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INTERNATIONAL ARCHIVES OF MEDICINE
SECTION: INFECTIOUS DISEASES
ISSN: 1755-7682

2015
Vol. 8 No. 138
doi: 10.3823/1737

Molecular Epidemiology of *Escherichia Coli* in HIV-Positive Individuals in South west Nigeria

ORIGINAL

Lawrence Ehis Okoror[1], Duna Christina Fashina[2], Titilayo Silifat Jimoh[2], Ezekiel Ayo Oisagah[1]

1 Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.
2 Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ife.

Contact information:

Lawrence Ehis Okoror.

Address: Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, PMB 5006, Ilesha, Osun State, Nigeria.

LEOkoror@gmail.com

Abstract

Introduction: *Escherichia coli* infection is a major health concern for human immunodeficiency virus (HIV)/AIDS patients because it is a substantial cause of diarrhoea-associated morbidity and mortality.

Materials and Methods: Stool and blood samples were collected from 879 HIV/AIDS patients in a tertiary hospital in southwest Nigeria. The blood samples were screened for HIV IgM using competitive ELISA, and the concentration of the IgM was determined. The stool samples were cultured on eosin methylene blue agar. The isolates were characterised based on the production of green metallic sheen on EMB agar. DNA was extracted from all the isolates, and the extracted DNA was analysed by PCR with primers specifically targeting the virulence genes of enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enteroinvasive *E. coli* (EIEC). Statistical analysis was conducted using the openepi online epidemiological package.

Results: All the blood samples tested positive for HIV IgM antibodies, and 80% had high concentration. All the stool samples were positive for *E. coli*, based on cell culture and DNA extraction. Of all the DNA tested by PCR using gene-specific primers targeting the 4 strains of *E. coli*, 222 samples were positive for EHEC shiga toxin 1 *(stx1)* gene, 212 samples were positive for EPEC intimin *(eaeA)* gene, 289 were positive for EHEC serotype O157:H7 using the *stx2a&b* gene, 125 were positive for EIEC haemolysin *(hlyA)* gene, and 31 were positive for ETEC *uid* gene.

Discussion: The high prevalence of *E. coli* O157:H7 is an indication that this strain, which has been previously linked with diarrhoea-related mortality in infants, may be responsible for most of the cases seen in HIV/AIDS patients. Though this strain is not common in adults,

Keywords
ELISA, PCR, IgM, Culture, Primers, DNA extraction.

its involvement in HIV/AIDS is indicative of suppressed immunity in this group of people. Other strains are also present in significant proportions, indicating that these strains also constitute a public health concern, because they may be transmitted to infants or other immunocompromised individuals.

Introduction

Chronic and persistent diarrhoea is a major cause of morbidity and mortality in people living with human immunodeficiency virus (HIV) and (AIDS). Diarrhoea occurs in almost 60-90% of people infected with HIV [1, 2]. Enteropathy is a common feature in HIV/AIDS patients [3] which can alter the lamina propia pre-disposing these patients to enteric pathogens and infections even when the pathogens are at low concentration, in contrast low pathogen levels in immune competent individuals will produce mild or asymptomatic infection [2].

A wide range of enteric pathogens has been implicated in diarrhoea in HIV/AIDS patients. Of these pathogens *E. coli* is strongly associated with persistence diarrhoea with different virulent factors such as adhesion; invasion; with production of toxins such as shiga-toxin and vibrio like toxins which are responsible for different types of diarrhoea produced by *E. coli* [4,5]. Six strains of *E. coli* are known and each strain is associated with a different type of diarrhoea-associated virulent factor encoded by different genes. Enterotoxigenic *E. coli* (ETEC) encode genes for heat labile and heat stable enterotoxins while the bundle forming pillus and intimin (attachment and effacing; eaeA) genes are encoded by enteropathogenic *E. coli* (EPEC). The invasion plasmid antigen H (*ipa*H) and haemolysin *(hlyA)*; vibrio-like toxin) genes are encoded by enteroinvasive *E. coli* EIEC). Enterohaemorrhagic *E. coli* (EHEC) encodes shiga-like toxin I (*slt*I) and shiga-like toxin II (*slt*II), Enteroaggregative *E. coli* (Eagg-EC), and diffusely adherent *E. coli* (DAEC) encodes the encodes the afimbrial adhesin gene [2]. The *stx*2A and *stx*2B genes are encoded by EHEC O157:H7 serotype (ECO157:H7) [6]. The *uid*A gene, which encodes the beta-glucuronidase can be used to identify EPEC.

