for various microorganisms. This dose ranges from just a few organisms of

Salmonella typhi to produce typhoid, several hundred organisms of *Shigella flexneri* to cause dysentery, several million cells of *Salmonella* serotype to cause gastroenteritis, to as many as a hundred million cells of *Vibrio cholerae* to produce cholera.

The minimum infectious dose also varies by the age, health, nutritional and immunological status of the exposed individual. As WHO notes, "Those at greatest risk of waterborne disease are infants and young children, people who are debilitated or living under insanitary conditions, the sick, and the elderly. For these people, infective doses are significantly lower than for the general adult population"[10]. The size of the minimum infectious dose does not directly translate into ease of prevention of the relevant disease [since concentrations of the pathogens in the water are variable, too). However, it does point to the reasonableness of the approach to minimize disease risk by defining a maximum allowable concentration of an indicator organism in drinking water.

The published studies in sub Saharan Africa reviewed by this current study, appears largely aimed at determining the microbial contamination of selected water sources. However, it is not known how the various water sources [both ground and surface waters) relate in terms of microbial distribution. Furthermore, most of these studies seldom investigated microbial status across seasons (wet and dry season). This is very much needed in tracking contamination sources as well as anthropogenic influences. Moreover, most of the studies had looked at the presence of microbes in the population, while providing little or no information on the routes by which these bacteria contaminates the water source. Furthermore, the diseases risk assessments of the various water sources were not carried out in most cases. The aim of this paper is to determine the bacteriological profile of bacteria flora in the drinking water sources as a measure of disease risk assessment.

METHODOLOGY

Sample Size and Sampling Frequency

Four hundred and sixty four (464) water samples were collected for the study. The sample collection period spanned the two seasons in Ghana: the dry and rainy seasons. Table 1 shows the details of water samples collection.

TABLE 1 WATER SAMPLE COLLECTION DETAILS

WATER SOURCE	WATER SAMPLE COLLECTION DETAILS		
	RAINING SEASON	DRY SEASON	TOTAL
Dams	60	60	120
Bore holes	32	32	64
Streams	68	68	136
Hand-dug wells	60	60	120
River	6	6	12
Canal	6	6	12
TOTAL			464

Prior to water sampling, important observations were made of sanitary conditions and possible sources of contamination, both anthropogenic and natural events that occur in the proximity of water bodies and are likely to influence water quality from all the sources sampled. For example, it was observed that in some places, refuse dumps, and places of convenience (toilets) were sited close to water bodies. In other cases, organic and inorganic waste as well as wastewater from various human activities had been disposed off near or into water bodies, which also served as sources of water for some communities.

The importance of accurate field records when conducting water sampling cannot be over emphasized. Recording site details and other environmental factors help when interpreting the sample results later on. Field notes including the following were therefore recorded: Date, Time of sampling, Water body type, Site code, etc.

The following environmental factors were also recorded: Water clarity/turbidity (visual clarity in the water i.e. leaves, debris, algae), Weather conditions (temperature, wind, rainfall), presence of animals (birds) and other comments (e.g. faecal accidents).

Water sample collection procedure

All water sampling and preservation procedures were performed according to Standard Methods for the examination of water and wastewater

(APHA, 1998; APHA, 1995), and WHO guidelines for drinking water quality (WHO, 1996, 1982). Sampling for bacteriological analysis was done aseptically with care, ensuring no external contamination of samples. In the process, sterilized plastic Polyethylene (PET) bottles were used. The bottles were cleaned and rinsed carefully, given a final rinse with distilled water and then sterilized at 121°C for 15 minutes. Sterilization effectiveness was checked by putting sterilization strips on each sampling bottle and glassware in each run.

During samples collection, enough air space was left in each sampling bottle (at least 3 cm) to aid thorough mixing by the electronic shaker prior to examination. Samples collected were representative of the water being tested.

