British Microbiology Research Journal
2(3): 175-186, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org

Contributions of *Escherichia coli* to Diarrhoea among HIV/AIDS Patients at a Hospital in Tropical West Africa

Esther D. Tyodugh[1], Ubleni U. Emanghe[2], Godwin T. Jombo[1*] and Ella B. Abraham[3]

[1]Department of Medical Microbiology and Parasitology, College of Health Sciences, Benue State University, PMB 102119 Makurdi, Nigeria.
[2]Department of Medical Microbiology and Parasitology, College of Medical Sciences, University of Calabar, PMB 1115 Calabar, Nigeria.
[3]Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author EDT designed the study and wrote methodology. Author EBA also designed the study and participated in laboratory procedures. Author UUE did statistical analysis and managed literature searches. Author GTJ worked on introduction and discussion. All authors read and approved the final manuscript.

Research Article

Received 26th January 2012
Accepted 4th April 2012
Published 8th December 2012

ABSTRACT

Aims: To ascertain the contributions of *Escherichia coli to* diarrhea among patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in a HIV endemic community.
Study Design: Hospital based cross-sectional study.
Place and Duration of Study: Mkar Christian hospital, Mkar, Gboko local government area of Benue state, Nigeria between January and June, 2009.
Methodology: Close ended questionnaires were administered and relevant information such as age, sex, marital status, educational background, occupation and regular intake of antiretroviral drugs were obtained from HIV/.AIDS patients with diarrhea and non-HIV/AIDS patients which served as control. Stool samples were collected, stored, transported and processed using standard procedures of microscopy, culture and sensitivity.

**Corresponding author: Email: jombogodwin@yahoo.com;*

Results: Bacteria were found to contribute 59.9% (209) of the diarrhoea among HIV/AIDS patients at Mkar of which *E. coli* accounted for 111 (43.4%) of the cases and was significantly higher among HIV/AIDS patients on irregular or absent anti-retroviral treatment (ART) than those on regular ART, 64.0% (87) versus 36.9% (24) respectively (P< 0.05). Other bacteria recovered were *Salmonella typhi* (30.6%) and *Shigella dysentheriae*/*flexneri* (26.0%).

Conclusion: *E. coli* should be accommodated in symptomatic management of diarrhoea among HIV/AIDS patients at Mkar while efforts should be made at provision of adequate antiretroviral drugs for the people while their strict intake and adherence enforced.

Keywords: Acquired immunodeficiency syndrome; diarrhoea; Escherichia coli; human immunodeficiency virus.

1. INTRODUCTION

Since the discovery of Human Immunodeficiency virus / Acquired immunodeficiency syndrome (HIV/AIDS) in 1981 in Atlanta USA, and the subsequent identification of the virus in Nigerians in 1986; the virus has so far claimed over 35 million lives worldwide (Lurie and Rosenthal, 2010; Onakewhor et al., 2011; Tanser et al., 2010). While the disease appears to be under control in the western hemisphere, same cannot be said about sub-saharan Africa specifically and other developing parts of the world (Yakasai et al., 2011; Agbelusi et al., 2011; Nuwagaba-Birisonwoha et al., 2012). Available statistics show that HIV/AIDS prevalence still stands at over 20% in some African countries constituting a nucleus of infection to other parts of the world (Nuwoha et al., 2011; Schull et al., 2011; Desmonde et al., 2011).

Association of *E. coli* with diarrhea among individuals with HIV/AIDS has received mixed reportage in different parts of the world including Nigeria. A collaborative study from Western Pacific, Southeast Asia and Africa showed varying degrees of significant contributions of *E. coli* associated diarrhea and mortalities (Abba et al., 2009). Studies from Mexico, Brazil and Zimbabwe showed that *E. coli* contributed significantly to diarrhea associated mortalities in HIV/AIDS patients (Fang et al., 1995; Amadi et al., 2001; Guerra-Godinez et al., 2003). On the other hand, findings from USA, Italy and Bangladesh did not show significant association of *E. coli* with diarrhea among HIV/AIDS patients (Akhtar and Shaheen, 2007; Assefa et al., 2009; Matin et al., 2011).

Diarrhoea which is often a common presentation of AIDS among patients in Benue state is often inadequately managed for lack of competent personnel as well as inadequate diagnostic facilities (Hilhorst et al., 2006; Olowoseun et al., 2008; Oboh and Tsue, 2010; BENSACA, 2010). This has perharps led to the assumption that diarrhoea in AIDS patients is almost inevitably caused by parasites, with the attendant wrong treatments and increased mortalities (Olusegun et al., 2009; Adesiji et al., 2007). This study was therefore set up to ascertain the contribution of *E. coli* and other *Enterobacteriaceae* to diarhoea among HIV/AIDS patients at Mkar, a sermi-urban community with high HIV/AIDS prevalence. The findings would be useful to health personnel as regards choices of drugs for management of diarrhoea in AIDS especially where facilities and personnel for proper laboratory diagnosis constitute a major management constraint (Appleton et al., 2009).