In Nigeria, there have been a couple of studies on *E. coli* but none have characterised *E. coli* using molecular methods except a study by Okeke *et al.* [7] using hybridization technique and there study was from a different population. Though some studies have screened for some strains of *E. coli*, using the culture and serological methods and DNA hybridization, this will be the first molecular characterization (e.g. genotyping) of *E. coli* in HIV positive individuals. Vieira *et al.,* [8] reported a high prevalence of EIEC in a remote town in Ecuador using PCR.

Methods

Patients

Blood (n=879) and stool (n=879) samples were collected from individuals screened for HIV/AIDS at a tertiary health institution. All the subjects gave informed consent by filling out questionnaire. Subjects were in four groups; HIV positive patients with diarrhoea (HIV^+Dia^+); HIV positive without diarrhoea (HIV^+Dia^-); HIV negative with diarrhoea (HIV^-Dia^+); HIV negative without diarrhoea (HIV^-Dia^-). Stool samples were collected from individuals positive and negative for HIV IgM antibodies, and negative samples acted as the control. The control subjects had no history of diarrhoea at least a month prior to

clinical screen whether or not they were HIV$^+$. Subjects were defined as HIV positive with diarrhoea (Dia$^+$HIV$^+$; diarrhoea cases were defined as having frequent stooling more than 3 times a day), HIV positive and diarrhoea negative (Dia$^-$HIV$^+$), HIV negative and diarrhoea positive (Dia$^+$HIV$^-$), HIV negative and diarrhoea negative (Dia$^-$HIV$^-$). Though this study did not include a matched case control study, questionnaire data collected from HIV$^+$ subjects included age, sex, marital status, socio-economic status, medical history, access to portable water, and contact with animals.

Blood processing

The blood samples were screened for HIV 1 and 2 positivity using the ELISA kit as per the manufacturer's instruction (WKEA Medical Supplies, China). The blood samples were collected into sterile vacutaners and immediately centrifuged at 3000 rpm at room temperature (Beckman 12), and sera were separated from all the samples and then stored at -4˚C. At the start of ELISA, the serum and the ELISA kits were allowed to come to room temperature. The IgM antibodies to both HIV 1 and 2 were detected by absorbance measurement in a Thermomax Microplate Reader (Molecular Devices, CA, USA); cut-offs were calculated as described by the manufacturer.

Stool processing

The stool samples were collected into sterile container; samples (1 g) were diluted in 9 mL of sterile water added to it to start a serial dilution process and 10-fold serial dilutions were carried out. From each of the dilutions, 0.1 mL was transferred onto eosin methylene blue agar using the pour-plate method and incubated at 37° C for 24 h. Those positive for *E. coli* were further confirmed by Gram staining, catalase, indole, coagulase, oxidase, urease, gelatinase, niterate reduction, methyl red and Voges-Proskauer, citerate utilization, motility, and sugar fermentation (glucose, lactose, maltose, sucrose, mannitol, fructose, and inositol). All confirmed *E. coli* culture were sub-cultured into Luria broth for DNA extraction.

DNA extraction and PCR

For DNA extraction, approximately 1000 µL of the broth was placed into Eppendoff microfuge tubes and centrifuged for 10 minutes at 10000 rpm for 5 minutes, and then pellets were re-suspended in TE buffer. DNA was extracted was using a DNA extraction kit for bacteria (Norgen Biotek, Canada) as directed by the manufacturer. Primers for PCR were custom designed using Biology Work bench with default parameters and all the gene sequences used for primer design were obtained from the Genbank. PCR was performed to amplify the following genes using the primers as follows:- for *uid*A (5'-GACGACTTGGTTGTGGAGGT-3' and 5'-ATCCCCCTAAATCGATCACC-3') encoded by ETEC generating a 921 bp product;, for *stx2a&b* (5'-TATATCAGTGCCCGGTGTGA-3' and 5'-TGTGACAGTGACAAACGCA-3') encoded by *E. coli* O157:H7 strain and designed from gene sequence SM-25(1) stx-2 subunit A (stx2A) and stx2B complete CDS, generating a 1241 bp product,; for shiga toxin 1 gene *(stx1)* (5'-TGGTTGCGAAGGAATTTACC-3'and 5'-TGTGAAAAATCAGCAAAGCG-3') encoded by the EHEC FE94195 strain and designed from partial *stx1* gene for shiga toxin 1 strain, generating 624 bp product;, for haemolysin *(hlyA)* (5'-ATGCCTATTTCCGTGAGTGG-3' and 5'-CATTCTTCGCATTACGCTCA-3') encoded by the Vibrio cholerae strain ATCC 14035 and designed from *hlyA* partial CDS, generating a 415 bp product;, and for *eaeA* (5'-CAGCAAATCGAGCCACAGTA-3' and 5'-ACGATCCAGACCGTATTTGC-3') encoded by EPEC strain E3-8- and designed for intimin gene partial CDS, generating a 918 bp product. Primers for Eagg (5'CTGGCGAAAGACTGTATCAT-3' and 5'-CAATGTATAGAAATCCGCTGTT-3') and *ipa*H (5'-GCTGGAAAAACTCAGTGCCT-3' and 5'-CCAGTCCGTAAATTCATTCT-3) were pre-desig-

