

Prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* in HIV patients in Ile-Ife, Osun State

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Abstract

Introduction: Diarrhoea is a clinical manifestation of HIV/AIDS infected people. These people have increased risk of Non-typhoidal *Salmonella* (NTS) and *Shigella* infections compared to immunocompetent people. The goal of this study is to determine the prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* in HIV Seropositive patients.

Methods: A total of 150 HIV patients attending Obafemi Awolowo University teaching hospital were enrolled in this study with age range of 18 – 70 years. Stool samples were collected and cultured on a selective medium for the isolation of *Salmonella* and *Shigella* strains. Isolates were subjected to antimicrobial susceptibility testing by disc diffusion method. Interpretation was done based on Clinical and Laboratory Standard Institute criteria.

Results: The prevalence of *Salmonella* and *Shigella* found in the stool samples examined were found to be 10(6.6%). *Salmonella* accounted for 2(1.3%). The species consisted of *Salmonella enterica* serovar Enteritidis (n=2). *Shigella* accounted for 8(5.3%). The species comprised of *S. flexneri* (n=7), *S. dysenteriae* (n=1). All *Salmonella* and *Shigella* strains were 100% resistant to Tetracycline and Ampicillin and 100% sensitive to Nitrofurantoin and Ciprofloxacin. It was observed that 90% of all the patients had a CD4 count >200 cells/mm³ as a result of the administration of antiretroviral therapy.

Conclusion: The high proportion of HIV patients who had diarrhoea in the absence of *Salmonella* and *Shigella* strongly indicate that other infectious agent are responsible for diarrhoea like *Campylobacter* or *Escherichia coli* especially Diarrhoeagenic *Escherichia coli*. Out of the antimicrobial drugs tested against the isolates, drugs of the class of Fluoroquinolones seemed to be the most effective.

Keywords: *Salmonella*, *Shigella*, Diarrhea, HIV/AIDS, CD4 count, antiretroviral drugs.

1. Introduction

HIV/AIDS is a global problem affecting both developing and developed countries. Globally, 35.0 million [33.2–37.2 million] people were living

with HIV at the end of 2013. Sub Saharan Africa remains most severely affected, with nearly 1 in every 20 adults living with HIV and accounting for

nearly 71% of the people living with HIV worldwide. In Nigeria, this disease has resulted to 210,000 estimated deaths in the year 2012[1]. HIV is now the leading cause of death worldwide in the age group of 15-24[2]. Worldwide, Nigeria has the second highest number of new infections reported each year, and an estimated 3.7 percent of the population are living with HIV[4, 5]. Approximately 210,000 people have died from AIDS in Nigeria in 2011[4].

Acquired immunodeficiency syndrome (AIDS) caused by HIV infection predispose individuals to several diarrhoeagenic bacterial diseases due to their low CD4 (Cluster derivative) count, bacteria like *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and diarrhoeagenic *Escherichia coli* [6, 7]. In addition to bacteria, viruses (Hepatitis B and C), parasites such as *Cryptosporidium parvum* and *Giardia lamblia* have been incriminated [8]. The pathogenesis of HIV/AIDS is that the primary target cell for HIV is the immune cells bearing CD4 marker at their surface. With the infectivity of HIV in the host, there is a gradual decrease of human immune cells bearing CD4 antigen receptor, the most important being T helper cells (CD4 T cells), B lymphocytes, macrophage and natural killer cells in fighting foreign antigens in the host [9].

Non – typhoidal salmonella (NTS) species are important food borne pathogens with acute gastroenteritis being the most common clinical manifestations [10]. As a larger proportions of patients have become immunocompromised either due to primary infections, malignancy or due to HIV/AIDS or terminal diseases appears to be on the increase [11,12]. Non-typhoidal salmonellae (NTS) are the commonest bacterial isolates from adult blood culture series in African countries where HIV prevalence is high [13,14].

