Antibiogram of enterovirulent *Escherichia coli* from stool specimens of HIV patients with diarrhea in Southwest Nigeria

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ABSTRACT

Diarrheal diseases are frequent complications seen in patients with Human immunodeficiency virus (HIV) infection. The etiological spectrum of enteric pathogens in these patients is broad, and these include, bacteria, parasites, fungi and viruses. *Escherichia coli (E.coli)* strains are well-known bacterial agents of diarrhea in these patients, and, these can be life-threatening. There have been reports of the emergence of multidrug resistant *E.coli* among these patients, and this necessitates the study of the antibiogram of strains of this organism in this environment. This study was therefore conducted to determine the antibiotic susceptibility or resistance pattern of enterovirulent *E.coli* strains from stools of HIV-infected patients with diarrhea in our environment. Ninety-eight strains of *E.coli* identified and confirmed as pathogens of diarrhea in HIV- seropositive patients were included in this study. They were subjected to antibiotic susceptibility test, using the disc diffusion method. High resistant rates were demonstrated towards tetracycline, cotrimoxazole, amoxicillin-clavulanate and amoxicillin, the antibiotics commonly used in this environment to treat diarrhea. This therefore calls for the enforcement of judicious use of antibiotics in this environment to control the situation.

Keywords: HIV patients, stools*, Escherichia coli*, antibiogram.

INTRODUCTION

Opportunistic infections play a major contributing role in the morbidity associated with Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). Among these are diarrheal diseases which are frequent complications associated with HIV infection. Moreover, people with HIV/AIDS have higher likelihood of developing diarrhea than those with competent immune systems (Hayes et al., 2003). Among AIDS patients in developing countries, as many as 95% may have diarrhea, which may become prolonged and life-threatening in a large proportion of this population (Guerrant et al., 1990). Chronic diarrhea has been reported to be an independent marker of poor prognosis in patients with AIDS (Lew et al., 1997).

Diarrhea in these patients have been attributed to progressive decline in their immunological responses, and, some 30%-60% of them, require medical attention during the course of the HIV-infection (Mukhopadhya et al., 1999). The etiological spectrum of enteric pathogens in these patients is broad, and these include, bacteria, parasites, fungi and viruses (Mitra et al, 2001). These produce intractable high volume watery diarrhea which has also proved to be extremely difficult to treat (Huang et al, 2007). As opportunistic infections, they may play a role in the final well-being of HIV-infected patients. In studies conducted in Maiduguri and other parts of Northern Nigeria, prolonged animal contact, contamination of the environment with animal feces, as well as consumption of unpasteurized milk and dairy products have been implicated as common sources of infections (Anietie, 2006). Although *Escherichia coli (E.coli)* is part of the normal fecal flora of humans and

animals, some strains can cause life-threatening diarrhea especially in immunocompromised patients (CSE, 2006). Different classes of enterovirulent *E.coli* have been implicated as causative agents of diarrhea in HIV/AIDS patients and these include enteropathogenic *E.coli* (EPEC), enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli,* and enteroaggregative *E.coli* (EAEC). Although antibiotics have been developed for the treatment of infectious diseases, multidrug resistance to these antibiotics are becoming a global concern. Furthermore, antibiotic susceptibility profiles of microorganisms have been documented to vary considerably (Sein et al., 2004). The emergence of multidrug resistant *E.coli* has also necessitated the study of this organism and its susceptibility in HIV-infected patients presenting with diarrhea.

MATERIALS AND METHODS

This study was carried out among confirmed HIV-seropositive patients with diarrhea who presented in two teaching hospitals in Osun State, Nigeria. These were Ladoke Akintola University of Technology Teaching Hospital (LTH), and Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife. The study commenced after ethical approval was obtained from the institution ethical committee. Informed consent was also obtained from each of these patients. The study period was between June and December 2009.

Ninety-eight strains of *E.coli* identified as causative agents of diarrhea in HIV-seropositive patients who presented at the clinics with diarrhea were included in this study. Diarrhea was diagnosed in a patient with passage of 3 or more watery stools in 24 hours. *Escherichia coli* strains were isolated and identified from the fecal samples of these patients using standard bacteriological techniques which included morphology on MacConkey agar, lactose fermentation, motility, positive catalase test, negative oxidase test, positive indole test and negative citrate test. The isolates were further confirmed as pathogens responsible for the diarrhea by serotyping. The *E.coli* strains were subjected to antimicrobial susceptibility testing according to the recommendation of Clinical and Laboratory Standard Institute (CLSI) for antibiotic disc diffusion susceptibility method (CLSI, 2006). The isolates were tested against the following antibiotics: Tetracycline(30ug), gentamycin(10ug), ofloxacin(30ug), cotrimoxazole(25ug), amoxicillin(25ug), nitrofurantoin (30ug), amoxicillin/clavu lanate(30ug), and nalidixic acid (30ug).