ned [2] for the virulent strains EaggEC and DAEC respectively and were obtained commercially (Inqaba, South Africa). DNA was amplified using PCR 2X Master Mix (Norgen Biotek, Canada) in a Hybaid Omnigene Thermocycler with the following condition: 1 cycle of denaturation at 95oC for 3 mins and 40 cycles of annealing for 30 s at 55oC, and enlongation at 72oC for 5 mins. and 1 cycle of final enlongation for 1 min.

Results

Of the 879 stool samples screened for 5 different strains of *E. coli* based on virulence genes, 574 (62.2%) were HIV$^+$ of which 390 (67.7%) were from Dia$^+$HIV$^+$ patients and 186 (32.3%) were from Dia$^-$HIV$^+$ patients. Another 301 stool samples were from HIV$^-$ subjects, where 134 (63.8%) were Dia$^+$HIV$^-$ and 76 (36.2%) were Dia$^-$HIV$^-$. The concentration of HIV IgM antibodies was higher in Dia$^+$HIV$^+$ samples than in Dia$^-$HIV$^+$ samples **(Table 1)**, with the likelihood of having diarrhoea increasing as IgM concentration increase (Confidence Interval (CI)=99%). All culture were positive for production of gas in glucose, galactose and lactose; they were positive for motility, indole, and methyl red. The finger print of all the PCR products was able to detect the genes targeted by each primer with varying prevalence **(Fig. 1A-E)**. Age and sex were not associated with prevalence of diarrhoea in HIV$^-$ positive individuals as was the case without HIV (CI=99%), nor did increased IgM depend on age or sex **(Table 2)**. The strain specific virulent genes detectable in HIV$^+$ and in some controls samples and strain O157:H7 more frequently isolated in both cases and control compared with other strains. EaggEC and DAEC were not detected while ETEC and EIEC were detected with low frequency. And ETEC was not extracted in Dia$^-$HIV$^-$ individuals. Multiple genes were detected in Dia$^+$HIV$^+$ as against only 2 in Dia$^+$HIV$^-$ **(Table 3)**. According to odds- based estimate, **(Table 4)** O157:H7 (Odd Ratio, OR=1.736), EPEC (OR=1.595) and EHEC (OR=1.338) were most frequently associated with diarrhoea in the population .OR for all the multiple genes could not be determined because of too few or lack samples bearing multiple of *E. coli* genes. The etiological fraction (EF) of the general population (EFp/OR) was 27.54% (lower limit= 6.605 and upper limit = 48.48) and the EF/OR in HIV$^+$ patients was 36.91% (lower limit=3.291 and upper limit= 58.84).

Table 1. Concentration of HIV IgM in infected individuals with or without diarrhoea.

IgM Conc.	No. Dia$^+$	No. Dia$^-$	Total	Odds of Exp.	Odds Ratio
20	70	80	150	0.88	1
30	90	50	140	1.8	2.06
40	120	30	150	4	4.57
50	70	21	91	3.33	3.81
60	30	5	35	6	6.86
80	10	0	10	'undefined'	'undefined'
Total	390	186	576		

Table 2. Co-founding different HIV IgM concentrations using Mantel-Haenszel Summary Odds Ratios (OR) and Crude OR for concentration using the lowest concentration as baseline.

IgM Concs.	MH Summary OR	Crude OR
20 vs. 20:	1	1
30 vs. 20:	2.057	2.057
40 vs. 20:	4.571	4.571
50 vs. 20:	3.81	3.81
60 vs. 20:	6.857	6.857
80 vs. 20:	'undefined'	'undefined'
Extended Mantel-Haenzel Chi square for linear trend = 46.08 P-value for one degree of freedom = <0.0000001		

Table 3. Distribution of *E. coli* strains identified by their virulence genes isolated from HIV-positive and HIV-negative individuals with or without diarrhoea.