2. MATERIALS AND METHODS

2.1 Setting

The study was hospital based and cross-sectional in nature and was carried out at Mkar, a sub-urban community in Gboko local government area of Benue state located at about 85 kilometres north east of Makurdi, the state capital. Based on 2006 national population census the community has a population of 35,000 inhabitants and houses a university, a mission hospital (NKST-Nongu U Kristu U Ken Sudan hen Tiv Hospital), and other higher institutions among others (Nigeria Min. of Information, 2006 National Population census). More than 90% of the inhabitants are subsistent farmers and over 98% of the populace is of Tiv ethnicity.

2.2 Sampling Procedure

The study was carried out at NKST Hospital Mkar which has a HIV/AIDS treatment centre which caters for the needs of host community and environs as well as people from other parts of the state. HIV/AIDS patients with diarrhoea attending the clinic between January and June 2009 were consecutively recruited into the study. Participation was voluntary, questionnaires were administered to obtain relevant information such as age, gender, educational level, occupation, marital status, and presence or otherwise of diarrhoea. Control subjects were obtained from non-HIV/AIDS in- and out-patients with diarrhoea.

2.3 Sample Collection and Processing

Diarrhoea was defined as passage of loose or watery stool for at least three times in 24 hours (Parry et al., 2004). Stool samples were collected using wide mouthed grease-free stool containers and were transported in trays within 10 minutes of collection to the laboratory sited about 30 metres away. Where delays of more than 30 minutes were envisaged before processing, samples were stored at 4^{o}C. Saline and iodine wet preparations, modified Ziehl-Neelsen staining procedure, and microscopy were carried out using standard procedures (Mkhize-Kwitshana et al., 2011). Concentration of stool was carried out using fomol-ether concentration technique (Mkhize-Kwitshana et al., 2011). Culture was carried out on MacConkey agar, Deoxycholate citrate agar and Cysteine Lactose electrolyte efficient (CLED) media and incubated at 36.5^{o}C over night. Biochemical tests such as oxidase test, indole test, motility test, citrate, urease and sugar fermentation were carried out using standard procedures (Appleton et al., 2009). Antimicrobial susceptibility test was carried out on nutrient agar medium using modified disk diffusion method with appropriate McFarland's ratio of turbidity (Appleton et al., 2009). Control stool samples were obtained from willing subjects who presented to the hospital during the study period who tested non-reactive to HIV infections. Control samples were collected, transported, stored and processed using the same procedure as that of the test samples (Mkhize-Kwitshana et al., 2011; Appleton et al., 2009).

2.4 Ethical Considerations

Ethical approval for the study was obtained from the management of the Mkar Christian hospital, voluntary consent of the subjects was obtained while patients confidentiality was ensured throughout the study.

2.5 Data Management and Analysis

Data obtained was analysed using simple descriptive methods of arithmetic sum, mean, mode and standard deviation. Epi Info 6 statistical software was also used to compare differences at 95% confidence intervals where applicable.

3. RESULTS

A total of 366 HIV/AIDS patients with diarrhoea were assessed during the study period comprising 168(46.0%) males and 198 (54.0%) females. The age range of the subjects was 17 and 66 years with a mean age of 34 years (±2 SD), median age of 31 and, bimodal ages of 27 and 33 years (Table 1).

Table 1. Age* and gender distribution of HIV/AIDS patients with diarrhoea at Mkar, north central Nigeria**

Age Interval (Years)	Male (%)	Female (%)	Total (%)
≤20	11 (3.00)	21 (5.70)	32 (8.70)
21-30	32 (8.70)	49 (13.40)	81 (22.10)
31-40	86 (16.70)	63 (17.20)	124 (33.90)
41-50	38 (10.40)	30 (8.20)	68 (18.60)
51-60	20 (5.50)	24 (6.50)	44 (12.00)
≥61	6 (1.70)	11 (3.00)	17 (4.70)
Total	168 (46.00)	198 (54.00)	366 (100)

X^2(Yates Corrected) = 0.23, df =5, p= 0.68
X^2(Yates Corrected) = 0.71, df =1, p= 0.91

A review of micro-organisms recovered from stool samples of HIV/AIDS patients with diarrhoea showed that 201 (54.8%) of them were associated with bacteria compared to the 15.0% (15) among the non-AIDS patients with significant difference ($P < 0.05$). Also 38.5% (141) of the stool samples from HIV/AIDS patients had protozoa as compared to the low 14.0% (14) from the non-AIDS patients, this difference is also statistically significant ($P < 0.05$). There was no significant difference in the rate of helminthic infections among the two groups ($P > 0.05$). The number of stool samples without any pathogenic microorganism among the non-AIDS patients was significantly higher than that of the HIV/AIDS patients, 54.0% (54) versus 7 (1.9%) respectively ($P < 0.05$) (Fig. 1).

