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Full Length Research Paper

Isolation and Antibiogram of Shiga Toxin-Producing *Escherichia coli* O157:H7 from Diarrhoeic HIV/AIDS Patients in Lafia, Central Nigeria

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Abstract

This study assessed the prevalence and antibiogram of Shiga Toxin-Producing *Escherichia coli* O157:H7 from Diarrhoeic HIV/AIDS Patients in Lafia, Central Nigeria. *E. coli* O157:H7 from stools of consenting HIV/AIDS patients were isolated by culture based, biochemical and serological assays. Confirmed isolates were tested for their susceptibility to commonly used antimicrobial agents using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI). The prevalence of *E. coli* O157:H7 from Diarrhoeic HIV/AIDS patients was 1.9% of which 33.3% and 66.7% were from males and females respectively. The cumulative antibiotic resistance frequency of the isolates was 56.7%. All the 3(100%) *E. coli* O157:H7 isolates were resistant to Amoxicillin and Augmentin, 2(66.7%) to Cotrimoxazole, Sparfloxacin, Ofloxacin and Streptomycin. The isolates showed lower frequencies of resistance to Chloramphenicol (33.3%), Ciprofloxacin (33.3%), Gentamicin (33.3%), and Pefloxacin (0.0%). Resistance phenotypes recorded were: Cotrimoxazole-Chloramphenicol-Spafloxacin-Ciprofloxacin-Amoxicillin-Augmentin-Streptomycin; Spafloxacin-Amoxicillin-Augmentin-Gentamicin-Ofloxacin and Cotrimoxazole-Amoxicillin-Augmentin-Ofloxacin-Streptomycin with the same frequency (33.3%). There was joint resistance of *E. coli* O157:H7 isolates to almost all antibiotics tested, with the Multiple Antibiotic Resistance (MAR) index of 0.5 and 0.7 which indicates that these isolates were exposed to these antimicrobial agents. The high degree of antibiotic resistance and MAR indices suggests the need for continuous surveillance of antimicrobial resistance trends in immuno-compromised patients who are highly susceptible to opportunistic infections with Shiga Toxin-Producing *E. coli* O157:H7.

Key words: Multiple antibiotic resistance, *Escherichia coli* O157:H7, HIV/AIDS, diarrhoea

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) or Vero-toxigenic producing strains of *Escherichia coli* (VTEC) are recognized as an important human pathogen of public health concern (Bettelheim and Beutin, 2003). Since its first recognition, this serotype and its isolation from stool samples have sharply increased, and ranked as the 3rd most common bacterial pathogen of the human gut after *Salmonella* and *Campylobacter* spp (Riley *et al*., 1983; Fitzpatrick, 1999; Adwan *et al*., 2002).

Although a variety of *E. coli* serotypes have been associated with human illness, the most important among these is O157:H7. Enterohaermorrhagic *E. coli* 0157: H7 is one of the six groups of *E. coli* recognized as an etiological agent of diarrhoea (Aboaba *et al*., 2006). Infection with this *E. coli* serotype is associated with a spectrum of illnesses including watery diarrhoea, bloody diarrhoea, and the haemolytic uremic syndrome, a potentially fatal condition characterized by acute renal failure (Griffin and Tauxe, 1991). Cattle are the principal reservoir for these organisms. Important sources of infection include consumption of undercooked hamburger, ground beef, raw milk, meat and dairy

products, vegetables, unpasteurized fruit juices and water, and other contaminated food products (Chapman *et al*., 1997; Wilson *et al*., 1997). Infection can also be acquired by direct contact with animals and by person-to-person spread (Cho *et al*., 2006).

Diarrhoea occurs in about 30 to 60% of HIV/AIDS patients in developed countries and in an estimated 90% of such patients in developing countries (Sapkota *et al*., 2004). Epidemiological investigations during outbreaks have identified *E. coli* O157:H7 as a pathogenic strain that causes severe and life-threatening diarrhoea (Galane and Le Roux, 2001). *E. coli* O157:H7 infections pose the greatest risk to immuno-suppressed individuals because it can easily invade the cells of HIV/AIDS patients due to a suppressed cell-mediated immunity (Morris and Potter, 1997; Hoffman, 2004).

