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Faecal Carriage and Antibiotics Resistance Patterns of Campylobacter Species from HIV/AIDS Patients in Ibadan, Southwest Nigeria

Olutayo Israel Falodun^{1*}, Emmanuel Adedapo Adesola¹, Ebenezer Adewuyi Ademola² and Solomon A. Bakarey³

¹Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria. ²Department of Biological Science and Biotechnology, Caleb University, Imota, Lagos, Nigeria ³Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OIF, EAA and SAB designed the study and the protocol. Author OIF supervised the study. Authors OIF and EAA managed literature search, data acquisition and wrote the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: As a result of the low immunosuppressive condition of people living with Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome (HIV/AIDS), they are at greater risk of *Campylobacter* infections but the use of antibiotics tends to reduce incidence. However, though this has reduced the incidence of the infection, there is likely development of antibiotic resistance with them thereby becoming asymptomatic carriers of drug resistant *Campylobacter* species.

Aim: This study was carried out to determine the faecal carriage and antibiotic susceptibility pattern of *Campylobacter* species isolated from HIV patients in Ibadan, Nigeria.

Methods: One Hundred stool samples were collected from HIV patients attending two Antiretroviral Clinics in Ibadan between February and March, 2017. *Campylobacter* species were

*Corresponding author: Email: falod2013@gmail.com, solution_falodun@yahoo.com;

isolated and identified using standard methods. Antibiotic susceptibility test of the isolates to amikacin, gentamycin, chloramphenicol, amoxicillin/clavulanate, cefixime, aztreonam, ciprofloxacin, cephalothin, nalidixic acid and ertapenem was done using disk diffusion method. **Results:** The prevalence of *Campylobacter* species in the stool samples of the HIV/AIDS patients was 68%. A total of one hundred and twenty isolates were identified as *C. upsaliensis* 32(26.7%), *C. jejuni* 30(25%), *C. lari* 23(19.2%), *C. coli* 20(16.7%), and *C. fetus* 15(12.5%). The antibiotics susceptibility pattern of these isolates showed that 97(80.8%), 88(73.3%), 82(68.3%), 9(7.5%), 9(7.5%) and 12 (10.8%) were resistant to gentamycin, chloramphenicol, cefixime, amikacin, ciprofloxacin and ertapenem respectively. Moreover, 102 (85.0%) of the isolates were found to be multidrug resistant.

Conclusions: The faecal carriage and high prevalence of antibiotic resistance of *Campylobacter* strains among the HIV/AIDS patients therefore present them as asymptomatic carrier and reservoir for dissemination of the bacteria.

Keywords: Campylobacter species; antibiotic resistance; HIV and AIDS; Ibadan-Nigeria.

1. INTRODUCTION

Campylobacteriosis is an infectious disease caused by Campylobacter species; and generally regarded as the most common type of diarrhoea worldwide, exceeding salmonellosis, shigellosis and E. coli infections [1]. It is regarded as water and food-borne diseases caused by the ingestion of contaminated water and food, including unpasteurized milk, undercooked chicken and meat products [2]. It is also a zoonotic disease transmitted from animals by direct or indirect contact such as consumption of their meat and products which are contaminated with C. jejuni, C. coli, and C. lari as major culprits of infection; however, C. fetus, C. concisus, C. upsaliensis, and C. hyointestinalis also have the ability to cause infection [3, 4].

The symptoms of campylobacteriosis include: watery or bloody diarrhoea, fever, nausea and vomiting, abdominal cramps, malaise, loss of weight and loss of appetite [5, 6]. At times, the disease mav produce extra-gastrointestinal manifestations such as bacteremia. meningoencephalitis. inflammatory bowel cholecystitis, diseases. colorectal cancer. Guillain-Barre and Miller Fisher Syndromes [6, 7]. Children that are less than two to five years of age, pregnant women, the elderly, international travellers and immunocompromised individuals are at higher risks of this infection [8, 9].

There are several reports on *Campylobacter* infections from developed and developing countries, but most of the reports were from children, pets, domestic animals, farm animals and poultry [9, 10]. Reports on people living with HIV/AIDS are scarce and limited, and to the best of our knowledge, there is no such report in Ibadan, Oyo State, Nigeria. Due to the

immunocompromised condition of HIV/AIDS patients, opportunistic secondary pathogenic organisms in the gastrointestinal tract and subsequently in the blood stream causing hospital admissions are frequently recurrent [10], [11]; and incidence of campylobacteriosis among HIV/AIDS patients have been reported to exceeds that of healthy population in multiple fold [12].