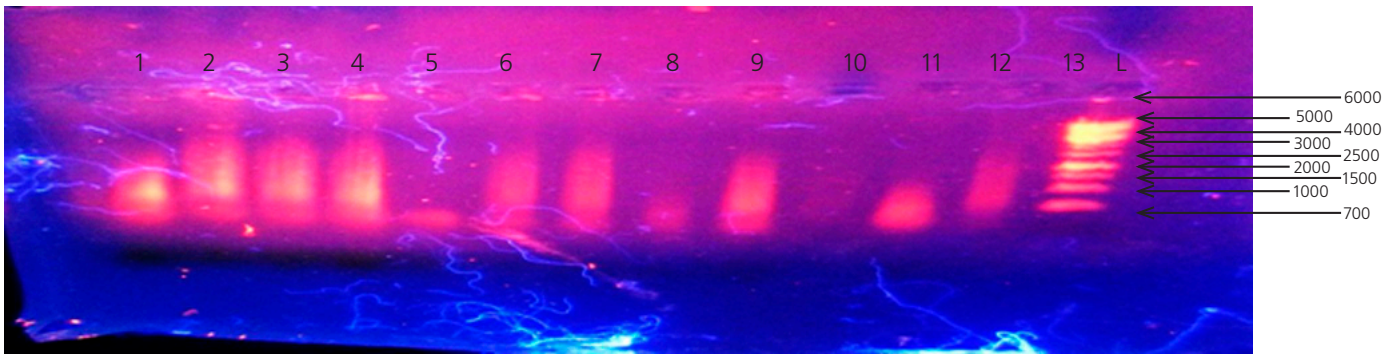
Virus strains	No. of Cases (%)		No. of controls (%)		Fisher Exact p value		Mid-Point Exact p value	
	$Dia^+ HIV^+$ (n = 490)	$Dia^+ HIV^-$ (n = 134)	$Dia^- HIV^+$ (n = 183)	$Dia^- HIV^-$ (n = 76)	1-tail	2-tail	1-tail	2-tail
Education								
EPEC	98(75.4)	32(24.6)	48(65.8)	25(34.2)	0.09698	0.1940	0.07481	0.1496
EHEC	142(74.3)	49(25.9)	52(68.4)	24(31.6)	0.2030	0.4060	0.1661	0.3322
ETEC	16(94.1)	1(5.9)	9(100)	0(0)	0.6538(P)	>0.9999999	0.3269(P)	0.6538
EIEC	30(85.7)	5(14.3)	9(90)	1(10)	0.5978(P)	>0.9999999	0.3986(P)	0.7971
ECO157:H7	204(81.3)	47(18.7)	65(71.4)	26(28.6)	0.03676	0.07353	0.02785	0.05570
Multiple								
EPEC and EHEC	11(91.7)	1(8.3)	-	-				
EPEC and EIEC	1(100)	-	-	-	0.8889(P)	>0.9999999		
EIEC and EHEC	1(100)	-	-	-				
EIEC and ECO157:H7	3(100)	-	-	-				
EPEC and ECO157:H7	31(88.6)	4(11.4)	1(100)	-				
EPEC, EHEC and ECO157:H7	7(77.8)	2(22.2)	1(100)	-	0.8000(P)	>0.9999999	0.4000(P)	0.8000
Crude	544(79.4)	141(20.6)	185(70.9)	76(29.1)	0.003827	0.007654	0.003060	0.006121
Adjusted					0.01353	0.02705	0.01108	0.02216

Table 4. Odds-based estimates and confidence limits of genes associated with different strains of diarrheagenic *E. coli*.

