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Research Article

Prevalence of Non-Typhoidal Salmonella and Aerobic Bacteria Causing Bacteraemia among HIV/AIDS Seropositive Patients Attending Aminu Kano Teaching Hospital, Kano State

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ABSTRACT

The study aimed at determining the prevalence of non-typhoidal *Salmonella* and aerobic bacteria causing bacteraemia among HIV/AIDS seropositive patients attending Aminu Kano Teaching Hospital, Kano, the relationship between the CD4 cell counts and prevalence of these bacterial infections among the study population. Two hundred and sixty (260) HIV patients of 77 (29.6%) males and 183 (70.4%) females of 22-75 years were enrolled for the study. Socio-demographic and clinical history were obtained. The CD4 cell counts were enumerated using Cytoflometer. Bacteriological blood culture, isolation, identification and characterization of isolates were carried out using standard microbiological methods. The Salmonella isolates identified were further subjected to biochemical reactions using Enterosystem 18R test kits. Data were analysed using Chi-squared method. P-value < 0.05 was taken as the level of significance. The overall prevalence of Non-typhoidal *Salmonella* (NTS) was 5.7% (n=2) with male and female having the same prevalence of 1 (2.9%) each. Other bacteria isolated were *Staphylococcus aureus* 19 (54.1%), *Klebsiella* spp 6(17.1%), *Escherichia coli* 3(8.6%), *Pseudomonas aeruginosa* 3(8.6%), *Salmonella typhi* 2(5.7%). *Staphylococcus aureus* had good sensitivities to vancomycin, chloramphenicol, ciprofloxacin and amikacin but were resistant mainly erythromycin (68%). The Gram-negative organisms isolated also showed 100% resistance to ampicillin, chloramphenicol, colistin and trimethoprim sulfamethoxazole while being highly sensitive to meropenem, amikacin, and ciprofloxacin .Though the association of NTS and bacteraemia among HIV infected patients was low in this study (13.5%), there is need for increased public enlightenment on the subject-matter to limit the spread of this infectious agents to reduce associated morbidity and mortality.

Keywords: Prevalence; Non-Typhoidal *Salmonella*; Aerobic Bacteria; Bacteraemia; HIV/AIDS; Seropositive Patients; Aminu Kano Teaching Hospital

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INTRODUCTION

Bacteraemia constitutes a significant public-health problem that constitutes an important cause of morbidity and mortality in HIV-infected patients. Despite the significant reduction in AIDS-related deaths and opportunistic infection rate after the introduction of combined antiretroviral therapy (cART), infection with the human immunodeficiency

virus (HIV) remains a cause of increased risk of bloodstream infection (BSI) (Huson *et al*., 2014). The genus *Salmonella* belongs to the family Enterobacteriaceae while Non-typhoidal *Salmonella* (NTS) include all *Salmonella enterica* spp. except for *S*. *enterica* serovar Typhi, Paratyphi A, Paratyphi B, and Paratyphi C (Shahunja *et al.*, 2015). Almost all the serotypes of Salmonella can cause bacteraemia, while *S. dublin and S. choleraesuis* are the two invasive NTS strains that are highly associated with the manifestations of bacteraemia (Shu-Kee *et al.,* 2015).

Bacteria commonly isolated in blood cultures of patients include *Acinetobacter spp., Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae* among Gram-negative bacteria and *Staphylococcus aureus,* coagulase-negative staphylococci (*CoNS),* Enterococci and alpha-hemolytic Streptococci among Gram-positive bacteria (Debananda *et al.*, 2015). Non-typhoidal *Salmonella* (NTS) are important bacterial foodborne pathogens that cause gastroenteritis. The clinical presentation does not have a clear relationship to the $CD4^+$ cell count and neither patient's $CD4^+$ cell count nor bacterial strain properties necessarily predicted the clinical presentation of HIV/AIDS patients with NTS infection (Michael *et al.,* 2012).