However. the incidence of opportunistic infections among HIV infected individuals has reduced drastically. This is because most people with HIV/AIDS take combination living antiretroviral therapy (ART). Continuous and prolong multiple course of ART and antibiotics for therapeutic and prophylactic purposes, a common practise among HIV/AIDS patients may serve as selection pressure for development of resistance among microorganisms including species Campvlobacter [1]. Isolates of Campylobacter with resistance to ampicilin, ciprofloxacilin, macrolides, tetracycline, nalidixic acid, kanamycin and gentamicin have been reported from developing and developed countries [6,13,14]. Added to this, is the concerns that infections with antibiotics resistant Campylobacter species are associated with a longer duration of illness which is an increased risk of invasive disease and death [14].

Asymptomatic *Campylobacter* infections are common in developing countries, whereby faecal specimens are positive for *Campylobacter* species without any symptoms and this could have impact on the transmission of campylobacteriosis [6,13]. Patients living with HIV/AIDS are likely to be asymptomatic due to drug usage that tends to reduce the incidence of secondary infections. This study therefore, was aimed at determining the faecal carriage and antibiotic susceptibility patterns of *Campylobacter* species isolated from HIV/AIDS patients attending ART clinic in Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Stool samples were collected from one hundred HIV infected participants accessing care at the Antiretroviral/Presidents' Emergency Plan for AIDS Relief (ARV/PEPFAR) Clinic at the University College Hospital and Adeovo Maternity Teaching Hospital, Ibadan, Nigeria. The samples were collected between the months of February and March, 2017 using sterile sample bottles with the help of trained nurses other counselors. and health professionals at the clinics. The samples were transported in ice-packs to the Pathogenic Laboratory of the Department of Microbiology. University of Ibadan for immediate processing. A structured questionnaire was administered to each of the HIV patients that met the inclusion criteria to obtain relevant information for the study including age and sex.

2.2 Isolation and Identification of *Campylobacter* Species

The stool samples were emulsified in sterile normal saline and serially diluted. Appropriate dilutions of the emulsified samples were aseptically plated out on Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) CM0739 with CCDA selective supplement (SR0155E, Oxoid) using pour plate technique. The plates were left to solidify, inverted, arranged in an anaerobic jar with CampyGen (Campygen Oxoid) to generate microaerophilic 3.51 condition and incubated at 37°C for 48 hours. Distinct colonies with characteristics presumptive of Campylobacter species were sub-cultured to obtain pure isolates. The isolates were identified using morphological and biochemical characteristics such as colonial appearance, Gram's reaction, catalase, oxidase, nitrate hydrolysis, reduction. urea and sugar fermentation. Isolates were suspended in nutrient broth with 15% glycerol and stored at -20°C for further analysis.

2.3 Antimicrobial Susceptibility Testing

This was carried out using disk diffusion method described by Clinical Laboratory Standard Institute (CLSI) [15] on Mueller Hinton agar (Oxoid, UK) supplemented with 5% sheep blood. The agar plates were inoculated with sstandardized (0.5 McFarland) cell suspension of the test isolates. The following antibiotics discs: amikacin (30µg), amoxicillin/clavulanic acid (30µg), aztreonam (30µg), cephalothin (30µg), nalidixic acid (30µg), gentamycin (30µg), cefixime (5µg), chloramphenicol (30µg), ciprofloxacin (5µg), and ertapenem (10µg) (Oxoid, UK) were placed on the agar plates. These plates were incubated in an anaerobic jar with CampyGen (Campygen 3.5L, Oxoid) to generate microaerophilic condition at 37°C for 48 hours. The diameters of the zone of inhibitions were measured and the values were interpreted using CLSI [15] standard.

2.4 Statistical Analysis

The prevalence of *Campylobacter* species was calculated and expressed in percentage (%). The association between prevalence rates of *Campylobacter* species and gender was analyzed using chi-square test. A p value of < 0.05 was considered statistically significant.