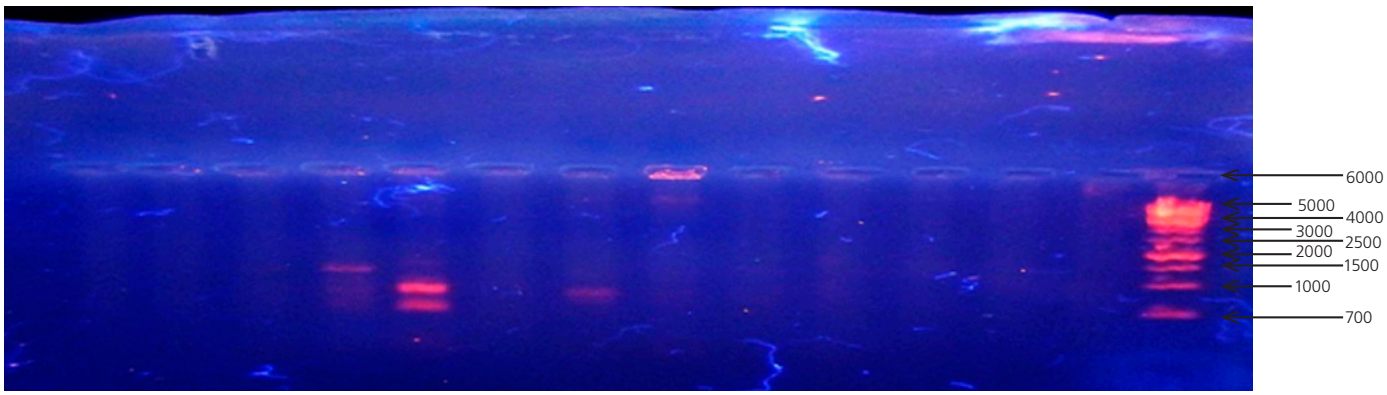
Virulence strains	Odd Ratio	Confidence Limit (99%)			
		Fisher Exact		Mid-Point Exact	
		Lower	Upper	Lower	Upper
EPEC	1.595	0.6617	3.799	0.69	3.647
EHEC	1.338	0.5867	2.964	0.6095	2.863
ETEC	0.0	0.0	375.9	0.0	187
EIEC	0.6667	0.002508	12.48	0.005014	10.16
ECO157:H7	1.736	0.7938	3.696	0.8221	3.581
EPEC and EHEC	undefined	-	-	-	-
EPEC and EIEC	0.0	0.0	1592	-	-
EIEC and EHEC	'undefined	-	-	-	-
EIEC and ECO157:H7	undefined	-	-	-	-
EPEC and ECO157:H7	undefined	-	-	-	-
EPEC, EHEC and ECO157:H7	0.0	0.0	796	0.0	396
All Strains	1.585	1.016	2.453	1.029	2.423

Virulence strains	Odd Ratio	Confidence Limit (99%)			
		Fisher Exact		Mid-Point Exact	
		Lower	Upper	Lower	Upper
Crude	CMLE Odds Ratio*	1.584	1.029, 2.423[1]	Mid-P Exact	
			1.016, 2.453[1]	Fisher Exact	
	Odds Ratio	1.585	1.034, 2.429[1]	Taylor series	
	Etiologic fraction in pop.(EFp|OR)	27.54%	6.605, 48.48		
	Etiologic fraction in exposed(EFe|OR)	36.91%	3.291, 58.84		
Adjusted	CMLE Odds Ratio*	1.476	0.9515, 2.276[1]	Mid-P Exact	
			0.9391, 2.304[1]	Fisher Exact	
	Directly Adjusted OR	'?'	'?', '?'[1]	Taylor series	
	Mantel-Haenszel OR	1.48	0.9574, 2.289[1]	Robins, Greenland, Breslow	
	Breslow-Day test for interaction of Odds Ratio over strata:		2.964	0.6095	
	chi square=	'?'	p =	NaN	
	p greater than 0.05 does not suggest interaction. Adjusted OR can be used.	0.002508	12.48	0.005014	

Figure 1: a: PCR amplification pattern of shiga toxin 2 (stx2A and stx2B) subunit genes encoded by *E. coli* O157:H7