Borehole water samples were taken from boreholes fitted with hand pumps. Before samples were taken, the pumps were continuously operated for about 5 minutes, after which the mouth of the borehole was cleaned with cotton wool soaked in 70% concentrated alcohol and then flamed for about 5 minutes. Water was again pumped out for a further 3 minutes to allow the metal to cool. Water samples were then collected by direct flow into sterilized bottles and carefully sealed. For hand-dug wells, a sterilized bottle was tied to a rope and lowered into the wells. The lid was first removed and the bottle lowered into the well to a depth of about 1m below the water surface. The bottle was removed and quickly covered. Immediately after collection, samples were placed in an insulated box (an ice chest) filled with ice cubes to keep the temperature below 4°C. Water samples from streams/river were also collected from depths of about 1m from the active part of the streams/river where people normally collected water for domestic purposes. Steps were taken at all times to avoid contamination using standard procedures. All other equipment used for the exercise was sterilized by autoclaving on the eve of each sampling day. All samples were transported to Noguchi Memorial Institute for Medical Research (NMIMR) of University of Ghana within 2 hours for analysis.

Bacteria isolation and identification

All gram-positive organism were identified by conventional methods, such as Gram reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNAse) test, Indole test, Methyl-red test, Voges-Proskauer test, Citrate utilization test, Triple sugar iron (TSI) agar test,

Motility test , Oxidase test etc, whiles an API 20E kit was used to identify the gram negative organism.

RESULTS

Figure 1 shows the distribution of gram-negative bacteria isolated during the rainy season. Dam water sources recorded the highest number of 57 gram-negative bacteria. This was followed by hand-dug wells with 43 gram-negative bacteria isolates.

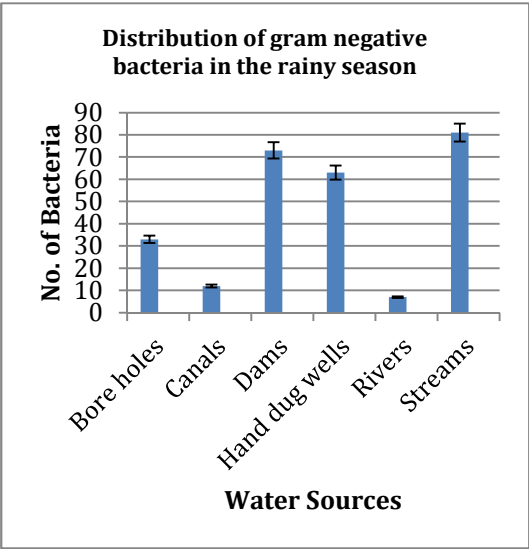


FIGURE 1: THE DISTRIBUTION OF GRAM-NEGATIVE BACTERIA ISOLATED IN THE RAINY SEASON

The least number of gram-negative bacteria isolates in the rainy season was obtained from river water sources with 12 isolates.

Figure 2. shows the distribution of gram-negative bacteria isolates during the dry season. Dam water sources recoded the highest (73) number of gram-negative bacteria isolates in the dry season. This was followed by Hand-dug wells with 63 isolates. The least number of isolates (7) was obtained from river water sources

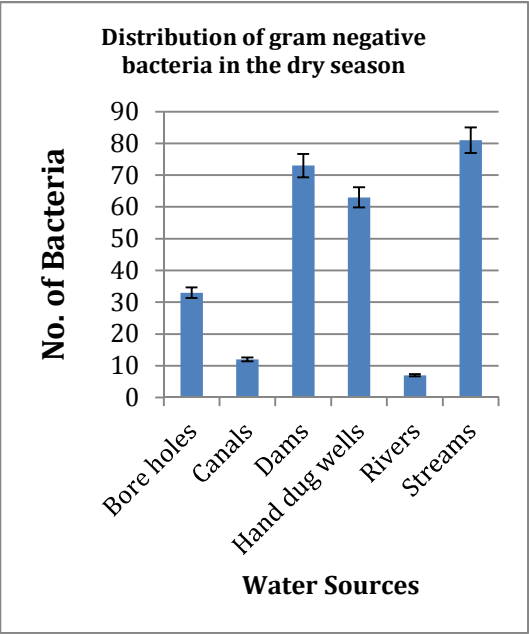


FIGURE 2: THE DISTRIBUTION OF GRAM-NEGATIVE BACTERIA ISOLATED IN THE DRY SEASON.

Figure 3 shows the distribution of gram-positive bacteria isolated during the rainy season. Stream water sources had the highest number (10) of gram-positive bacteria in the rainy season.