Awareness of the clinical and therapeutic aspects of diarrhoea suspected to have been caused by *E. coli* O157:H7 in HIV/AIDS patients is therefore vital in directing diagnostic evaluation of these patients and further research to improve human health. This strain of *E. coli* is a threat to human health, especially to immuno-compromised persons such as HIV/AIDS patients, as the bacteria can be contracted from foods and water consumed by these patients. Also because of a compromised immune system, such patients may develop diarrhoea attributed to *E. coli* O157:H7 than it would be the case for immuno-competent persons (Abong *et al*., 2008).

Although, antibiotics are not recommended for treatment of *E. coli* O157:H7 infections in humans, there is evidence that bacterial isolates are resistant to some antibiotics (Aibinu *et al*., 2007). However, such treatments may be recommended for cystitis and pyelonephritis other than haemorrhagic colitis all caused by *E. coli* O157:H7 (Griffin and Tauxe, 1991). For those limitations of using antimicrobial agents in *E. coli* O157:H7 cases, the generally accepted belief is that *E. coli* O157:H7 may still be susceptible to most antimicrobials. In addition to their epidemiological importance, the studies of antimicrobial susceptibility of *E. coli* O157:H7 may have more therapeutic significance as recent studies have indicated a possible role of early administration of antimicrobials in preventing the progression of haemolytic uremic syndrome and haemorrhagic colitis both caused by *E. coli* O157:H7 (Molbak *et al*., 2002).

To the best of our knowledge, there is no study that has elucidated the presence and antibiogram of *E. coli* O157:H7 from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria. Unfortunately, the diagnosis of *E. coli* O157: H7, in spite of its role in diarrhoea and its potentially severe outcome, is not considered a routine laboratory test in the study area, and there are no comprehensive and documented data on the incidence and prevalence of diarrhoea caused by this particular strain. This study thus investigated if *E. coli* O157:H7 is one of the bacteria involved in diarrhoea that habitually characterize HIV/AIDS patients. The antimicrobial susceptibility profiles of *E. coli* O157:H7 isolates obtained from the stools of these patients were also investigated.

METHODS

Study Population and Area

This study was carried out among confirmed HIV-seropositive patients with diarrhoea who presented in Dalhatu Araf Specialist Hospital (DASH) Lafia, Central Nigeria. The study area was selected based on the researchers' familiarity with the area, HIV prevalence and the presence of a referral hospital, DASH, which is situated in Lafia, Nasarawa State capital and caters for HIV/AIDS patients of various races, gender and age groups from various Local Government Areas, districts and rural areas within and outside the State. According to the recent National HIV/AIDS Reproductive Health Survey, Nasarawa State has a very high prevalence (8.1%) of HIV/AIDS in Nigeria after Kaduna, Taraba and Rivers States with the prevalence of 9.2%, 10.5% and 15.2% respectively (NARHS, 2012).

The study commenced after ethical approval was obtained from the hospital ethical committee. Informed consent was also obtained from each of these patients. The study period was between May, 2014 and June, 2015.

Sample Collection

One hundred and sixty (160) fresh stools specimen were obtained from confirmed HIV/AIDS positive patients visiting DASH for treatment, using sterile plastic universal container (Sterilin, UK), and returned to the laboratory immediately. The confirmed HIV/AIDS patients had already been tested for HIV at the HIV/AIDS clinic of DASH and were known by the hospital clinicians to be carriers of the virus. The patients' diarrhoeal condition and HIV/AIDS status were recorded. Anonymity of the patients was protected as much as possible. Diarrhoeic stools in this study were diagnosed in the case of patients experiencing three or more watery stools in 24 hours. Patients included children, adults and the elderly. Detailed history to define age, sex and location of patients were documented.

Selective Plating and Identification of *E. coli* O157:H7 Colonies

A loop full of each stool specimen was cultured for *E. coli* on Eosin Methylene Blue Agar (Oxoid CM 0069) and incubated at 44^{O}C for 24 h as described by Okeke *et al*.

Table 1. Prevalenceof *E. coli* O157: H7 from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria

Feature	No. (%) of Samples Tested	No. (%) Positives
Age		
< 10	9 (5.6)	1 (33.3)
11- 20	18 (11.3)	0 (0.0)
21- 30	47 (29.4)	0 (0.0)
31- 40	53 (33.1)	0 (0.0)
41- 50	17 (10.6)	1 (33.3)
51>	16 (10.0)	1 (33.3)
Gender		
Male	71 (44.4)	1 (33.3)
Female	89 (55.6)	2 (66.7)
Location		
Urban	58 (36.3)	2 (66.7)
Suburban	69 (43.1)	1 (33.3)
Rural	33 (20.6)	0 (0.0)
Total	**160 (100.0)**	**3 (1.9)**

(2001) and screened for *E. coli* O157:H7 on Sorbitol MacConkey Agar (Oxoid, CM813) enriched with Cefixime-Tellurite supplement (Oxoid SR 172).*E. coli* colonies have green metallic sheen appearance on EMB while typical *E. coli* O157:H7 appeared as non-sorbitol fermenter colonies (NSFC) which are characterized as having a slightly transparent, almost colourless with a weak pale brownish appearance on CT-SMAC. Discrete colonies were randomly selected and then examined for the presence of gram-negative rods using Gram staining technique (Prescott *et al*., 2005).