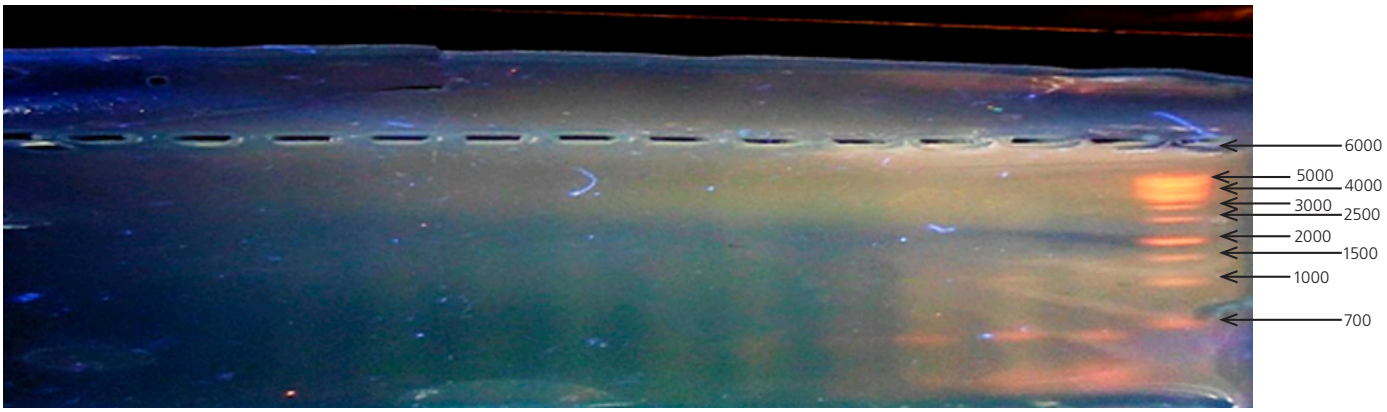


b: PCR amplification pattern of shiga toxin 1 gene (stx1) encoded by strain EHEC FE94195.

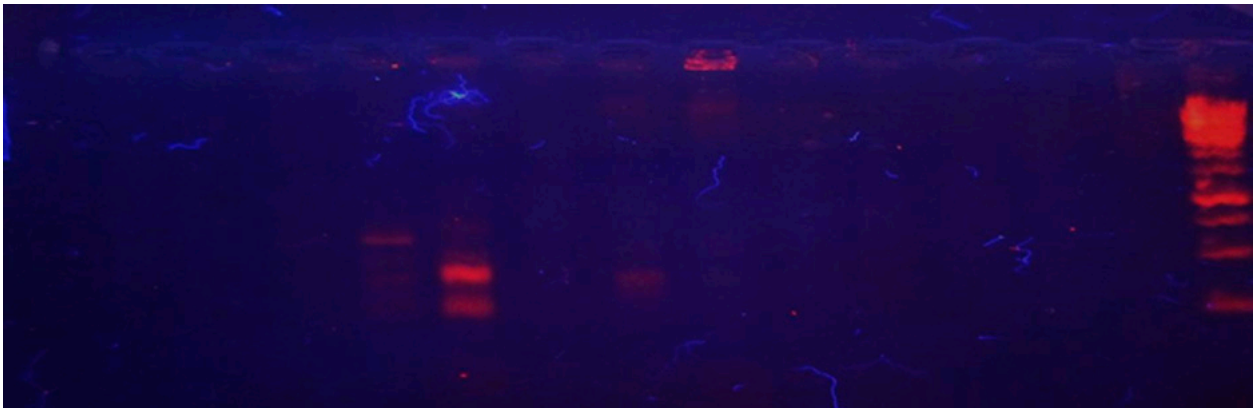


INTERNATIONAL ARCHIVES OF MEDICINE
SECTION: INFECTIOUS DISEASES
ISSN: 1755-7682
2015
Vol. 8 No. 138
doi: 10.3823/1737

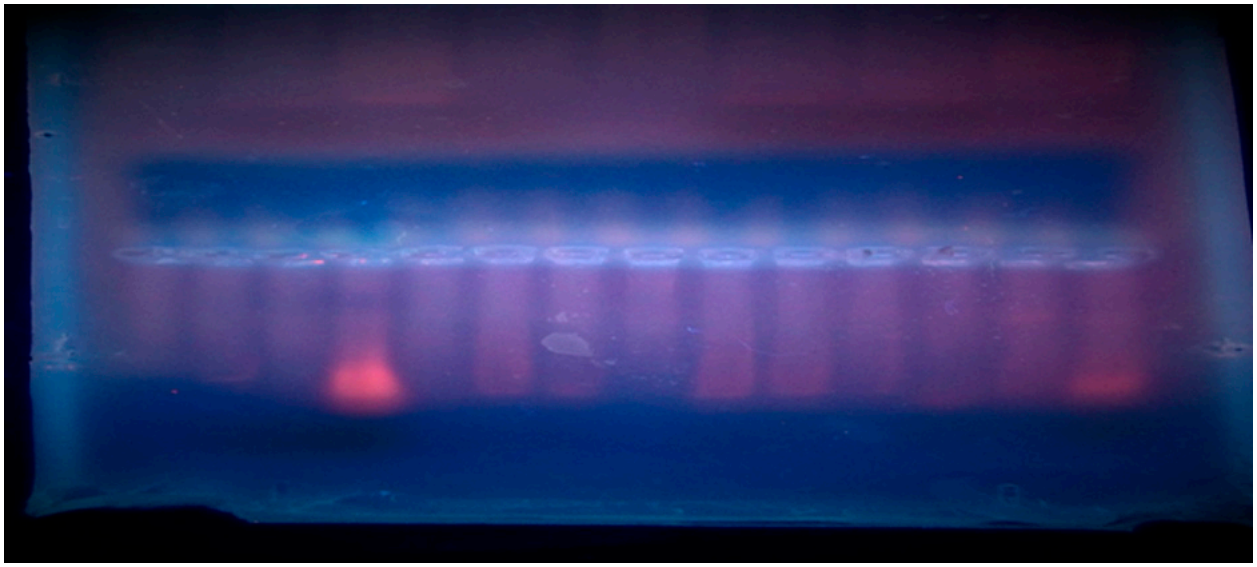
Figure 1: c: PCR amplification pattern of haemolysin gene (hlyA) encoded by ETEC.