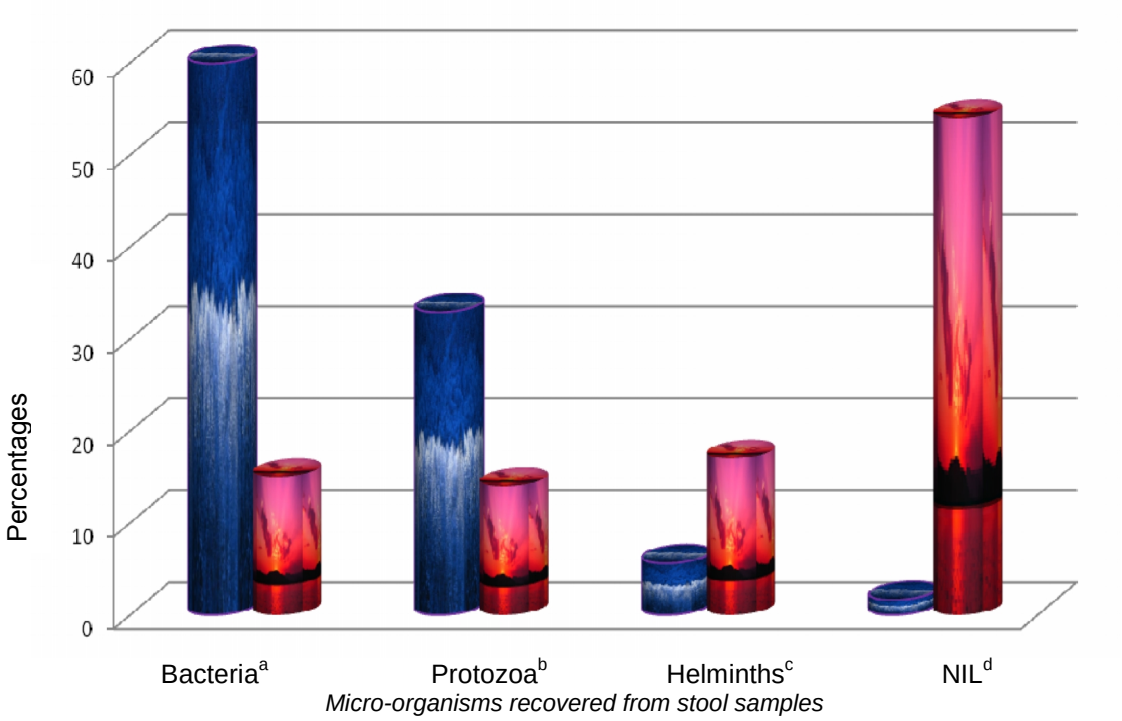

Fig. 1. Spatial grouping of Micro-organisms recovered from stool samples of HIV/AIDS patients with diarrhea at Mkar, north central Nigeria

Key:

Diarrhoeal patients with HIV/AIDS (n=429)

Diarrhoeal patients without HIV/AIDS (n=100)

a X^2 (Mantel-Haenszel)= 16.68, df=1, P < 0.0001
b X^2 (Mantel-Haenszel)= 8.54, df= 1, P< 0.05
c X^2 (Mantel-Haenszel)= 3.71, df=1, P > 0.05
d X^2 (Mantel-Haenszel)= 39.19, df= 1, P < 0.0001

Analysis of the association of the type of microorganism with diarrhea among the patients showed that 52.1% (187) and 19.6% (9) were associated with diarrhea among the HIV/AIDS and non-AIDS respectively with statistically significant difference (P < 0.05). The rates of parasitic infections only among the non-AIDS patients on the other hand were significantly higher than that among the HIV/AIDS patients, 58.7% (27) versus 32.0% (115), (P < 0.05). There was no significant difference in rate of bacteria and parasites co-infections among the two groups (P > 0.05) (Fig. 2).

