Prevalence of *Escherichia Coli* Pathotypes in Stools of HIV-Positive Adults Attending a HAART Clinic Ileife, Nigeria

[1]Eniola, K. I. T., [2]Awoniyi, L. M., [3]Torimiro, N, [1]Ajayi O. O. and [1]Adeyemo-Eleyode V. O.

[1]Environmental and Public Health Research Laboratory, College of Agriculture and Natural Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.

[2]Virology Research Clinic, Obafemi Awolowo University Teaching Hospital Complex, Ile Ife.

[3]Department of Microbiology, Obafemi Awolowo University, Ile Ife

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ABSTRACT

Morbidity and mortality among HIV/AIDS patients are often exacerbated by opportunistic infections (OIs). *Escherichia coli* has emerged as a significant agent of OIs in HIV-positive patients. The use of highly active antiretroviral therapies (HAART) has improved health outcomes, including a reduction in OIs like diarrhoea. This study aimed to identify and characterize the pathotypes of *E. coli* present in the stools of HIV-positive adults attending the HAART Clinic in Ile-Ife, Nigeria. *E. coli* was isolated and pathotyped based on the presence of specific virulence genes: *eaeA*, *bfpA*, *stx1*, *stx2*, *eltB*, *estA*, *ipaH*, *pCVD*, and *EcoRI-PstI*. Out of 271 stool samples examined, *E. coli* was detected in 27 (9.96%). Among the 72 *E. coli* strains isolated, 21 were identified as diarrheagenic *E. coli* (DEC): 11 (52.38%) were Enteropathogenic *E. coli* (EPEC), 7 (33.33%) were Shiga toxin-producing *E. coli* (STEC), and 3 (14.29%) were Enterohemorrhagic *E. coli* (EHEC) pathotypes. Notably, five stool samples contained more than one DEC pathotype. The study found a low prevalence of DEC, with three major pathotypes prevalent among the HIV-positive adults sampled. Identifying specific *E. coli* pathotypes in HIV/AIDS patients is crucial for understanding potential complications and associated risks, which can guide clinicians in selecting appropriate treatments and managing bacterial co-infections more effectively.

Keywords: Diarrheagenic *Escherichia coli (*DEC), Opportunistic infections (OIs), HAART (Highly Active Antiretroviral Therapy).

INTRODUCTION

Opportunistic infections (OI) are a significant cause of morbidity and mortality in people with HIV infection due to their weakened immune systems (Wingfield and Wilkins, 2010). HIV destroys the immune system and renders patients susceptible to opportunistic infections (Elfstrand and Floren, 2010). HIV Infection causes a decline in the levels of CD4 T cells and affects critical cells (macrophages and dendritic cells) in the body system (Kumar, 2018; Cunningham *et al.*, 2020; Ponnan *et al.*, 2021; Chang *et al.*, 2022). There is a progressive loss of protective antibodies, which often causes the patients to become a microbial zoo (Geoff et al., 2022; Arora and Arora, 2009).

Kibwengo *et al.* (2022) opined that opportunistic infections (OIs) are the first clinical manifestations that alert clinicians to the occurrence of acquired immunodeficiency syndrome (AIDS). In their study, they reported that delay in antiretroviral treatment (ART) initiation after positive test results, poor drug adherence and moderate malnutrition are major risk factors that affect the occurrence of OI. The establishment of opportunistic infection in all circumstances depends on quality of treatment and exposure to infectious agents (*Mycobacterium tuberculosis*, *Candida albicans*, *Escherichia coli*, *Cryptosporidium parvum*, *Toxoplasma gondi* and so on) (Kibwengo *et al.*, 2022; Shrestah *et al*., 2022; Tan *et al*., 2012)

The microbial agents associated with OI include bacteria, fungi, viruses, and protozoa, which can cause opportunistic infections, such as pulmonary, oropharyngeal, gastrointestinal, dermatological, neurological, ophthalmic and even multi-system infections. Among these, gastrointestinal infections represent a serious public health risk (Wingfield and Wilkins, 2010). Diarrhoea is a common problem in HIV-infected individuals in many developing countries (Carcamo *et al.*, 2005; Sun *et al.*, 2022). Dysentery and bacterial gastroenteritis are among opportunistic infections (OIs) often associated with HIV patients (Carcamo *et al.,* 2005; Okeke, 2009 and DeWitt *et al.*, 2019). E. coli is widely associated with diarrhoea. Braz *et al*. (2020) categorised *E. coli* into diarrheagenic *E. coli* (DEC), non-pathogenic *E. coli*, and extraintestinal pathogenic *E. coli*. They further subdivided the extraintestinal pathogenic *E. coli* into uropathogenic *E. coli* (UPEC), sepsis-causing *E. coli* (SEPEC) and neonatal meningitis-associated *E. coli* (NMEC). DEC has been consistently implicated as a cause of diarrhoea in adults infected with HIV14- (Oluma and Richard, 2008; Zhu *et al.*, 2019 and Verma *et al*., 2022).