d: PCR amplification pattern of intimin gene (eaeA) encoded by EPEC.



e: PCR amplification pattern of uid encoded by EIEC.



Discussion

In sub-saharan Africa and most developing and underdeveloped world, diarrhoea- causing- *E. coli* are a significant cause of morbidity and mortality in young infants. In contrast, adults are usually asymptomatic visible infection in adults is usually self-limiting. However, in the HIV/AIDS patients diarrhoea due to *E. coli* has become a leading cause of morbidity and mortality in adults as well, which is well documented [9]. High levels of HIV IgM in patients with diarrhoea confirm that these patients are in the acute or early stage of HIV infection prior to seroconversion [10]. In this study, these patients were also among those with multiple genes (strains) of *E. coli*. The aetiological fraction of those positive for HIV was more than that of the general population, hence serostatus of the HIV$^+$ individuals is likely responsible for diarrhoea in this group of people, and the different *E. coli* virulence genes accounts for this diarrhoea. This result supports the idea that proper management of diarrhoea in the acute phase of HIV infection is necessary, as diarrhoea itself is a serious source of morbidity in those living with HIV/AIDS especially presenting in the early phase of the infection of which this study further confirms an earlier study by Gassama-Sow *et al.,* [2].

There was no significant association between patients' age or sex and *E. coli* strain-specific virulence genes which correlates earlier reports on infants [11]. However, results of a study in Lima, Peru surprisingly deviated from these earlier reports. Medina *et al.,* [12] reported a significant association in both age and sex in E coli carriage when they studied diarrhoea-causing *E. coli* strains in infants. Reports indicate that diarrhoeagenic *E. coli* are moslyt isolated from immunocompromised patients with diarrhoea [13] but strains could vary from regionally; consequently, regional interpretation of results must be taken into account, especially in clinical settings, to enhance proper management of diarrhoea in HIV$^+$ patients. Of patients in Nigeria, this is the first study to genotypically characterize *E. coli* in HIV patients by *E. coli* strain-specific virulent genes. Earlier studies characterized diarhoeagenic *E. coli* serotypically, and though Nweze [14] identified some of these strains using PCR, the strains were not characterized using the method described here. Okeke *et al.,* [7] used a DNA hybridization technique characterized *E. coli* strains but this does not underscore PCR relative to sensitivity and specificity.

EHEC serotype ECO157:H7 has been detected from various parts of the world from specific outbreaks in the normal population of which infants have been worst hit, including outbreaks reported by Riley et al,. [15] in the United States, by Morgan *et al.,* [16] in Britain, by Effler *et al.,* [17] Swatziland, by Koyange *et al.,* [18] in Democratic Republic of Congo. In Nigeria, a prevalence study of ECO157H7 included report from southwest Nigeria by Ogunsanya *et al.,* [19]; Okeke *et al.,* [7] and; Olorunshola *et al.,* [20].Esumeh *et al.,* [21] also reported a similar high prevalence of ECO157:H7 in Benin City, Nigeria. In this study high prevalence of ECO157:H7 serotype was associated with a high risk population. Although other studies also showed a high prevalence of ECO157:H7, these studies were mainly on infants and not on immunocompromised individuals. We confirm that ECO157:H7 accounted for 81.3% of diarrhoea cases among HIV$^+$ patients even when ECO157:H7 is an emerging EHEC serotype that is not well documented in Nigeria or in other parts of developing world. According to odd- based analysis, ECO157:H7 has the highest ability to diarrhoea among HIV$^+$ patients which correspond to a similar result from an earlier report [21]. Hence, isolation of ECO157:H7 depended on the HIV status of the subjects in this study.