In the past few years, there have been an increase in the occurrence of antimicrobial resistance, among *Salmonella* and *Shigella* has been observed in many countries, such as Asia, Africa and China that includes resistance to quinolones and third generation cephalosporin's [15]. The antimicrobial resistance of NTS has become a large threat to public health. The emergence of antimicrobial resistance and the transmission of resistant strains from animals to humans are associated with the nontherapeutic use of various classes of antimicrobials in large quantities in food animals. Recurrent episodes of NTS in HIV/AIDS population are mainly associated with *Salmonella enterica* serotype Enteritidis or *Salmonella enterica* serotype Typhimurium. The goal of this study is to determine the prevalence of NTS and *Shigella* HIV/AIDS patients attending Obafemi Awolowo University Teaching Hospitals Complex,

Ile Ife in Nigeria and to determine their susceptibility patterns.

2. Methods

This study was performed between July 2013 and September 2013. It involved HIV Seropositive patients admitted to Obafemi Awolowo University Teaching Hospital Complex, a public tertiary health care institution. A total of 150 patients were used for this study. This research was given ethical clearance by the Research Committee. A total of 150 stool samples from HIV/AIDS patients having diarrhoea were received and processed at the Department of Medical Microbiology and Parasitology of Obafemi Awolowo University during the study period. After taking verbal consent, they were interviewed to fill up pre structured questionnaire. The variables include socio demographic characteristics of the patient, age, sex, antiretroviral therapy drug history, clinical history.

2.1 Isolation of Stool Pathogens

The collected stool samples were cultured on MacConkey agar (Oxoid, UK) and Deoxycholate Citrate agar (Oxoid, UK) after inoculation with a sterile wire loop. All inoculated plates were incubated at 37°C for 24 hours. Organisms were identified physically on the plate as whether they are lactose fermenter or non-lactose fermenter before subjected to further biochemical tests. The isolates were subjected to susceptibility testing by disc diffusion technique described by Kirby-Bauer according to the Clinical Laboratory Standards International (CLSI) guidelines with quality controls [16]. The antimicrobial tested against isolates included Ampicillin (10µg), Nitrofurantoin (300µg), Nalidixic acid (30µg), Ciprofloxacin (5µg), Streptomycin (10µg), Tetracycline (10 µg) and Cotrimoxazole (10µg) and Gentamycin (30µg). Antimicrobial sensitivity patterns were determined on the basis of the size of the zone of inhibition and were interpreted at sensitive, intermediate or resistant.

3. Results

A total of 150 HIV seropositive patients having diarrhea participated in this study. The age group of the participants ranged from 18 – 70. Age group 40 – 50 had the highest prevalence of *Salmonella* while age group of 51 – 61 had the highest prevalence of *Shigella* (Table 1). Out of the 150 study participants, 29 (19.3%) were males and 121 (80.7%) were females resulting in an overall male to female ratio 4.2:1.

Table 1: Frequency distribution of bacterial pathogens isolated from fecal sample of HIV seropositive among age groups.

Age Group	Salmonella (n=2)		Shigella (n=8)	
	+VE	-VE	+VE	-VE
18 – 28	0(0%)	11(100%)	0(0%)	11(100%)
29 – 39	0(0%)	63(100%)	4(6.4%)	59(93.6%)
40 – 50	2(4.3%)	45(95.7%)	1(2.3)	42(97.7%)
51 – 61	0(0%)	19(100%)	3(15.8%)	16(84.2%)
62 – 72	0(0%)	10(100%)	0(0%)	10(100%)
Total	2(1.3)	148(98.7%)	8(5.3%)	142(94.7%)

Table 2: Frequency distribution of bacterial pathogens isolated from fecal sample of HIV seropositive among sex.