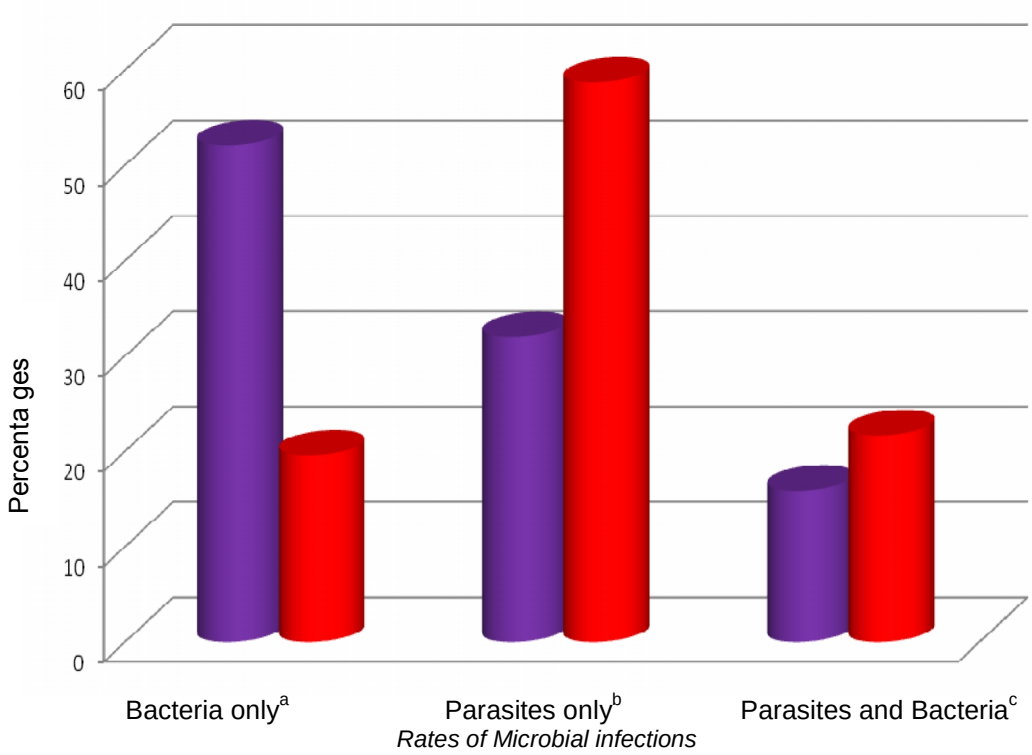

Fig. 2. Association of micro-organisms with diarrhea among patients with HIV/AIDS in relation to those without HIV/AIDS at Mkar, north-central Nigeria

a X^2 (Mantel-Haenszel)= 10.57, df=1, P < 0.01
b X^2 (Mantel-Haenszel)= 5.53, df= 1, P < 0.05
c X^2 (Mantel-Haenszel)= 0.57, df= 1, P > 0.05

A comparison of the bacterial species from stool samples of HIV/AIDS patients with diarrhea as compared to the non-AIDS patients showed that *E. coli* was significantly higher among the HIV/AIDS patients compared to their non-AIDS counterpart, 48.4% (111) versus 6.7% (1), (P < 0.05). The rate of Shigellae infections among the non-AIDS patients on the other hand was significantly higher among the non-AIDS patients compared to the HIV/AIDS individuals, 80.0% (12) versus 26.0% (67), (P < 0.05). There was no significant difference in rates of isolation of *Salmonellae species* compared to the other bacteria among the two study groups (P > 0.05) (Fig. 3).

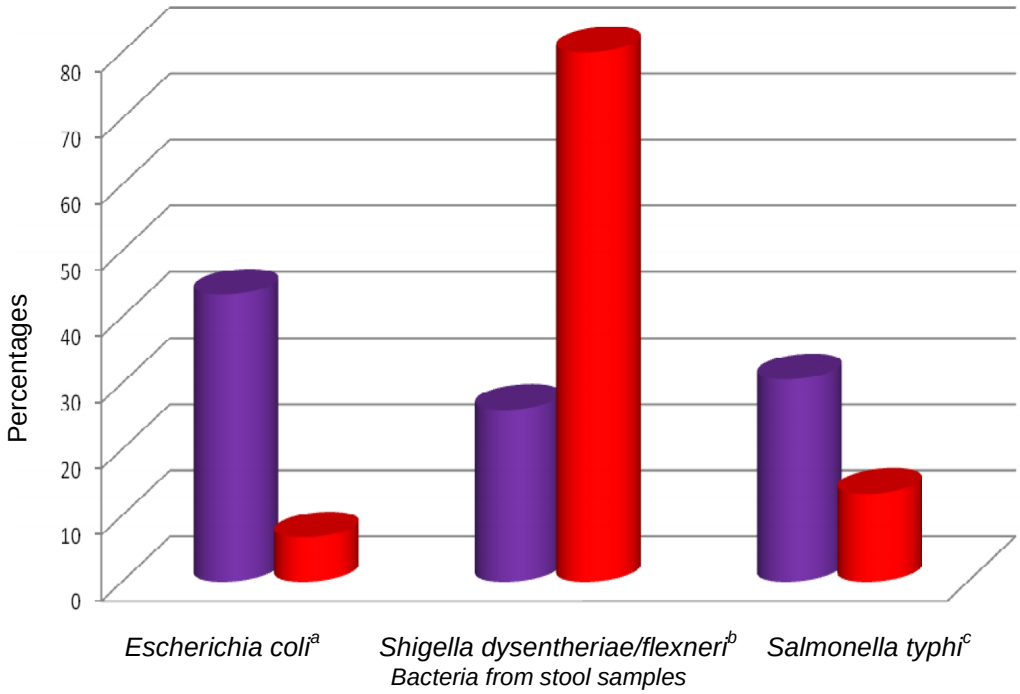

Fig. 3. Bacteria species recovered from stool samples of HIV/AIDS patients with diarrhoea in relation to patients without HIV/AIDS at Mkar, north-central, Nigeria

Key:

HIV/AIDS Patients with diarrhea (n=257)

Diarrhoea patients without HIV/AIDS (n= 15)

a X^2 (Mantel-Haenszel)= 24.51, df= 1, P < 0.0001
b X^2 (Yates Corrected)= 17.52, df= 1, P < 0.00001
c X^2 (Mantel-Haenszel)= 5.54, df= 1, P > 0.05

Among the 366 HIV/AIDS patients studied, 65 (17.8%) were found to be on regular anti-retroviral treatment (ART) while 301 (82.2%) were either on irregular or absent medications. *E. coli* was recovered from 64.0% (87) of the stool samples of persons on nil or irregular ART compared to 36.9% (24) from those on regular ART. This difference is statistically significant (P < 0.05) (Fig. 4).