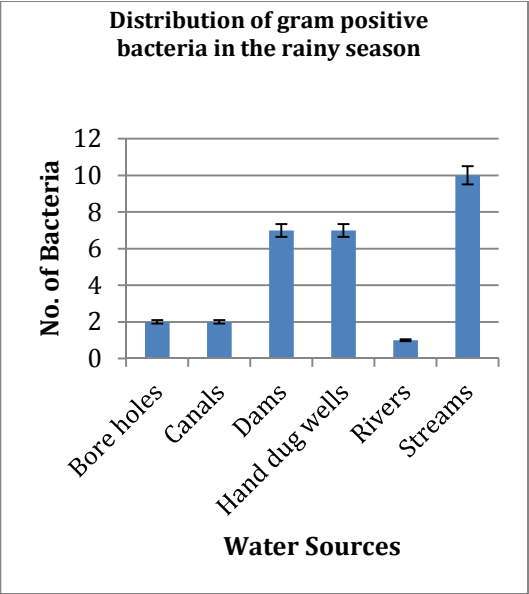


FIGURE 3: THE DISTRIBUTION OF GRAM-POSITIVE BACTERIA ISOLATED IN THE RAINY SEASON

his was followed by hand-dug wells and dam water sources with (7) bacteria isolate each. The least number (1) of gram-positive bacteria were obtained from river water sources.

Figure 4 shows the patterns of gram-positive bacteria isolated in the dry season. Hand-dug wells presented with the highest number (11) of bacteria. The second highest number (10) of gram-positive bacteria isolates in the dry season was observed in dam water sources. The least number (1) was recoded in river water sources.

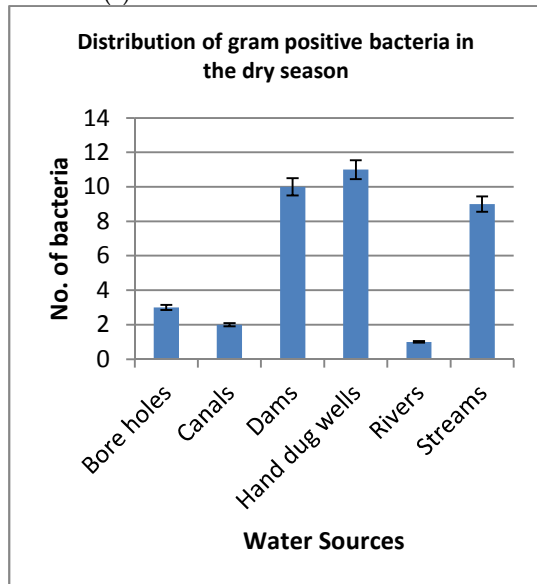


FIGURE 4: THE DISTRIBUTION OF GRAM-POSITIVE BACTERIA ISOLATED IN THE DRY SEASON

Generally, there were more gram-positive bacteria isolated in the dry season as compared to the rainy season.

Figure 5 shows the overall percentage distribution of bacteria isolated from the various water sources in terms of gram stain reactions. Five hundred and twenty (520) bacteria were isolated. Four hundred and fifty two (452) representing 87.5% were found to be gram negative; while sixty eight (68) representing 12.5% were gram positive.

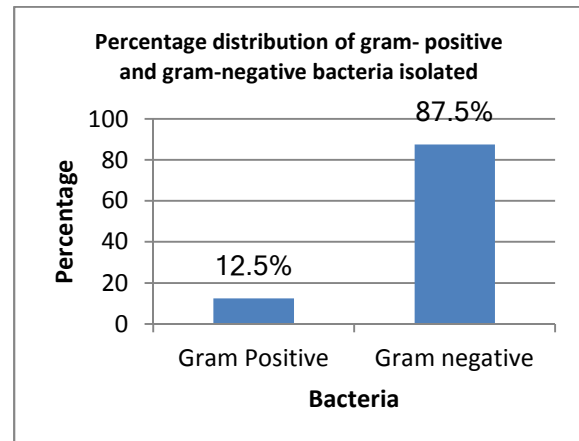


FIGURE 5: PERCENTAGE DISTRIBUTION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA ISOLATES

Microbiological pathogens that are transmitted by the faecal-oral route, especially those originating from human feces are of particular concern for public health. Result from the bacteria isolation analysis (tables 2 and 3), indicates the presents of these oral-faecal pathogens in the various drinking water sources. Bacteria that cause faecal-oral infections include *Escherichia coli* (diarrhoeal infection or dysentery), *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerae* (cholera), were isolated.