3. RESULTS

Of 100 stool samples from HIV/AIDS patients examined for this study, 32 (32%) were male and 68(68%) were female participants, age ranged 16 years to 85 years. Majority (77%) were in the age range of 26 to 65 years while, age range 10 (10%) was unknown. Sixty-eight (68%) of the stool samples were culture positive for *Campylobacter* species, with 24 (35.3%) from male and 44(64.7%) from female participants. The prevalence of Campylobacter species is different among the age ranges with the highest 21/68 (30.9%) in age range 36-45 years. However, there was no significant difference in the prevalence between male and female gender (p=0.213) and among all the age ranges (p=0.213)0.130) (Table 1). The frequency of occurrence of the Campylobacter species (n=120) isolated from the stool samples showed that 32 (26.7%) were identified as C. upsaliensis, 30 (25%) as C. jejuni while, 23(19.2) were identified as C. lari, 20 (16.7%) as C. coli and 15 (12.5%) as C. fetus (Table 2).

The antibiotics susceptibility pattern of the isolates showed that 97 (80.8%), 88 (73.3%), 82 (68.3%) and 73 (60.8%) were resistant to gentamycin, chloramphenicol, cefixime and cephalothin while 9 (7.5%), 9 (7.5%) and 12 (10.8%) were resistant to amikacin, ciprofloxacin and ertapenem respectively. All (100%) the *C. jejuni, C. lari* and *C. coli* showed resistance to

cephalothin but none (0%) of the *C. fetus* and *C. upsaliensis* exhibited resistance to cephalothin. Similarly, 25 (83.3%) of the *C. jejuni* were resistant to gentamycin while, none of the isolates was resistant to nalidixic acid. It was also observed that while 19 (95.0%) of the *C. coli* exhibited resistance to gentamycin, none was resistant to both ciprofloxacin and nalidixic acid. Moreover, 16 (69.5%) of *C. lari* were resistant to both chloramphenicol and cefixime, 25 (78.1%) of *C. upsaliensis* showed resistance to both chloramphenicol and gentamycin and 1 (3.1%) was resistant to nalidixic acid, ciprofloxacin and amikacin among *C. upsaliensis, C. upsaliensis* and *C. fetus* respectively (Table 3).

The antibiotype of the *Campylobacter* species is shown in Table 4. Of the 120 isolates, 102 (85%) showed resistance to three or more antibiotics while, 22 (18.3%) showed resistance to three antibiotics, 37 (30.8%), 24 (20.0%), 13 (10.8%) and 6 (5.0%) showed resistance to four, five, six and seven antibiotics respectively.

4. DISCUSSION

In this study, the prevalence of Campylobacter species isolated from the stool samples of people living with HIV and AIDS was 68%. This is comparable to previously reported prevalence of 62.67% in feacal samples of humans and pigs in Kebbi State, northern part of Nigeria [16]. However, this is slightly higher than the 55% prevalence from different poultry and human samples in Sokoto, Nigeria [17]. Similarly, it is higher than the reported 26% rate in samples from gastroenteritis patients in Ghana [18], 19.6% in diarrhoeic HIV patients in Nigeria [1] and 15.5% among diarrhoeic children in Ethiopia [19]. The variation in the prevalence may be due in part to differences in the sensitivity of detection methods, geographical location, study population, population-level immunity, scope of the case profile/disease condition studied, food practises and study period among others [6].

By gender, the prevalence rate of *Campylobacter* species in this study was higher in male patients compared to their female counterparts, with no statistical significant difference (p=0.13). This observation is in concordance with the reports of previous studies on diarrheic children in Osun State Nigeria and Ethiopia in East Africa [20, 21]. However, the observation is contrary to the reports of some other studies where it was observed that the prevalence of *Campylobacter* species was higher in females than their male counterparts [1, 16, 19, 22]. Also in this study,

Campylobacter species was detected in all age groups and there was no statistical significance in the sample positivity and age group. This may be attributed to the immunocompromised state of the patients and lack of no consideration for any infection or symptom (e.g. diarrhoea), but rather a case of faecal carriage only. The higher prevalence observed in ages 26 to 45 years, is slightly similar to a report of higher prevalence in the age range 30 - 39 [1]. Generally, Campylobacter is commonly recovered in children in developing countries, but elderly and immunocompromised patients have also been identified to be predisposed to Campylobacter infections [6]. The high prevalence may be due to resistance of Campylobacter to Zidovudine, the Anti-retroviral drugs commonly administered to HIV/AIDS patients which have been observed to inhibit Gram negative bacterial growth. This can also be supported by the assertion that a higher incidence of HIV/AIDS can increase the number of cases of campylobacteriosis in the adult population in developing countries [8].