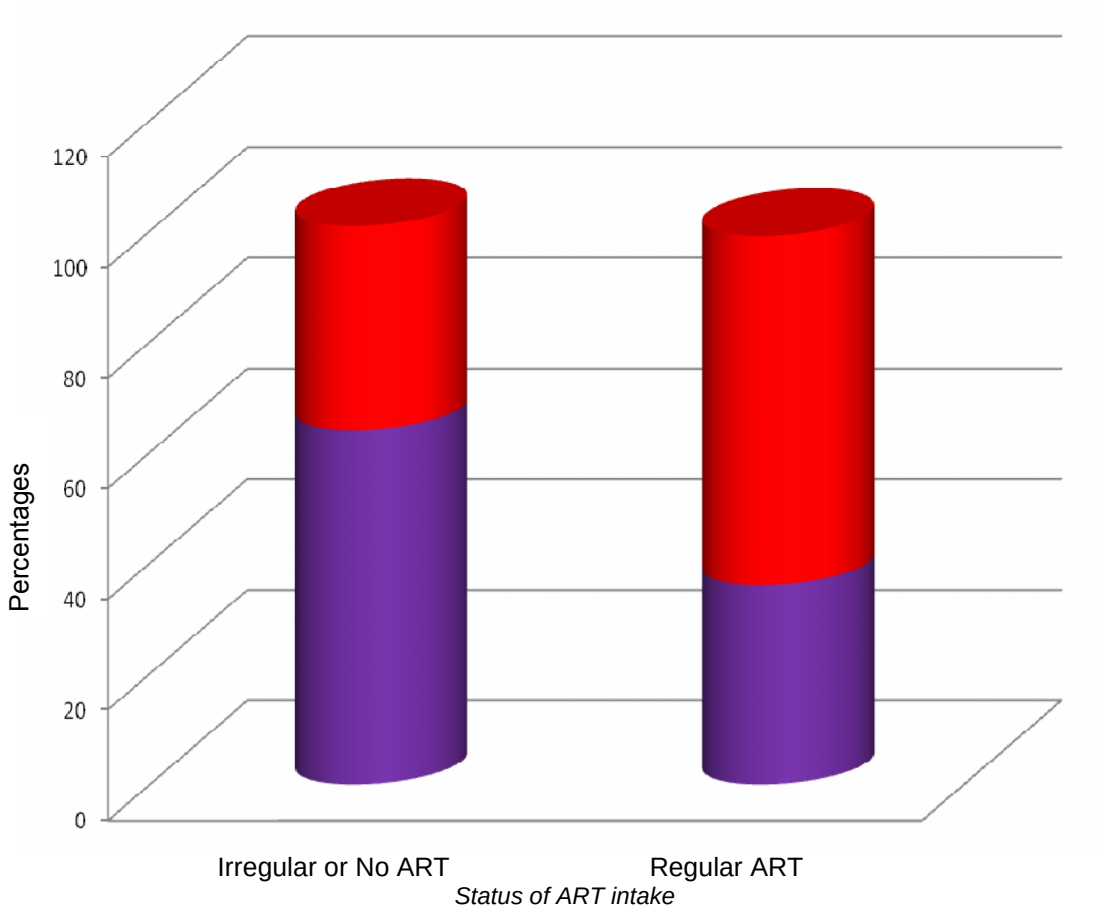

Fig. 4. Isolation of *E. coli* from HIV/AIDS patient with diarrhea in relation to intake of ART

Key:

Escherichia coli (n=111)

Other Bacteria (n=100)

ART= Anti-retroviral treatment

X^2 (Yates Corrected) = 5.27, OR= 1.05-3.00, RR= 1.05-2.07, df= 1, P < 0.05

The most active antibiotics against the bacterial isolates were ciprofloxacin, ofloxacin, ceftriaxone, amikacin, and clavulanic acid/amoxicillin combination (57.0%-98.0%) while tetracycline, ampicillin, co-trimoxazole, erythromycin and chloramphenicol were the least (< 35.0%)

4. DISCUSSION

The study was set up to ascertain the contribution of *E. coli* to diarrhoea among HIV/AIDS patients. Bacteria were found to contribute 59.9% (209) of the diarrhoea among HIV/AIDS

patients at Mkar of which *E. coli* accounted for 111 (43.3%) of the cases and was significantly higher than in the control population (5.0%, 5 out of 100) (P< 0.01). Other bacteria recovered were *S. dysentheriae*/flexneri (30.6%) and *S. typhi* (26.0%).