Biochemical Test

The strains were characterized biochemically using Microbact 12E (MB1130A+, Oxoid) according to the manufacturer's instruction. Identification of *E. coli* strains was done following a series of 12 biochemical tests.

Serological Test

Presumptive *E. coli*O157:H7 colonies were serologically confirmed by using *E. coli* O157:H7 latex agglutinations assay (R30959601, Oxoid), containing latex particles coated with antibodies specific for *E. coli* O157 and *E. coli* H7 antigen. Isolates were tested separately with anti-O157, and H7 antisera. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instruction, hence colonies that agglutinated to the separate antisera were considered to be *E. coli* O157:H7.

Antimicrobial Susceptibility Test

All *E. coli* O157: H7isolates were tested for antimicrobial susceptibility by disk diffusion technique in accordance to Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2007) using multi-antibiotic discs (Maxicare Medical Laboratory, Nigeria) containing the following antimicrobials and disc content (in µg): Cotrimoxazole (30µg), Chloramphenicol (30µg), Ciprofloxacin (10µg), Amoxicillin (30µg), Augmentin (30µg), Gentamicin (10µg), Pefloxacin (µg), Ofloxacin (10µg), Streptomycin (30µg), Sparfloxacin (10µg). *E. coli* ATCC 25922 was used as control organism, and the results were interpreted using the CLSI criteria (CLSI, 2007).

RESULTS

Prevalence of *E. coli* O157:H7

The prevalence of *E. coli* O157:H7 from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria is 1.9%, with 33.3 and 66.6% from males and females respectively. With respect to age groups, patients within the age group < 10, 41- 50 and 51> had the prevalence of 33.3 % while those within 11- 20, 21- 30, and 31- 40 age groups had 0.0%. The prevalence of *E. coli* O157:H7 among urban, suburban and rural dwellers was 66.7, 33.3 and 0.0% respectively (Table 1).

Antimicrobial Resistance

All the isolates were resistant to most of the antibiotics.

Table 2. Antimicrobial susceptibility of *E .coli* O157:H7 isolates from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria.

	Susceptibility (n = 3)		
	R	I	S
Antibiotic	**No. (%)**	**No. (%)**	**No. (%)**
Cotrimoxazole	2 (66.7)	0 (0.0)	1 (33.3)
Chloramphenicol	1 (33.3)	0 (0.0)	2 (66.7)
Sparfloxacin	2 (66.7)	1 (33.3)	0 (0.0)
Ciprofloxacin	1 (33.3)	0 (0.0)	2 (66.7)
Amoxicillin	3 (100.0)	0 (0.0)	0 (0.0)
Augmentin	3 (100.0)	0 (0.0)	0 (0.0)
Gentamicin	1 (33.3)	1 (33.3)	1 (33.3)
Pefloxacin	0 (0.00)	2 (66.7)	1 (33.3)
Ofloxacin	2 (66.7)	0 (0.0)	1 (33.3)
Streptomycin	2 (66.7)	0 (0.0)	1 (33.3)
Total	**17 (56.7)**	**4 (13.3)**	**9 (30.0)**

Legend: R, Resistant; I, Intermediate; S, Sensitive.

Table 3. Distribution into various resistance phenotypes of antibiotic resistant *E .coli* O157:H7 isolates from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria.

S/N	Resistance Pattern*	No. (%) of Isolates	Location	Age Group	Sex**
1	Cot, Amo, Aug, Ofl, Str	1 (33.3)	Urban	<10	M
2	Spa, Amo, Aug, Gen,Ofl	1 (33.3)	Rural	50>	F
3	Cot, Chl, Spa, Cip, Amo, Aug, Str	1 (33.3)	Urban	41-50	F

Symbols: * Cot, Cotrimoxazole; Chl, Chloramphenicol; Cip, Ciprofloxacin; Amo, Amoxicillin; Aug, Augmentin; Gen, Gentamicin; Ofl, Ofloxacin; Str, Streptomycin; Spa, Sparfloxacin.
**F, Female; M, Male.