Table 2 shows the numbers and distribution of bacteria isolated during the rainy season. The results show that 215 bacteria were isolated from the different water sources during the rainy season. *Klebsiella* spp. was the highest isolated bacteria (45) representing 20.9% of the total bacteria isolated. *E. coli* followed with 39 isolates representing 18.1% of the total isolates in the rainy season. This was followed by: *Pseudomonas auriginosa* (15.8%); *Enterobacter* spp. (14.0%); *Proteus vulgaris* (12.6%); *Enterococcus faecali* (910.7%); *Streptococcus* spp. (2.8%); *Salmonella typhi* (21.4%).

TABLE 2: DISTRIBUTION OF BACTERIA SPECIES ISOLATED FROM DIFFERENT WATER SOURCES IN THE RAINY SEASON

Type of Bacteria	Number of bacteria isolates from each water source						Total (%)
	Bore holes	Canals	Dams	Hand-dug wells	Rivers	Streams	
<i>E. coli</i>	4	3	12	10	0	10	39 (18.1)
<i>Enterobacter</i> spp.	2	1	11	8	0	8	30 (14.0)
<i>Klebsiella</i> spp.	4	3	12	10	1	15	45 (20.9)
<i>Salmonella typhi</i>	2	0	0	1	0	1	4 (1.9)
<i>Streptococcus</i> spp.	0	1	2	2	0	1	6 (2.8)
<i>Proteus vulgaris</i>	2	0	10	5	0	10	27 (12.6)
<i>Vibrio cholerae</i>	0	0	1	1	0	2	4 (1.9)
<i>Shigella</i> spp.	0	0	1	0	0	2	3 (1.4)
<i>Pseudomonas aeruginosa</i>	2	1	10	8	1	12	34 (15.8)
<i>Enterococcus faecalis</i>	2	1	5	5	1	9	23 (10.7)
Total							215 (100)

The least isolated organism was *Vibrio cholerae* (1.9%) and *Shigella* spp. (1.4%). The three most significant bacteria isolates in terms of public health importance isolated during the rainy season were *E. coli*, *Vibrio cholerae* and *Shigella* spp. *E. coli* was isolated in all the water sources except river. *Vibrio cholerae* was isolated in two (2) water sources namely: streams, and dams, whilst *Shigella* spp. was isolated in streams, and dams water sources only. The highest occurring gram positive organism isolated was *Enterococcus faecalis* (23) representing 10.7% whilst that of gram negative organisms *Klebsiella* spp. (45) 20.9%. Generally, the patterns of bacteria isolated in the dry season (table 4.12) did not differ much from that observed in the rainy season. However,

the total bacteria isolated in the dry season were 305.

The highest occurring bacteria isolated during the dry season were *Klebsiella* spp. (59), accounting for 19% of the total bacteria isolates of 305. *E. coli* followed with (58) 19.3%, thus almost the same percentage as that of *Klebsiella* spp. The occurrences of the other bacteria were: *Pseudomonas aeruginosa* (15.4%); *Enterobacter* spp. (8.2.0%); *Proteus vulgaris* (13.4%); *Enterococcus faecalis* (910.7%); *Streptococcus* spp (2.8%); *Salmonella typhi* (21.4%). The least isolated organism was *Vibrio cholerae* (0.7%) and *Shigella* spp. (1.0%). *Vibrio cholerae* was isolated from bore hole and stream sources, whilst *Shigella* spp. were isolated from stream sources only.

TABLE 3: DISTRIBUTION OF BACTERIA SPECIES ISOLATED FROM DIFFERENT WATER SOURCES IN THE DRY SEASON

Bacteria	Number of bacteria isolates from each water source						Total (%)
	Bore holes	Canals	Dams	Hand-dug wells	Rivers	Streams	
<i>E. coli</i>	7	3	16	14	2	16	58 (19.0)
<i>Enterobacter</i> spp.	6	2	15	13	1	13	50 (16.4)
<i>Klebsiella</i> spp.	6	3	16	14	2	18	59 (19.3)
<i>Salmonella typhi</i>	2	1	2	2	0	2	9 (3.0)
<i>Streptococcus</i> spp.	0	0	7	3	0	1	11 (3.6)
<i>Proteus vulgaris</i>	5	1	12	9	0	14	41 (13.4)
<i>Vibrio cholerae</i>	1	0	0	0	0	1	2 (0.7)
<i>Shigella</i> spp.	0	0	0	0	0	3	3 (1.0)
<i>Pseudomonas aeruginosa</i>	6	2	12	11	2	14	47 (15.4)
<i>Enterococcus faecalis</i>	3	2	3	8	1	8	25 (8.2)
Total							305 (100)