Findings from the present study probably present the existing regional and geographic variations in stool bacteriology among symptomatic HIV carriers with diarrhoea. The present findings deviate from available literature in which protozoa and helminthes are mostly reported to be responsible for diarrhoea in this group of patients (Mkhize-Kwitshana et al., 2011; Appleton et al., 2009; Mariam et al., 2008). The non-availability and irregular intake of antiretroviral drugs among the respondents which was placed at 93.3% may also have contributed to this microbial picture (Udeh et al., 2008; Adams et al., 2006; Carcamo et al., 2005).

The findings from the present study have also been documented in Central African Republic where *E. coli* contributed to 30% of the diarrhoea in HIV/AIDS patients, 29.5% in South Africa, and 19.0%-42.0% in Lima, Peru (Okeke, 2009; Samie et al., 2007; Medina et al., 2010; Garcia et al., 2010). Provision of potent antiretroviral drugs to the people as well as ensuring strict adherence could eliminate the role of non-specific diarrhoea including *E. coli* to diarrhoea among the patients. Also symptomatic management of diarrhoea among HIV/AIDS patients in the community in the absence of appropriate laboratory reports should make provision for the likelihood of *E. coli* as a causative agent.

The availability of antiretroviral drugs as well as documented evidence of 90 to 98% (average 95%) adherence among studied populations in Venezuela[37], Kuwait[38], Japan[39], and Nicaragua[40] may have on the contrary contributed to the near absence of *E. coli* as a sole causative agent of diarrhea among the HIV/AIDS patients (Hannaoui et al., 2010; Albert et al., 2009; Fujihera et al., 2009; Vilchez et al., 2009). This stresses the need for urgent provision of adequate antiretroviral drugs for the residents of Mkar community and proper control of the disease through education and counseling.

The present study is limited by the fact that not all culture media suitable for potential organisms such as fastidious bacteria and viruses were included. Also no characterization or toxin screening of *E. coli* was conducted to determine their pathogenicity. The research findings therefore should be viewed in the context of these limitations as they have the potential of contributing to the over-representation of *E. coli* in the studied diarrhoeal stools.

5. CONCLUSION

The present study has shown that *E. coli* contributes significantly to diarrhoea among HIV/AIDS patients at Mkar and should be considered at symptomatic management of the patients in the community. Also antiretroviral drugs should be made readily available to the people in the community in order to limit the contribution of *E. coli* to the morbidity of HIV/AIDS patients.

ACKNOWLEDGEMENTS

The authors wish to express their sincere appreciation to those who made it possible for this work to see the light of the day. Notably, the Management of Mkar Christian Hospital for giving us permission to carry out the study in the hospital; the head of the Laboratory, Mr JI Ugor for his logistic support; Mr. MI Mbasugh, Mrs. Iortyour Hannah, Mr. Afer EM, Miss Gwa

Patricia, Mrs. Kalaga Ngohemba and numerous others who assisted immensely in questionnaire administration, sample collection and processing.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

REFERENCES

Abba, K., Sinfield, R., Hart, C.A., Garner, P. (2009). Pathogens associated with persistent diarrhoea in children in low and middle income countries: systematic review. BMC Infectious Diseases, 9, e88. Doi: 10.1186/1471-2334-9-88.

Adams, V.J., Markus, M.B., Kwitshana, Z.L., Dhansay, M.A., Van der Merwe, L., Walzl, G., Fincham, J.E. (2006). Recall of intestinal helminthiasis by HIV-infected South Africans and avoidance of possible misinterpretation of egg excretion in helminth/HIV co-infection analyses. BMC Infectious Diseases, 6(88), 1-8.

Adesiji, Y.O., Lawal, R.O., Taiwo, S.S., Fayemiwo, S.A., Adeyeba, O.A. (2007). Cryptosporidiasis in HIV infected patients with diarrhea in Osun state Southwestern Nigeria. European Journal of General Medicine, 4(3), 119–122.

Agbelusi, G.A., Adeola, H.A., Ameh, P.O. (2011). Knowledge and attitude of PLWHA concerning oral lesions of HIV/AIDS among patients of PEPFAR clinic in Lagos University Teaching hospital (LUTH) Lagos, Nigeria. Nig Postgraduate Medical Journal, 18(2), 120-125.

Akhtar, A.J., Shaheen, M. (2007). Increasing incidence of *Clostridium difficile*-associated diarrhoea in African-American and Hispanic patients: association with the use of proton pump inhibitor therapy. Journal of National Medical Association, 99(5), 500–504.

Albert, M.J., Rotimi, V.O., Dhar, R., Silpikurian, S., Pacsa, A.S., Molla, A.M., Szucs, G. (2009). Diarrhoeagenic *Escherichia coli* are not a significant cause of diarrhoea in hospitalized children in Kuwait. BMC Microbiology, 9, e62. Doi: 10. 1186/1471-2180-9-62.