Table 4. Frequency of multiple antibiotic resistance (MAR) and multiple antibiotic resistance indices of *E.coli* O157:H7 isolates from diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria.

Number of antibiotic to which isolates are resistant	Number of isolates with MAR	Frequency (%)	MAR indices
5	2	66.7	0.5
7	1	33.3	0.7

The cumulative antibiotic resistance frequency of the isolates was 56.7% as shown in Table 2. The highest (100%) resistance was obtained to amoxicillin and Augmentin; while the least was to Pefloxacin (0.0%) (Table 2).

Antibiotic Resistance Phenotypes

The distribution of the *E. coli* O157:H7 isolates into different antibiotic resistance phenotypes observed is as given in Table 3. The resistance phenotypes as shown by the isolates are: Cot, Amo, Aug, Ofl, Str; Spa, Amo, Aug, Gen, Ofl and Cot, Chl, Spa, Cip, Amo, Aug, Str with all having the frequency of 33.3% with respect to patients' gender, location and age group.

Multiple Antibiotic Resistance (MAR) and MAR indices

Table 4 shows the MAR of the isolates. MAR occurs in all the isolates at different frequencies. MAR indices obtained from this study are 0.5 and 0.7.

DISCUSSION

Results from the present study showed a low prevalence (1.9%) of *E.coli* 0157: H7 infection in diarrhoeic HIV/AIDS patients in Lafia, Central Nigeria, thus confirming the presence of this emerging pathogen in this locality. This is the first report of a systematic surveillance study on the prevalence of *E. coli* 0157: H7 isolated from diarrhoea

patients in the study area. HIV/AIDS patients have a higher likelihood of developing diarrhoea than people with competent immune systems (Mitchell *et al*., 1998; Hayes *et al*., 2003). Studies of diarrhoeal cases have been reported in both immuno-competent and immuno-suppressed persons such as those suffering from HIV/AIDS (Obi and Bessong, 2002; Obi *et al*., 2003; 2006; 2007). However, it is important to recognize that *E. coli* O157:H7 infections pose the greatest risk to immuno-suppressed individuals (Morris and Potter, 1997; Hoffman, 2004).

The prevalence of *E. coli* O157:H7 in patients by gender was of profound epidemiological interest. Out of the 3 patients who were infected with *E. coli* O157:H7, 66.7% (2/3) were females whereas only a single male (1/3) 33.3% was infected. The higher prevalence of *E. coli* O157 in females than in males has been observed in most epidemiological studies. A similar trend was reported in Cameroon in 1998 when an epidemic of diarrhoea due to *E. coli* O157:H7was investigated (Cunin *et al*., 1999). Similarly Obong *et al*. (2008), revealed the same trend among diarrhoeic HIV/AIDS patients in South Africa.

Age distribution among *E.coli* O157:H7 positive HIV/AIDS patients indicated that age groups from <10, 41- 50 and 50> had the same prevalence (33.3%) of *E. coli* O157:H7 infection. This finding is in consonance with the reports by Cunin *et al.* (1999) and Obong *et al*. (2008). With respect to locations, prevalence was higher in urban area (66.7%) followed by suburban area (33.3%). This was probably because of the proximity and readily access urban inhabitants have to the hospital. More so, the pool of the HIV/AIDS patients in the hospital is from the urban and suburban areas. This could be a reflection of rural-urban migration, which is known to affect most cities universally.

Antimicrobial susceptibilities of the *E. coli* O157:H7 isolates from HIV/AIDS patients to some commonly used antibiotics revealed that the 3 isolates were resistant to Cotrimoxazole, Chloramphenicol, Ciprofloxacin, Amoxicillin, Augmentin, Gentamicin,Ofloxacin, Streptomycin and Sparfloxacin with the cumulative resistance of 56.7%. High degree of antibiotic resistance by the *E. coli* O157:H7 isolates were recorded to Cotrimoxazole, Sparfloxacin, Ofloxacin and Streptomycin (66.7%) whereas 100% resistance were recorded to Amoxicillin and Augmentin.

A very high resistance (84.2 and 100%) of *E. coli*O157:H7to Amoxycillin was also reported by Reuben and Owuna (2011) and Isibor *et al*. (2012). Ngwai *et al*. (2011) reported that *E. coli* isolates from HIV/AIDS patients in Keffi, Nigeria showed a very high degree of resistance (99.2%) to Augmentin as recorded in this study.