Sex	Salmonella (n=2)		Shigella (n=8)	
	+VE	-VE	+VE	-VE
Male	1(3.4%)	28(96.6%)	6(20.7%)	23(79.3%)
Female	1(0.8%)	120(99.2%)	2(1.7%)	119(98.3%)
Total	2(1.3%)	148(98.7)	8(5.3%)	142(94.7%)

The frequency distribution of *Salmonella* and *Shigella* among different occupations showed the prevalence of salmonella to be 2(1.3%); one was a trader and the other was a civil servant. The prevalence of *Shigella* was high in artisans followed by traders (Table 3)

Table 3: Frequency distribution pathogens isolated from fecal sample of HIV seropositive across occupations

Occupation	Salmonella (n=2)		Shigella (n=8)	
	+VE	-VE	+VE	-VE
Farmer	0(0%)	8(100%)	0(0%)	8(100%)
Trader	1(1.3%)	75(98.7%)	5(6.6%)	71(93.4%)
Civil Servant	1(3.8%)	25(96.2%)	0(0%)	26(100%)
Student	0(0%)	2(100%)	0(0%)	2(100%)
Artisan	0(0%)	30(100%)	3(10%)	27(90%)
Retiree	0(0%)	5(100%)	0(0%)	5(100%)
No Job	0(0%)	3(100%)	0(0%)	3(100%)
Total	2(1.3%)	148(98.7%)	8(5.3%)	142(94.7%)

Table 4: Frequency of pathogens isolated from fecal sample of HIV seropositive.

Organism isolated	Frequency Distribution		Total
	+ve	-ve	
Shigella	8(5.3%)	142(94.7%)	150
Salmonella	2(1.3%)	148(98.7%)	150

Clinical data from the ten cases who had *Salmonella* (n=2) and *Shigella* (n=8) revealed that there were more infections in females compared to males. The age group infected ranged from 33-60, with the mean age of 46.5. Our data also revealed 90% of the infected patients CD4 count level were >200 cells/mm³.

Table 5: Clinical data from the ten reported cases of *Salmonella* and *Shigella* infections in HIV patients

Case No	Age	Sex	Source of Organism	Organism isolated	ART	CD4 cell count
1	39	F	Stool	<i>Shigella flexneri</i>	Nil	252
2	50	F	Stool	<i>Salmonella enterica</i> serovar Enteritidis	Zidovudine	665
3	37	F	Stool	<i>Shigella flexneri</i>	Zidovudine	562
4	60	F	Stool	<i>Shigella flexneri</i>	Zidovudine, Zidovudine	390
5	42	M	Stool	<i>Shigella flexneri</i>	Lamivudine, Efavirenz	400
6	58	F	Stool	<i>Shigella flexneri</i>	Zidovudine	420
7	33	F	Stool	<i>Shigella flexneri</i>	Zidovudine, Zidovudine,	1015
8	57	F	Stool	<i>Shigella flexneri</i>	Lamivudine, Efavirenz	42
9	48	M	Stool	<i>Salmonella enterica</i> serovar Enteritidis	Zidovudine, Lamivudine, Efavirenz	538
10	36	M	Stool	<i>Shigella dysenteriae</i>	Lamivudine, Efavirenz	408

Table 6: Drug resistance pattern of bacteria isolated to the antibiotics.

	<i>Salmonella</i> (n=2)			<i>Shigella</i> (n=8)		
	R	I	S	R	I	S
Nitrofurantoin	0 (0%)	0(0%)	2(100%)	0(0%)	0(0%)	8(100%)
Nalidixic acid	2(100%)	0(0%)	0(0%)	4(50%)	2(25%)	2(25%)
Streptomycin	2(100%)	0(0%)	0(0%)	5(62.5%)	2(25%)	1(12.5%)
Tetracycline	2(100%)	0(0%)	0(0%)	8(100%)	0(0%)	0(0%)
Ampicillin	2(100%)	0(0%)	0(0%)	8(100%)	0(0%)	0(0%)
Co-trimoxazole	2(100%)	0(0%)	0(0%)	4(50%)	2(25%)	2(25%)
Gentamycin	1(50%)	1(50%)	0(0%)	3(37.5%)	2(25%)	3(37.5%)
Ciprofloxacin	0(0%)	0(0%)	2(100%)	0(0%)	0(0%)	8(100%)