The patients' biographical information such as gender and age as well as their HIV/AIDS status were obtained and recorded.

RESULTS

A total of 98 strains of *E. coli* recovered from stools of HIV-seropositive patients, and confirmed as causative agents of diarrhea in them were analysed during the period of study. The *E.coli strains* were obtained from 26(26.5%) male and 72(73.5%) female patients. The highest incidence of diarrhea was recorded among patients in the age group 21-30 years 35(35.7%)) while the least incidence was recorded among the age group 1-10 years 2(2.04%) (Table 1). The age range of these patients was between 1 and 60 years.

High resistance rates were demonstrated by these isolates towards co-trimoxazole 98(100%), tetracycline 96(98%), amoxicillin 91(92.9%), and amoxicillin/clavula-nate 70(71.4%).

However, resistance rates to ofloxacin, gentamycin, and nitrofurantoin were quite low, and these were 27.6%, 22.5% and 1% respectively (Table 2). Some of these isolates also demonstrated resistance to 3 or more classes of antibiotics (Table3).

DISCUSSION

In the age distribution of study patients, highest incidence of diarrhea was found among the age-groups 21-30 years (35.7%) and 31-40 years (33.7%) while lowest was found among the age group 1-10 years (2.04%) with mean age of 32.6 years. A similar result was obtained in a study conducted by Okodua in 2003 where the incidence was highest in the mean age 30years (Okodua et al, 2003).In addition, the incidence of pathogenic *E.coli* in stools of HIV-patients was higher in females (73.4%) than in males (26.5%). This is in keeping with a study carried out by Abong et al in 2008 (Abong et al, 2008).

High resistance rates to antibiotics were recorded among the pathogenic fecal isolates of *E.coli* in this study. Many of them also demonstrated multiple antibiotic resistance, an observation that has serious implications as regards the management of *E.coli* enteric infections in these patients. It is also pertinent to state here that high resistance (100%) was demonstrated against co-trimoxazole, a drug of choice in the management of this group of patients in our environment. This may be due to the fact that this drug has been abused in this environment as many people use it for prophylaxis and non-prescription treatment for respiratory and gastrointestinal tract infections.

Resistance to many of the older and less expensive antibiotics was also observed in this study. These included tetracycline, cotrimoxazole, amoxicillin-clavulanate and amoxicillin.This may be connected with the indiscriminate use of these antibiotics that is rampant in our environment. This is likely to have a far reaching

Table 1. Age and sex distribution of HIV patients with diarrhea.

Age (years)	Frequency	Percentage (%)
1-10	2	2.04
11-20	6	6.12
21-30	35	35.71
31-40	33	33.67
41-50	16	16.33
51-60	6	6.12
Total	**98**	**99.99**
SEX		
Male	26	26.53
Female	72	73.47
Total	**98**	**100.0**

Table 2. Antibiotic resistance pattern of pathogenic *E.coli* isolates from HIV patients with diarrhea.

Antibiotics	No of resistant isolates	Percentage (%)
Tetracycline	96	97.96
Cotrimoxazole	98	100.0
Gentamycin	22	22.45
Ofloxacin	27	27.55
Nalidixic acid	53	54.08
Nitrofurantoin	1	1.02
Amoxicillin/clavulanate	70	71.43
Amoxicillin	91	92.86

Table 3. Resistance to multiple antibiotics by pathogenic *E.coli* isolates from HIV patients with diarrhea

No. of classes of antibiotics	No of resistant isolates	Percentage
3	14	14.29
4	30	30.61
5	16	16.33

effect on the cost of treatment option for these infections. The resistance to more than three classes of antibiotics in these pathogens may be something to worry about because such strains can become endemic within the environment and pose serious public health threats (Obi et al, 2007).

CONCLUSION AND RECOMMENDATIONS

The present study has demonstrated the reality of high antibiotic resistance in *E.coli* enteric infections among HIV-infected patients. This calls for effective interventions to halt the trend and spread of resistance among etiological agents of enteric infections. There is also an urgent need for the formulation and implementation of effective antibiotic use policy in order to tackle this problem of resistance and reduce the burden. Control of antibiotic resistance is necessary to conserve the usefulness of the remaining drugs. The use of cotrimoxazole, tetracycline, amoxycillin and amoxycillin-clavulanate can be suspended for some time as a measure of controlling resistance to these antibiotics.

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