In summary, analysis of results from Tables 2 and 3 show that five hundred and twenty bacterial isolates (520) were obtained during the period of study. More of the Isolates (305) representing 58.65% of the total were obtained during the dry season, as against (205) representing 41.35% in the rainy season. The most commonly occurring organism in the water samples was *Klebsiella spp.* (20%). The next most occurring bacterial isolate after *Klebsiella spp.* was *E. coli* (18.7%) of the total bacterial Isolates. This was followed by: *Pseudomonas aeruginosa* (15.61%); *Enterobacter spp.* (15.4%); *Proteus vulgaris* (13.1%); *Enterococcus faecalis* (9.2%); *Streptococcus spp.* (3.1%); *Salmonella typhi* (2.4%). The least isolated organism was *Vibrio cholerae*

(1.2%) and *Shigella spp.* (1.2%). *Vibrio cholerae* was isolated in four (4) water sources namely: stream, borehole, hand-dug wells and dam water sources, while *Shigella spp.* was isolated in stream, borehole and dam water sources only.

Table 4 presents the statistical summary of the bacteria species isolated from different water sources. Generally there was a statistical differences ($P > 0.05$) of the microbes isolated seasonally. The highest occurring was *Klebsiella spp.* (9.83 ± 6.99 , $P > 0.05$) in the dry season and the least *Shigella spp.* $P > 0.05$. negative; while sixty eight (68) representing 12.5 % were gram positive.

Bacteria	Rainy season				Dry season				P value
	Mean $\pm$ SD	Min	Max	d.f	Mean $\pm$ SD	Min	Max	d.f	
<i>E. coli</i>	6.5 $\pm$ 4.81	0	10	5	9.67 $\pm$ 6.47	2	16	5	0.01
<i>Enterobacter spp.</i>	5 $\pm$ 4.56	0	11	5	8.33 $\pm$ 6.12	1	15	5	0.00
<i>Klebsiella spp.</i>	7.5 $\pm$ 5.61	1	15	5	9.83 $\pm$ 6.99	2	18	5	0.01
<i>Salmonella typhi</i>	0.67 $\pm$ 0.82	0	2	5	1.5 $\pm$ 0.84	0	2	5	0.02
<i>Streptococcus spp.</i>	1 $\pm$ 0.89	0	2	5	1.83 $\pm$ 2.79	0	3	5	0.20
<i>Proteus vulgaris</i>	4.5 $\pm$ 4.64	0	10	5	6.83 $\pm$ 5.78	0	14	5	0.01
<i>Vibrio cholerae</i>	0.67 $\pm$ 0.82	0	2	5	0.33 $\pm$ 0.52	0	1	5	0.20
<i>Shigella spp.</i>	0.5 $\pm$ 0.84	0	2	5	0.5 $\pm$ 1.23	0	3	5	0.50
<i>Pseudomonas aeruginosa</i>	5.67 $\pm$ 4.93	1	12	5	7.83 $\pm$ 5.23	2	14	5	0.00
<i>Enterococcus faecali</i>	3.83 $\pm$ 3.13	1	9	5	4.16 $\pm$ 3.06	1	8	5	0.33

TABLE 4: STATISTICAL SUMMARY OF BACTERIA SPECIES ISOLATED FROM DIFFERENT WATER SOURCES

SD= standard deviation, df= degree of freedom, Min= minimum, Max= maximum

Figure 6 shows the number of bacteria that was isolated from each water source across seasons. The highest number (70) of bacteria isolated in the rainy season was obtained from stream water sources. This was followed by dam water sources with 64 bacteria isolates.

The least number (3) of bacteria isolated was obtained from river sources. The highest number (90) of bacteria isolated in dry season was obtained from stream water sources. This was followed by dam water sources with 83 isolates. The least (3) num 155 bacteria isolated in the dry season was from river water sources. The highest number of bacteria isolated per water source across both the dry and

rainy season was 160 representing (21%) this was obtained from stream water sources. The least was 12 (2.1%) obtained from river water sources. Figure 7 shows the percentage distribution of coliform bacteria and non-coliform isolated from the various water sources. Coliform bacteria are generally lactose fermenters and belong to the family enterobacteriaceae. Out of the five hundred and twenty (520) bacteria isolated, three hundred (300) representing 57.7 % were found to be coliform bacteria; while two hundred and twenty (220) representing 42.3 % were non-coliform bacteria.