Amadi, B., Kelly, P., Mwiya, M., Mulwazi, E., Sianongo, S., Changwe, F., Thomson, M., Hachungula, J., Watuka, A., Walker-Smith, J., Chintu, C. (2001). Intestinal and systemic infection, HIV, and mortality in Zambian children with persistent diarrhoea and malnutrition. Journal of Pediatric Gastroenterology and Nutrition, 32, 550–4. doi: 10.1097/00005176-200105000-00011.

Appleton, C.C., Mosala, T.I., Levin, J., Olsen, A. (2009). Geohelminth infectio and re-infection after chemotherapy among slum-dwelling children in Durban, South Africa. Annals of Tropical Medicine and Parasitolology, 103(3), 249-261.

Assefa, S., Erko, B., Medhin, G., Assefa, Z., Shimelis, T. (2009). Intestinal parasitic infections in relation to HIV/AIDS status, diarrhoea and CD4 T-cell count. BMC Infectious Diseases, 9, e155. doi: 10.1186/1471-2334-9-155.

Benue State AIDS Control Agency (BENSACA). (2010). Benue state HIV/AIDS response: MAP 1 close-out report (February 2002- March 2010). BENSACA Assessment Report, Nigeria Makurdi, 1-61.

Carcamo, C., Hootan, T., Wener, M.H., Weiss, N.S., Gilman, R., Arevalo, J., Carrasco, J., Seas, C., Caballero, M., Holmes, K.K. (2005). Etiologies and manifestations of persistent diarrhoea in adults with HIV-1 infection: a case control study in Lima, Peru. Journal of Infectious Diseases, 191, 11-19.

Desmonde, S., Coffie, P., Aka, E., Amani-Bosse, C., Messou, E., Dabis, F., Alioum, A., Ciaranello, A., Leroy, V. (2011). Severe morbidity and mortality in untreated HIV-infected children in a paediatric care programme in Abidjan, Côte d'Ivoire, 2004-2009. BMC Infectious Diseases, 11, 182. doi: 10.1186/1471-2334-11-182.

Fang, G.D., Lima, A.A.M., Martins, C.V., Nataro, J.P., Guerrant, R.L. (1995). Etiology and epidemiology of persistent diarrhoea in northestern Brazil: a hospital-based, prospective, case-control study. Journal of Pediatric Gastroenterology and Nutrition, 21, 137–44. doi: 10.1097/00005176-199508000-00003.

Fujihara, S., Arikawa, K., Aota, T., Tanaka, H., Nakamura, H., Wada, T., Hase, A., Nishikawa, Y. (2009). Prevalence and properties of diarrhoeagenic *Escherichia coli* among healthy individuals in Osaka city, Japan. Japanese Journal of Infectious Diseases, 62, 318-323.

Garcia, C., Chincha, O., Leon, M., Iglesias, D., Barlette, F., Mercado, E., Ochoa, T. (2010). High frequency of diarrhoeagenic *Escherichia coli* in human immunodeficiency virus (HIV) patients with and without diarrhoea in Lima, Peru. American Journal of Tropical Medicine and Hygiene, 82(6), 1118-1120.

Guerra-Godinez, J.C., Larrosa-Haro, A., Coello-Ramirez, P., Tostado, H.R.A., Rivera-Chavez, E., de Leon, Y.A.C., Bojorquez, R., Ma del C., Aguilar-Benavides, S. (2003). Changing trends in prevalence, morbidity, and lethality in persistent diarrhea in infancy during the last decade in Mexico. Archives of Medical Research, 34, 209–13. doi: 10.1016/S0188-4409(03)00027-4.

Hannaoui, E., Villalobos, L., Martinez, R., Maldonado, A., Hagel, I., Bastardo, J. (2010). Diarrhoeagenic *Escherichia coli* associated with acute diarrhoea in children of Cumana, Venezuela. Invest Clin, 51(4), 489-500.

Hilhorst, T., Liere, M.J., Ode, A.V., de Konong, K. (2006). Impact of AIDS on rural livelihoods in Benue state, Nigeria. J Social Aspects of HIV/AIDS. 3(1), 381-393.

Lurie, M.N. (2010). Rosenthal S. Concurrent partnerships as a driver of the HIV epidemic in sub-saharan Africa? The evidence is limited. AIDS Behavior, 14, 17-24.

Mariam, Z.T., Adebe, G., Mulu, A. (2008). Opportunistic ad other intestinal parasitic infections in AIDS patients, HIV seropositive healthy carriers and HIV seronegative individuals in southwest Ethiopia. East African Journal of Public Health, 5(3), 169-172.

Matin, N., Shahrin, L., Pervez, M.M., Banu, S., Ahmed, D., Khatun, M., Pietroni, M. (2011). Clinical Profile of HIV/AIDS-infected Patients admitted to a New Specialist Unit in Dhaka, Bangladesh—A Low-prevalence country for HIV. Journal of Health Population Nutrition, 29(1), 14–19.