R=resistant, I= intermediate, S= sensitive

4. Discussion

Opportunistic infections in HIV/AIDS have been on a rise in the recent years and it is a global challenge. We were interested in the prevalence of *Salmonella* and *Shigella* as an enteric pathogen responsible for diarrhea in immunocompromised patients. It is certain that these defects in both cellular and humoral immunity caused by HIV infection make these patients prone to salmonellosis and shigellosis among others. Our findings showed that the prevalence of *Salmonella* and *Shigella* recovered from the stool samples examined were found to be 10(6.6%). *Salmonella* and *Shigella* accounted for 2(1.3%) and 8(5.3%) respectively. The species of *Salmonella* consisted of *Salmonella enterica* serovar Enteritidis. Studies have documented that *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serotype Typhimurium are the most common serovar implicated in HIV/AIDS patients living in Sub Saharan African countries [11,17-21].

Shigella accounted for 8(5.3%). The species comprised of *S. flexneri* (n=7), *S. dysenteriae* (n=1). These findings were higher than Andualet *et al*[7] who reported a frequency of 3.5% isolation of *Shigella* isolates in HIV/AIDS Patients. HIV infection has several effects in the transmission of *Shigella*. Immunocompromised persons may have extended carriage of *Shigella* species and may have prolonged symptomatic or asymptomatic shedding at high titer. A high CD4 cell count was observed in 90% of the patients having shigellosis and salmonellosis; this can be because all are on antiretroviral drug except one. The age of HIV/AIDS patients having *Salmonella* and *Shigella* recovered from their stool samples ranged from 33- 60 years of age.

Females had higher number of shigellosis compared to males, this can be because they perform most household chores like fetching water from polluted streams or well water and as a result of that, drinking such polluted water from streams is inevitable. Our research showed a low prevalence of *Salmonella* and *Shigella* to be 1.3% and 5.3% respectively from HIV seropositive patients. We can conclude that diarrhoea must have been as a result of other diarrhogenic pathogens. Padmavathy *et al*[22] and Gassama-Sow *et al*[23] have reported high cases *Escherichia coli* in HIV Seropositive patients, Lehman *et al*[8] and Sarkar *et al*[24] have recovered *Candida albicans* recovered from stool of HIV patients, Lehman *et al*[8] also recovered high prevalence of *Cryptosporidium parvum* in patients too.

Salmonella and *Shigella* showed a 100% resistance to Ampicillin and Tetracycline in this

study. This work correlates with Andualet *et al.* 2006 who found 100% resistance to *Shigella* and 55% resistance to Co-trimoxazole, very similar to our result of 50%. Morapeth *et al*[25] has also reported high antimicrobial resistance to ampicillin, co-trimoxazole, and chloramphenicol among Non-Typhi *Salmonella* strains. The high rate of resistance of these conventional antibiotics used in our hospitals to combat infections are due to the extensive and abuse use of antibiotics for the treatment of diarrhea and other diseases. Self-medication has a major role to play for this increasing trend of resistance to antibiotics because patients do not know whether the sources of the infection are not caused by bacteria but viruses or parasites. However, *Salmonella* and *Shigella* has 100% sensitivity to ciprofloxacin and nitrofurantoin, a Fluoroquinolone. The rising trend is the increasing resistance of *Salmonella* and *E.coli* to third generation cephalosporin's and Fluoroquinolones.

5. Conclusion

In this study, our results showed a low prevalence rate of salmonella and Shigella in HIV patients who had diarrhoea. This strongly indicates that other infectious agents are responsible for diarrhoea which may be caused by parasites, fungi, viral or other bacteria like *Campylobacter* or *E. coli* especially Diarrhoeagenic *E. coli*. Out of the antimicrobial drugs tested against the isolates, drugs of the class of Fluoroquinolones seemed to be the most effective.

Competing interests

The authors have no competing interests to declare.

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