High resistance to cotrimoxazole is in agreement with the finding of Shroeder *et al*. (2001), who reported that among 189 *E. coli* O157:H7 isolates recovered from various sources between 1985 and 2000, 19 (10%) were resistant to this antibiotic. This antimicrobial agent is commonly used to treat respiratory infections, diarrhoea, mastitis, and other infections in beef and dairy cattle which may be consumed by HIV/AIDS patients.

High degree of resistance to quinolones (Sparfloxacin, Ofloxacin) is alarming since they are known to be very potent antimicrobial agents. However some workers have recently reported resistance to the quinolones in Nigeria (Daini *et al*., 2005; Umolu *et al*., 2006) as well as in other countries (Oteo *et al*., 2005). Nevertheless, the relatively lower resistance recorded to Pefloxacin and Ciprofloxacin (fluoroquinolones) shows that they can be used as antibiotic of choice against *E. coli* O157:H7 infections. Prior to their use, resistance was rare (Barry *et al*., 1990). A previous assessment of some six antibiotics against clinical isolates of *E. coli* showed them to be the most efficacious (Isibor *et al.,* 2003).

The lower resistance of the isolates to gentamicin (an aminoglycoside) could be attributed to its requirement for parenteral administration which hinder their misuse and abuse due the discomfort associated with injections. This finding agrees with that of Reuben and Owuna, (2013) who reported that none of the *E. coli* O157:H7 isolates tested was resistant to gentamicin. The widespread and inappropriate use of antibiotics is a significant contributing factor to the development and spread of bacterial resistance to antimicrobial agents (Mincey and Parkulo, 2001). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance (Granizo *et al*., 2000).

The observation that some isolates were resistant to Streptomycin but not to gentamicin could be explained by the fact that gentamicin, in addition to binding to a specific S12 protein in the 30S ribosome, also binds to the L6 protein of the 50S ribosome to inhibit protein synthesis (Tripathi, 2003). Hence, a possible alteration of the S12 protein target alone in the streptomycin-resistant isolates is incapable of affecting its action.

In this study, all the *E. coli* O157:H7 isolates tested showed multidrug resistance to the antibiotics at various percentages. This result is in agreement with the findings by other researchers, who reported multidrug resistance among *E. coli* O157:H7 isolates (Kim *et al*., 1994; Schroeder *et al*., 2002**).** The 3 isolates were resistant to 5 and 7 of the antibiotics tested. The multiple antibiotic resistance (MAR) indices obtained from this study were 0.5 and 0.7. The frequency of multiple antibiotic resistance (MAR) has been defined as joint resistance of *E. coli* isolates to more than two antibiotics (Ngwai *et al*., 2011). MAR was determined using the formula MAR=x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for susceptibility (Krumperman, 1983; Akinjogunla and Enabulele, 2010).

According to Krumperman (1983), MAR indices above 0.2 indicate that such isolates originate from environment where antimicrobial agents are freely available and accessible with high potential for abuse. This is true of the study area where people access antibiotics over-the-counter, almost without restrictions. This poses a serious public health concern.

The high MAR indices as recorded in this study also agree with that recorded by Ngwai *et al*. (2011) and Osibor *et al*. (2012) in their investigations of multidrug resistant *E.coli* from HIV patients in Keffi, Nigeria and *E. coli* O157:H7 from Edo State, Nigeria. Over the last two decades antimicrobial resistance has been reported for all classes of diarrhoeagenic *E.coli* and specifically from African isolates (Vila *et al.*, 1999). Similar to the high antibiotic resistance pattern observed in this study, Tobih *et al*. (2006) have also shown that pathogenic isolates of *E. coli* have relatively high potentials for developing resistance.

This study highlights *E. coli* O157:H7, an emerging bacterial pathogen as an aetiologic agent of diarrhoea among HIV/AIDS patients in Lafia, Central Nigeria. The prevalence of *E. coli* O157:H7 was ubiquitous with respect to patients' gender, age and locations. It is recommended that HIV/AIDS patients with prolonged diarrhoea should be routinely screened for this bacterium. The high degree of antibiotic resistance and MAR indices suggests the need for continuous surveillance of antimicrobial resistance trends in immuno-compromised patients who are highly susceptible to opportunistic infections. Proper implementation and legislation of antibiotics use strategies at all levels will decrease the risk and the clinical threat posed by antimicrobial resistance due to use and misuse of antibiotics.

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