Medina, A.M., Rivera, F.P., Romero, L.M., Kolevic, L.A., Castillo, M.E., Verne, E., Hermandez, R., Mayor, Y.E., Barlette, F., Mercado, E., Ochoa, T.J. (2010). Diarrhoeagenic *Escherichia coli* in human immunodeficiency virus (HIV) paediatric patients in Lima, Peru. American Journal of Tropical Medicine and Hygiene, 83(1), 158-163.

Mkhize-Kwitshana, Z.L., Taylor, M., Jootse, P., Mabaso, M.L.H., Watzl, G. (2011). The influence of different helminth infection phenotypes on immune responses against HIV in co-infected adults in south Africa. BMC Infectious Diseases, 11, e273. doi: 10. 1186/1471-2334-11-273.

Nigeria Ministry of Information: 2006. National population census, National Bureau of statistics, Ministry of Information Headquarters, 2006 Abuja, Nigeria.

Nuwagaba-Biribonwoha, H., Mayon-White, R.T., Okong, P, (2012). Brocklehurst P, Carpenter LM. The Impact of HIV on Maternal Morbidity in the Pre-HAART Era in Uganda. Journal of Pregnancy, 2012, e508657. doi: 10.1155/2012/508657.

Nuwaha, F., Babirye, J., Ayiga, N. (2011). Why the increase in under five mortality in Uganda from 1995 to 2000? A retrospective analysis. BMC Public Health, 11, e725. doi: 10.1186/1471-2458-11-725.

Oboh, V.U., Tsue, P.T. (2010). Awareness of HIV/AIDS pandemic among rural farmers in Vandeikya local government area of Benue state, Nigeria. Ethno Medicine, 4(3), 183-189.

Okeke, I.N. (2009). Diarrhoeagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. Journal of Infection in Developing Countries, 3(11), 817-842.

Olowoseun, T., Sule, A.M., Sanni, O.A., Onimisi, H.U., Olowosegun, O.M. (2008). Awareness of HIV/AIDS pandemic in selected fishing communities in north-central Nigeria. Asian Journal of Epidemiology, 1(1), 17-23.

Olusegun, A.F., Okaka, C.E., Machado, R.L.D. (2009). Isosporiasis in HIV/AIDs patients in Edo state Nigeria. Malaysian Journal of Medical Science, 16(3), 43–46.

Onakewhor, J.U., Olgbujo, B.N., Ande, A.B., Ezeanochie, M.C., Olkor, O.E., Okonofua, F.E. (2011). HIV-AIDS related maternal mortality in Benin-city, Nigeria. Ghana Medical Journal, 45(2), 54-59.

Parry, E., Godfrey, R., Mabey, D., Gill, G. (2004). Principles of Medicine in Africa, 3RD Edn, UK Cambridge University Press, 273-278.

Samie, A., Obi, C.L., Dillingham, R., Prinkerton, R.C., Guerrant, R.L. (2007). Enteroaggregative *Escherichia coli* in Venda, South Africa: Distribution of virulence-related genes by multiplex polymerase chain reaction in stool samples of human immunodeficiency virus (HIV)-positive and HIV-negative individuals and primary school children. American Journal of Tropical Medicine and Hygiene, 77(1), 142-150.

Schull, M.J., Cornick, R., Thompson, S., Faris, G., Fairall, L., Burciul, B., Sodhi, S., Draper, B., Joshua, M., Mondiwa, M., Banda, H., Kathyola, D, (2011). Bateman E, Zwarenstein M. From PALSA PLUS to PALM PLUS: adapting and developing a South African guideline and training intervention to better integrate HIV/AIDS care with primary care in rural health centers in Malawi. Implement Science, 6, e82. doi: 10.1186/1748-5908-6-82.

Tanser, F., Barnighausen, T., Hund, L., Garnett, G.P., McGrath, N., Newell, M.L. (2011). Effect of concurrent sexual partnerships on rate of new HIV infections in a high-prevalence, rural south frican population: a cohort study. Lancet, 378(9787), 247-255.

Udeh, E.O., Goselle, O.N., Popova, D., Abelau, M., Popov, T.V., Jean, N., David, J.S. (2008). The prevalence of intestinal protozoans in HIV/AIDS patients in Abuja, Nigeria. Science World Journal, 3(3), 1-4.

Vilchez, S., Reyes, D., Paniagua, M., Bucardo, F., Mollby, R., Weintraub, A. (2009). Prevalence of diarrhoeagenic *Escherichia coli* in children from Leon, Nicaragua. Journal of Medical Microbiology, 58(5), 630-637.

Yakasai, A.M., Muhammed, H., Babashai, M., Jumare, J., Abdulmumuni, M., Habib, A.G. (2011). Once-daily anti-retroviral therapy among treatment-experienced muslim patients fasting for the month of Ramadan. Tropical Doctor, 41(4), 233-235.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=140&id=8&aid=713.