One of the goals of Highly Active Antiretroviral Therapy (HAART), a combined therapy, is to improve immune function (Eggleton and Nagalli, 2022). The recovery of immune status with HAART has markedly improved the long-term outcome for patients with adequately treated HIV infection because it inhibits viral replication and greatly reduces the chances of treatment failure or development of multidrug resistance (Eggleton and Nagalli, 2022).

Although HAART has resolved diarrhoea cases caused by opportunistic pathogens previously considered untreatable; MacArthur and Duport (2012) indicated that most diarrhoea cases in HIV-infected patients on HAART are not due to opportunistic infection but are due to protease inhibitors. Treating diarrhoea in HIV-infected patients will be difficult if the specific cause is not known. Therefore, this study was designed to investigate the pathotypes of *E. coli* in adult HIV-positive patients on HARRT.

MATERIALS AND METHODS

Study population and sample collection

The study involved 271 consented HIV positive adult patients aged between 18 and 60 years who are on HAART. Ethical clearance (number: IRB/IEC/0004553 NATIONAL: NHREC/27/02/2009a) was obtained for the study from the Ethics and Research Committee of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife.

Stool samples were collected from participants during their clinic days into sterile universal bottles. The stool samples were analysed within 30 minutes at the Virology Research Clinic (VRC) of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) between August and October 2021.

Isolation and identification of *Escherichia coli*

E. coli was isolated by streaking the stool sample onto sterile Eosin Methylene Blue (EMB) agar plates (Oxoid Ltd, Basingstoke Hampshire, England) using the quadrant streak plate method (Poyil *et al*., 2022) and incubated at 37°C for 24 hours. Typical *E. coli* colonies with greenish metallic sheen on EMB agar were sub-cultured and further characterised by standard biochemical tests. Genomic DNA of each isolate was extracted as described by Dashti *et al*. (2009) and their identity confirmed by PCR using specific primer (New England *BioLabs*) (Bej *et al.*,1991; Aranda *et al.,* 2020)

Screening for diarrheagenic *Escherichia coli*

All the *E. coli* isolates were screened for virulence genes characteristic of Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Enterohaemorrhagic *E. coli* (EHEC) (Aranda *et al*., 2004; Abongo et al., 2018; Momba *et al*., 2008). The target genes were *eaeA* (structural gene for intimin of EHEC and EPEC), *bfpA* (structural gene for the bundle-forming pilus of EPEC), *stx1* and/or *stx2* (shiga toxins 1 and 2 of EHEC and STEC), *eltB* and/or *estA* (enterotoxins LT and ST of ETEC), *ipaH* (invasion-associated locus of the invasion plasmid found in EIEC and *Shigella*) and pCVD (the nucleotide sequence of the *Eco*RI-*Pst*I DNA fragment of pCVD432 of EAEC). *E. coli* strains

E2348/69, O42, H10407, EDL 933 and E137 served as positive controls for EPEC, EAEC, ETEC, EHEC and EIEC respectively.

Statistical analysis

Data were analysed using WinPepi version 11.65. Descriptive statistics (Percentage, Frequency and Mean) of data was presented. Chi-square test was used to test for the association between the groups and a P <0.05 was considered statistically significant.

RESULTS

E. coli was isolated from 27 (9.96 %) of the 271 stool samples analysed, some of the samples yielded more than one *E. coli* isolate and a total of 72 *E. coli* strains were confirmed. Among the 72 *E. coli* strains, 21 were found to be DEC strains while the other 51 were non-DEC strains. Three pathotypes of DEC: EPEC, STEC and EHEC were detected. The most prevalent was EPEC (11; 52.38 %), two of its sub-pathotype: Typical EPEC (7: 33.33 %) and Atypical EPEC (4: 19.05 %) were detected (Table 1). More than one DEC pathotype was recovered from 5 of the stool samples. All the Atypical EPEC strains carried the *eae* genes, while six of the Typical EPEC carried the *bfp* genes, and one of the Typical EPEC carried the *bfp* and *eae* genes. Two of the STEC strains carried *stx1* genes, three of them carried *stx2* genes, one carried *stx1* and *bfp,* while another one carried *stx2* and *bfp*. Two of the EHEC carried *bfp, eae.* and *stx2* genes while one other carried *eae,* and *stx2* genes.