The frequency of occurrence of Campylobacter isolated in this study showed that the most prevalent species were C. upsaliensis, C. jejuni and C. lari. This is in agreement with previous reports on these organisms [7, 16, 17, 18]. Campylobacter species most commonly isolated in cases of human infections are C. jejuni and C. coli. Others such as C. lari and C. upsaliensis have also been isolated from patients with diarrhoeal diseases but less frequently reported [7, 23]. The prevalence rates of C. jejuni (25.0%) and C. coli (16.7%) observed in this study were respectively higher than 8.9% and 0.7% reported from a study on children with diarrhoea in Poland [24], but lower than 33.3% C. jejuni and 44.4% C. coli in diarrhoeic HIV/AIDS patients in Nigeria [1]. The reason for this disparity may be as a result of the different study subjects in the studies and case/symptom studies. Isolation of C. fetus in this study is in concordance with the previous assertion that it has been found to be associated with immunocompromised individuals [3, 7, 25]. This is also an indication that the severity of Campylobacter infection caused by C. fetus in people living with HIV and AIDS might lead to cases of bacteremia (campylobacteremia), cholecystitis, Guillain Barre Syndrome, Miller Fisher Syndrome, meningitis, septic shock and death as previously noted [3, 6, 11]. The isolation of Campylobacter upsaliensis in this study is significant as this species has been listed as parts of clinically relevant Campylobacter species in human;

Age (years)	No examined	Male		Female		Total	P value (gender	P-value (over all
		No examined	No positive (%)	No examined	No positive (%)	-	prevalence within age range)	age prevalence)
16-25	4	3	3(100)	1	0(0)	3 (75)	0.046	
26-35	22	5	5(100)	17	14(76.5)	19(86.4)	0.312	
36-45	29	8	6(75)	21	15(71.4)	21(72.4)	0.847	
46-55	16	7	6(85.7)	9	4(44.4)	10(62.5)	0.091	
56-65	10	3	0(0)	7	4(57.1)	4(40)	0.091	0.130
66-75	6	4	2(50)	2	2(100)	4(66.7)	0.221	
76-85	3	0	0	3	1(33.3)	1(33.3)	ND	
Unknow	10	2	2(100)	8	4(50)	6(60)	0.197	
n						. ,		
Total	100	32	24 (75)	68	44(64.7)	68 (68)	0.213	

Table 1. Distribution of Campylobacter species in the stool samples of HIV/AIDS patients in relation to age and gender

ND - Not determined

Table 2. Frequency of occurrence of Campylobacter species from the stool samples of HIV/AIDS patients

Campylobacter species	Number isolated	Percentage (%)	
Campylobacter jejuni	30	25.0	
Campylobacter coli	20	16.7	
Campylobacter fetus	15	12.5	
Campylobacter upsaliensis	32	26.7	
Campylobacter lari	23	19.2	
Total	120	100	

Antibiotics	<i>C. jejuni</i> (n=30)	<i>C. coli</i> (n=20)	<i>C. lari</i> (n=23)	<i>C. fetus</i> (n=15)	<i>C. upsaliensis</i> (n=32)	Total (n=120)
Amikacin (30µg)	2 (6.7)	3 (15)	1 (4.3)	1 (6.6)	2 (6.2)	9 (7.5)
Amoxicillin/Clavulanate	9 (30)	5 (25)	9 (39.1)	3 (20)	9 (28.1)	35 (29.2)
(30µg)						
Aztreonam (30µg)	11 (36.7)	7 (35)	9 (39.1)	8 (53.3)	19 (59.3)	54(45)
Cefixime (5µg)	21 (70)	15 (75)	16 (69.5)	6 (40)	24 (75)	82 (68.3)
Cephalothin (30µg)	30 (100)	20 (100)	23 (100)	0 (0)	0 (0)	73 (60.8)
Ciprofloxacin (5µg)	4 (8)	0 (0)	2 (8.7)	2 (13.3)	1 (3.1)	9 (7.5)
Chloramphenicol (30µg)	21 (70)	14 (70)	16 (69.5)	12 (80)	25 (78.1)	88 (73.3)
Ertapenem (10µg)	2 (6.7)	2 (10)	6 (26.1)	0 (0)	2 (6.2)	12 (10.8)
Gentamycin (30µg)	25 (83.3)	19 (95)	17 (73.9)	11 (73.3)	25 (78.1)	97 (80.8)
Nalidixic acid (30µg)	0 (0)	0 (0)	23 (100)	13 (86.6)	1 (3.1)	38 (31.6)

 Table 3. Antibiotic Resistance Pattern of the Campylobacter species isolated from the stool samples n(%)

Table 4. The Antibiotype of the isolated Campylobacter species n (%)