Percentages of coliform and non-coliform bacteria Isolated

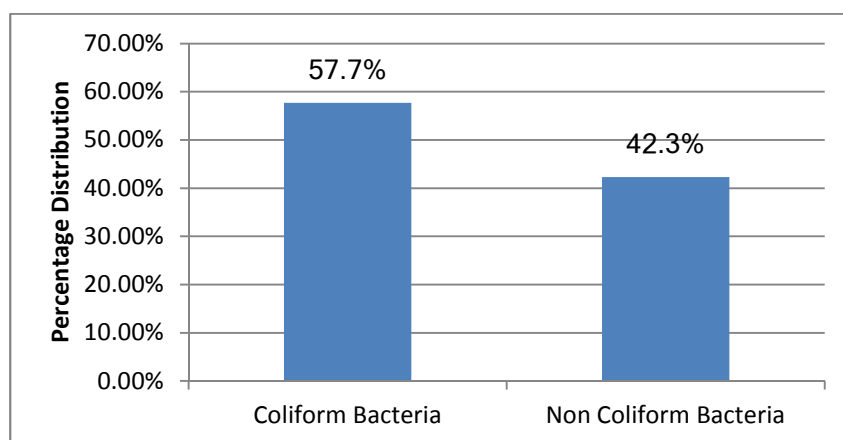


FIGURE 7: PERCENTAGE DISTRIBUTION OF COLIFORM AND NON-COLIFORM BACTERIA OBTAINED FROM WATER

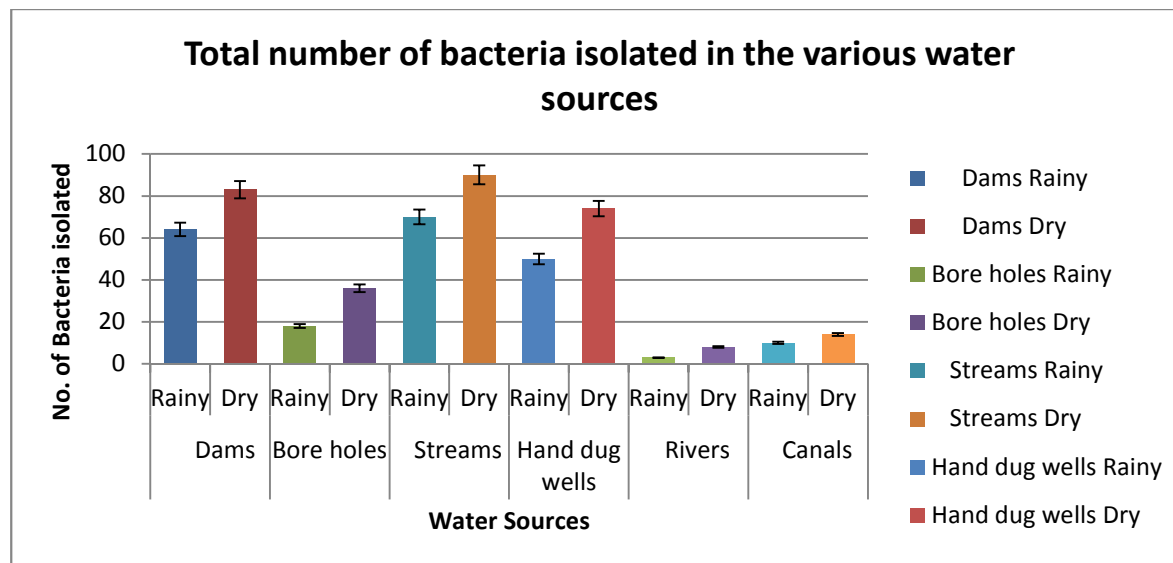


FIGURE 6: TOTAL NUMBER OF BACTERIA ISOLATED ACROSS SEASONS, PER WATER SOURCE

The coliform bacteria (tables 1 and 2) isolated included: *E. coli*, *Enterobacter spp*, *Klebsiella spp*, *Proteus vulgaris*, *Salmonella typhi*, and *Shigella spp*. The non-coliform bacteria isolated were: *Streptococcus spp*, *Vibrio cholera*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

DISCUSSION

There is increasing recognition that continual surveillance has a legitimate place in the consideration of options for water quality management. This is because they are sensitive indicators of changes or deterioration in overall water quality, providing a useful addition to physical, chemical and biological information. The effects of

the high bacteriological contaminants in the drinking water sources are cause for concern. They could trigger outbreaks of epidemics and isolated water borne diseases in the very near future if measures are not taken to get the water decontaminated before consumption.