Table 1 Pathotypes of Diarrheagenic *E. coli* (DEC) in Stool of HIV Positive Adults

Sample Code	***Number of DEC in samples***	***Pathotypes***	***Virulence Genes***
10	2	Atypical EPEC	*Eae*
		Typical EPEC	*Bfp*
12	2	STEC	*stx2*
		Typical EPEC	*Bfp*
37	1	Typical EPEC	*Bfp*
68	1	Atypical EPEC	*Eae*
70	1	Atypical EPEC	*Eae*
88	2	EHEC	*stx2, eae, bfp*
		Typical EPEC	*eae,bfp*
99	1	STEC	*stx2, bfp*
102	2	STEC	*stx1, bfp*
		Typical EPEC	*Bfp*
103	1	STEC	*stx2*
133	1	STEC	*stx1*
169	1	Typical EPEC	*Bfp*
184	1	STEC	*stx1*

186	1	STEC	*stx2*
207	2	Atypical EPEC	*Eae*
		EHEC	*stx2, eae*
268	1	EHEC	*stx2, eae,bfp*
271	1	Typical EPEC	*Bfp*

Keys: EPEC: Enteropathogenic *E. coli* EHEC - Enterohemorrhagic *E. coli*

STEC: Shiga toxin-producing *E. coli*

DISCUSSION

The frequency of *E. coli* in the stools of the HIV patients (9.00%) in this study is higher than the 1.9% reported by Reuben and Gyar (2015). However, it was lower than the 12.16%, 42% and 18% reported by Abongo *et al*. (2008), Garcia *et al*. (2010), and Ngalani, *et al*. (2019) respectively. Although most strains of *E. coli* are commonly facultative bacteria in the lower intestine of humans and warm-blooded animals, they may act as opportunistic pathogens (Braz *et al*., 2020). *E. coli* is gradually becoming a serious public health concern as an opportunistic infection associated with people living with HIV in developing countries (Okeke, 2009; DeWitt *et al*., 2019 and Chabala *et al*., 2020), the low frequency of its detection in the subjects suggests that it is either not yet a widespread problem or is being managed successfully.

DEC constituted a smaller proportion (29.17%) of *E. coli* isolated in this study compared with 39% obtained by Olaru etc al., 2021. The three pathotypes of DEC detected in this study have been associated with HIV positive patients by Alizade *et al*. (2017). EPEC which is the most frequent pathotype (15.28%), in the diarrheagenic adult HIV patients involved in this study, was also the most prevalent (30.77%) in the study by Medina *et al*. (2010), This appears to buttress the view that EPEC strains are becoming significant enteropathogens in immunocompromised adults (Alizade *et al*., 2017). STEC which is the second most prevalent (9.72%) DEC pathotype detected in this study has been reported by Alizade *et al*. (2019). The low frequency of EHEC pathotype detection in this study is similar to the result obtained by Kebede *et al*. (2020); which detected only two EHEC strains in 102 stool samples from HIV-positive adults. In the study by Llorente *et al.* (2023); Okeke and Nataro (2001) reported EAEC pathotype in the stools of HIV infected adults, however, the pathotype was not isolated in this study.

CONCLUSION

The study provides valuable data on the prevalence and types of *E. coli* pathotypes among HIV/AIDS patients around Ile-Ife, Nigeria, which is useful for targeted interventions by public health authorities to reduce bacterial infections among this vulnerable population. Information on specific *E. coli* pathotypes in HIV/AIDS patients, which the study provides is useful in understanding the potential complications and risks associated with these infections and can guide clinicians in selecting appropriate treatments and managing bacterial co-infections more effectively. This study reported a low prevalence of DEC in HIV-positive adults with a preponderance of EPEC and STEC pathotypes. Most of the isolates were resistant to a range of antibiotics. The study also highlights the need for continuous monitoring of bacterial infections in HIV-positive individuals, particularly in regions with high HIV prevalence.

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