No of Antibiotics	No of Antibiotype	<i>C. jejuni</i> n=30	<i>C. coli</i> n=20	<i>C. lari</i> n=23	<i>C. fetus</i> n=15	<i>C. upsaliensis</i> n=32	Total Isolate n=120
2	4	2 (6.67)	3 (15)	0 (0)	0 (0)	2 (6.3)	7 (5.8)
3	12	6 (20)	2 (10)	3 (13)	3 (20)	8 (25)	22 (18.3)
4	17	11 (36.7)	6 (30)	4 (17.4)	5 (33.3)	11 (34.4)	37 (30.8)
5	14	7 (23.3)	4 (20)	6 (26.1)	3 (20)	4 (12.5)	24 (20)
6	12	4 (13.3)	2 (10)	5 (21.7)	2 (13.3)	0 (0)	13 (10.8)
7	6	0 (0)	1 (5)	4 (17.4)	0 (0)	1 (3.13)	6 (5)
Total	65	30 (100)	18 (90)	22 (95.7)	13 (86.7)	26 (81.3)	109 (90.8)

and has been termed an emerging pathogen in gastroenteritis, and also found in breast abscess, blood, and placenta [6].

The observed resistance of the Campylobacter isolates in this study to ciprofloxacin (7.5%) is similar to 6.5% reported in human isolates [26]. This supports the fact that fluoroguinolones are one of the drugs of choice in the treatment of campylobacteriosis. However, the observed resistance is lower when compared with previously reported rates of 16% [19], 24.0% on commercially supplied layer chicken in South Africa [27], 50% in human (Canada) [28], and 55.9% in clinical isolates from Poland [29]. Similarly, resistance of C. coli (0%) and C. jejuni (8.0%) to ciprofloxacin in this study is contrary with the 71.4% and 65.2% reported for isolates from diarrhoea children [24]. Resistance to gentamycin (80.8%) was also observed to be higher compared to 18.2% [19], 13.2% [21] and 1.3% (26) previously reported. The reason for the disparity in resistance patterns is not clear but may be due to widespread use of the drugs in human and animals for prophylaxis and treatment of enteric infections as antibiotics are readily available for purchase in open market in

Nigeria where self-medication is also a common practise.

Furthermore, resistance of the isolates in this study to nalidixic acid (31.6%), cephalotin (60.8%) and chloramphenicol (73.3%) is generally lower compared to the reported resistance of the isolates recovered from the faeces and carcasses of healthy livestock which were in the range of 13.0%-69.0% (nalidixic acid), 97.0% -100% (cephalotin) and 83.0%-(chloramphenicol) [18]. 86.0% The high resistance reported from the latter study may be as a result of misuse of the antibiotics as growth promoters in the animal husbandry, and the fact that most Campylobacter species have inherent resistance to cephalothin [21]. However. resistance to ertapenem, amikacin, amoxicillin/clavulanic acid (augmentin) and nalidixic acid in this study was lower compared to the resistance that were previously reported to these antibiotics [22].

Furthermore, 85% of the *Campylobacter* isolates were found to show resistance to more than two antimicrobials. This is higher than the one from reports in previous studies [18, 21, 22]. This observation is an indication of multi-drug

resistant (MDR) strains which is of public health significance because, there are fewer or even sometimes no effective antimicrobial agents available for the control of infection caused by such strains. The high percentage of resistant strains and multiple drug resistant strains of *Campylobacter* spp. to most frequently used antibiotics may be due to uncontrolled use of antibiotics such as self-medication and access without prescription [21].

5. CONCLUSION

This study presents high prevalence of faecal carriage of multidrug resistance Campylobacter species from HIV/AIDS patients. This is a clear indication that these patients are at greater risk of campylobacteriosis; with gastroenteritis, septicemia and neutropenia complication, despite antimicrobial therapy. This study also found cases of asymptomatic campylobacteriosis HIV infected individuals. among which showed that they are reservoirs of antibiotic resistant Campylobacter strains which are of public health concern. Hence, screening of Campylobacter infection among those living with HIV and AIDS should be routinely carried out.

CONSENT AND ETHICAL APPROVAL

Ethical approval for this study was obtained from the University of Ibadan/University College Hospital (UI/UCH) Ethical Committee, with the approval number UI/EC/17/0072. The consents of the HIV patients were sought before sample collection, and all the samples were given laboratory codes without any traceable information of the patients on the sample containers to prevent disclosure of their identities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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