In the bacteriological analysis of the water sources, the study found that there are significantly high counts in total coliforms, faecal coliforms, and *E. coli* across seasons but specifically higher in the dry season as against the rainy season. This was observed despite, run-off, and heavy rain during the rainy season. Second, was the observation of a correlation between faecal coliform and total coliform counts in

the dry season and the rainy season.

What could account for the high *E. coli* counts observed in the water sources? Human activities as well as faecal discharges from animals may be major contributing factors. However the relative importance of specific animals as contributors to the high faecal coliform numbers observed here is difficult to assess with confidence and was not formally examined in this study. However, it is probably related to factors such as animal population density and utilization of the territory adjacent to the sample sites.

However, some observations in the district suggest the reasons for human faecal contaminations of the waters sources. For example, lack of proper and permanent disposal sites for both solid and liquid wastes in the district may result in the use of streams as receptacles for these untreated wastes. In addition, some residents resort to insanitary practices such as defecating or urinating into open space, gutters which ultimately find their way into bodies of water. Furthermore, the groundwater (wells) did not have proper physical barriers. For example the wells were observed to have missing covers, lockable sanitary lids and well linings, which could prevent overland runoff containing human, animals and domestic wastes from contaminating the water sources. This could account for the detection of bacteria of faecal origin in groundwater in the study area.

WHO [15] reported that groundwater is less vulnerable to contamination due to the barrier effect, and that once the protective barrier is breached direct contamination may occur. In the cases of boreholes, Chapman [16] noted that due to the relatively slow movement of water through the ground, once polluted, a groundwater body could remain so for decades, or even centuries.

Another interesting and important observation of this study was the apparent predominance of *Klebsiella* spp. (Table 3). *Klebsiella pneumoniae* is a rod shaped non-motile, gram negative, lactose fermenting and facultative anaerobic bacterium, which are usually found in the normal flora of skin, mouth, and intestines. *Klebsiella* spp. is responsible for pneumonia (the destructive lung inflammation disease). Besides *Klebsiella* is found to cause infections in the urinary and lower biliary tract [17,18]. *Klebsiella* is an opportunistic pathogen that primarily attacks immune-compromised individuals and hospitalized patients [19]. The predominance of *Klebsiella* spp. as opposed to *E. coli* is because *Klebsiella* spp. can survive and remain physiologically active under diverse environmental conditions under which they are exposed [20]. Second, they multiply to high numbers

in waters rich in nutrients, such as pulp mill wastes, etc. The environmental condition of the water sources in the area under study therefore made it conducive for their growth and survival than *E. coli*. Earlier works done though inconclusive appears to support the observation in the current study [21].

Furthermore dam water sources was observed to poses a high disease risk among the five water sources investigated, whiles borehole water sources possess a lower diseases low risk. Even much more alarming was the observation of the presence of bacteria of public health importance in the water 158 . These included *Shigella* spp. (dysentery), *Salmonella typhi* (typhoid fever and acute diarrhoeal infection), *Salmonella typhi* (typhoid fever), and *Vibrio cholerae* (cholera).

The various observation made above led to the conclusion that majority of the water sources used for drinking and domestic purposes in the study area are usually highly contaminated with faecal coliforms above the recommended standards (WHO, GSA) for drinking water. Both animals and humans are the possible sources of faecal bacteria contamination of the drinking water sources. Most of the faecal coliform isolates identified are opportunistic pathogens capable of causing infection and disease.

The implication and importance of this finding is momentous and cannot be overemphasized. Findings from this study indicate that rural folks residing in the Dangme West District of Ghana are at high risk and are highly vulnerable to waterborne diseases resulting from the presence of pathogenic bacteria in the water. This results from several activities, which include increased pollution from various human activities.