EPEC is an important diarrhoeagenic pathogen, which has been recognized as a highly prevalent cause of diarrhoea in Nigeria [7,14]. This study also confirmed that EPEC is a major cause diarrhoea since it was detected in 75% of all the cases. EPEC is an important strain because it is linked to acute

watery diarrhoea and persistent diarrhoea in infants and in immuno compromised patients, and it leads to high morbidity and mortality rates greater than 50% in HIV+ patients, especially in developing countries [2]. Medina *et al.,* [12] reported that EPEC strains could be a concern for HIV+ patients but the study examined only children. Gassama-Sow *et al.,* [2] also showed a high prevalence of EPEC in HIV+ individuals in Senegal which is in agreement with our study and another study in the Netherlands [22].

EHEC which was isolated with high frequency in the HIV cases and in some of the controls was also incriminated in this study as a cause of diarrhoea in HIV+ individuals, the odds of causing diarrhoea in the HIV cases was 1.338 (CI=99%) though data are scarce with regards of EHEC causing diarrhoea in Nigeria in both adults and infants. Okeke *et al.,* [7] used DNA hybridization and detected EHEC in infants for only a small proportion (0.5%) of total positive individuals. The high prevalence in this study may be connected with the fact that the population in this study was immunocompromised and that the PCR method used in this study is more sensitive than the hybridization techniques used by Okeke *et al.,* [7]. The virulence pattern of EHEC has changed with the advent of HIV/AIDS. However, earlier studies by Okeke *et al.,* [7] and Nweze [14] did not distinguish the serotype ECO157:H7 from the EHEC strain.

ETEC, which causes dehydrating diarrhoea because of heat-stable and heat-labile enterotoxins was isolated with low frequency in Nigeria and in HIV+ individuals and was less likely to cause diarrhoea in HIV+ individuals (CI=99%). ETEC has also been isolated with low frequency in infants with diarrhoea in several studies from several geographical locations [2, 7, 12,23].

Results from this study also incriminated EIEC as a causal strain for diarrhoea in those infected with HIV. Although not much is known about EIEC in Nigeria, deteriorating immune statuses in HIV/AIDS patients have made EIEC an emerging strain, though a study by Gassama-Sow *et al.,* [2] observed that EIEC infection was not dependent on HIV infection according to serostatus, because no asymptomatic carriage was found in their study. They reported that 95 screened HIV+ individuals did not present EIEC of the total 182 stool samples. In this study, 30 of the total 833 stool samples yielded EIEC from Dia+HIV+ individuals as compared to 5 from Dia- HIV+ individuals. Coupled with the fact that EIEC is scarcely reported in Nigeria, it is then reasonable to assume that EIEC was a causative agent of diarrhoea in HIV+ individuals. Vieira *et al.,* [8] also reported a high prevalence of EIEC in costal Ecuador, though they did not stratify the population. Further, Okeke *et al.,* [7] reported the possibility of EIEC in childhood diarrhoea in Nigeria but very little has been reported about EIEC in Nigeria after 2000. EaggEC and DAEC was not implicated as a probable cause of diarrhoea in this study. Although this strain is also said to be emerging and is not common to Nigeria, more researches should be conducted in the general population.

Multiple infections by different strains did not prove to be a cause of diarrhoea in HIV+ individuals or in the general population, as there were too few samples in which multiple strains were detected to determine their collective role in diarrhoea causation. This result supports an earlier report by Gassama-Sow *et al.,* [2] regarding, subjects from Senegal which is also a West African country where geographical data may be similar.

High resistance to tetracycline, cotrimoxazole and ampicillin was observed for EIEC, ECO157:H7, and ETEC strains. This resistance may be due to exposure of the strains to selective pressure since most of these antibiotics are used heavily in the treatment of diarrhoea and are heavily abused.

In conclusion, strains of *E. coli* possess great concern in HIV+ especially at the acute phase of HIV infection even before seroconversion and probably through the course of infection. Proper management is highly required in HIV+ individuals to redu-

ce morbidity and mortality. And such management should be geographically considered as different strains may dominate different geographical locations. This will reduce multiple drug resistance currently seen in most strains.

Acknowledgements

We wish to thank all those involved in the collection of sample in the tertiary health institution. Edittage editing company is highly recognized for a well done job in editing this manuscript. We do not regret patronizing edittage and advised other prospecting authors to look towards edittage.

We however declare no competing interes.

List of abbreviations

DAEC, diffusely adherent Escherichia coli; Dia^-, diarrhoea negative; Dia^+, diarrhoea positive; eaeA, attachment and effacing; EaggEC, enteroaggregative *E. coli*; EF, etiological fraction; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; HIV, human immunodeficiency virus; HIV^-, HIV negative; HIV^+, HIV positive; *hlyA*, haemolysin A; *ipa*H, invasion plasmid antigen H; OR, odds ratio; *slt*, shiga-like toxin; stx, shiga toxin

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