Cyclic assessment of the quality of water available to the rural communities may not only be deemed expedient but also fitting. Since many rural people usually rely chiefly on untreated water sources, the presence of coliform bacteria in all the water bodies then calls for concern from the government, corporate bodies as well as the council of elders of the respective communities involved in rural water provision. Taking into account the socio-economic significance of access to safe and potable water, it may be deemed necessary to consider all the water sources for rural communities rather than concentrating on only a single source such as boreholes which may not only serve a handful of the residents but also be accompanied by high drilling costs.

In a nutshell, to reduce the level of bacterial contamination of drinking water sources there should

be an incessant education on issues such as: environmental awareness, (cultivation sanitation habits and ensure that their surroundings and water sources are not indiscriminately polluted), causes, modes of transmission and prevention of water and sanitation related diseases. Furthermore, education on

modes of storing water in proper storing facilities, proper handling of stored water, the treatment of collected water and hand-washing, etc. to help reduce the consumption of contaminated water should be done.

REFERENCES

1. Spellman, F. R. (2017). *The drinking water handbook*. CRC Press.
2. Routledge, D. and Stewart, D. (1998). *Water: Essential for Existence*. Explore Magazine, (Volume 8/Number 5)
3. Kløve, B., Kvitsand, H. M. L., Pitkänen, T., Gunnarsdottir, M. J., Gaut, S., Gardarsson, S. M., ... & Miettinen, I. (2017). Overview of groundwater sources and water-supply systems, and associated microbial pollution, in Finland, Norway and Iceland. *Hydrogeology Journal*, 25(4), 1033-1044.
4. Johnson, C. C. (2016). Drinking Water Policy Problems Background Of The Current Situation. *Safe Drinking Water: Current and Future Problems*.
5. Fawell J and Nieuwenhuijsen M (2003) Contaminants in drinking water British Medical Bulletin 2003; 68: 199-208
6. Fawell J, Standfield G. (2001) Drinking water quality and health. In: Harrison RM (ed.) *Pollution: Causes, Effects and Control*, 4th edn. London: Royal Society of Chemistry,
7. Odonkor, S.T., & Ampofo, J.K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, 4(2), 5-8.
8. Pandit, A. B., & Kumar, J. K. (2015). Clean water for developing countries. *Annual review of chemical and biomolecular engineering*, 6, 217-246.
9. WHO (World Health Organization). (2000). WHO annual report on infectious disease: overcoming antimicrobial resistance; World Health organization: Geneva, Switzerland. (<http://www.who.int/infectious-disease-report/2000/>),
10. WHO (World Health Organization) (1993). *Guidelines for Drinking Water Quality*, Vols. 1-3. Geneva, Switz: WHO
11. APHA. 1998. *Standard Methods for the Examination of Water and Wastewater* 20th Edition. United Book Press, Inc., Baltimore, Maryland
12. APHA, 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th edition. American Public Health Association, American water works association and Water environment Federation Washington, D. C.. Beachwatch
13. WHO. 1996. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO scientific working group meeting. Berlin, Germany, 1-28.
14. WHO. 1982. Vaccine research and development. New strategies for accelerating *Shigella* vaccine development. *Wkly. Epidemiol. Rec.* 72:73-79.
15. WHO. (2006). *Guidelines for drinking-water quality: incorporating first addendum*. Vol. 1, Recommendations, 3rd (ED); 1, 136, 143, 222, 282.
16. Chapman, D. (1996). *Water Quality Assessments. A guide to use of biota, sediments and water in environmental monitoring*, 2nd (ED). E&FN Spon: 417
17. Ryan, K.J., & Ray, C.G. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385- 8529-9
18. Lopes, A. C. D. S., Rodrigues, J. F. & Morais, M. A. D. (2005). Molecular typing of *Klebsiella pneumoniae* isolates from public hospitals in Recife, Brazil. *Microbiological Research* 160:37-46.
19. Podschun, R., & Ullmann, U. (1998). *Klebsiella spp.* as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews* 11(4): 589-603.
20. Lopez-Torres, A. J., Terr, C.H., & Gary, A.T. (1987). Distribution and In situ Survival and Activity of *Klebsiella Pneumoniae* and *Escherichia coli* in a Tropical rain forest Watershed. *Current Microbiology* vol. vol 12 pp. 213-218.
21. Podschun, R., Pietsch, S., Höller, C., & Ullmann, U. (2001). Incidence of *Klebsiella Specie* in Surface Water and Their Expression Virulence factors. . *Appl Environ Rev Microbiol.* 2001 July; 67(7): 3325-3327.