In Africa and most other developing regions, multidrug resistance, particularly to commonly available antibiotics, remains a major challenge for the healthcare system (Ogunleye and Carlson, 2011), and multidrug-resistant NTS have caused life-threatening invasive disease outbreaks in many African countries, including Nigeria (Kingsley *et al*., 2009; Feasey *et al.,* 2012). Although NTS infections have been widely studied in medical literature, few studies have focused specifically on NTS bacteraemia. There is lack of information regarding the epidemiologic profile, antibiotic susceptibility pattern associated with NTS bacteraemia serogroups and data on its prevalence are limited especially among HIV patients, making it difficult to estimate the true burden of the disease in Kano State.

Salmonella are separated into two species, *Salmonella enterica* and *Salmonella bongori*, with the former being further classified into six subspecies (I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*) (*Brenneret al*., 2000). Within the seven subspecies, more than 2,500 serotypes (or serovars) have been reported (Tindal *et al*., 2005). A serovar or serotype is a grouping of microorganisms (or viruses) based on their cell surface antigens, allowing differentiation below the level of species.

Salmonellae causing human disease are traditionally divided into a small number of human-restricted invasive typhoidal serotypes (e.g, *Salmonella enterica*var Typhi [*S.* typhi] and *Salmonella enterica* var Paratyphi A [*S*Paratyphi A]) and thousands of non-typhoidal salmonella serotypes [commonly known as NTS serotypes]), which typically have a broad vertebrate host range and cause various presentations that usually include diarrhoeal disease (Feasey *et al.*, 2012).

Salmonellae can be presumptively identified biochemically using KIA (Kligler iron agar) medium and individual biochemical tests or commercially produced enterobacteria identification systems. Among the commercially available identification systems, Enterosystem 18R test kit which relies on biochemical substrate utilization set to identify the members of oxidase-negative Enterobacteriaceae, has often been used for identification of members of the family Enterbacteriaceae. Commonly used biochemical tests for the identification of *Salmonella* and their results are as follows (Cheesbrough, 2000; Tuhin *et al.*, 2013); NTS are differentiated from *S. typh i*based on the following biochemical reactions: ornithine decarboxylase (ODC), citrate (CIT), rhamnose (RHA), and arabinose (ARA) Negative with Weak H_2S production. While *S. Paratyphi*are differentiated from NTS based on the following biochemical reactions: lysine decarboxylase (LDC), citrate (CIT), H_2S, and melibiose (MEL) negative and arginine dihydrolase (ADH) positive (Bopp *et al.,* 2003; Bola and Oluyege, 2015).

Salmonella bacteraemia is a condition whereby the bacteria enter the bloodstream after invading the intestinal barrier. The clinical manifestation of bacteraemia is more commonly seen in NTS infections than in typhoid Salmonella infections. For non-typhoidal salmonellosis, the infectious dose is approximately 10^3 bacilli (Arciniega, 2017). In infants and persons with certain underlying conditions, a smaller inoculum can produce diseases, so that direct person-to-person transmission, although uncommon, sometimes occurs (Chen *et al.,* 2013). To be fully pathogenic, salmonellae must possess a variety of attributes called virulence factors. These include (1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). The severity of the infection and whether it remains localized in the intestine or disseminates to the bloodstream may depend on the resistance of the

patient and the virulence of the Salmonella isolate (Atek *et al.,* 2017).

Infection due to NTS has several clinical presentations ranging in severity from self-limited enteritis to fatal septicemia. One model for NTS infection in HIV/AIDS patients is that gastrointestinal infection, if left untreated, will disseminate. In this model, *Salmonella* infection begins in the intestinal tract, and local immune responses in healthy hosts are generally able to contain the disease (Michael *et al.*, 2012). However, in the very young or in patients with immunosuppression, the infection typically spreads beyond the gastrointestinal tract and results in bacteraemia (Michael *et al.*, 2012). Invasive NTS (iNTS) infections, which are often associated with patients with immunodeficiency, disease more closely resembles enteric fever in that patients often suffer from high fever, hepatosplenomegaly, and have respiratory complications with intestinal symptoms often being absent (Ohad *et al*., 2014). The main risk factor in adults is undoubtedly advanced HIV infection. So-called primary non-typhoidal salmonella bacteraemia, without associated diarrhoea, occurs in at-risk groups—such as patients who are immunosuppressed because of HIV infection, steroid use, malignancy, chronic renal or liver disease, diabetes, or sickle-cell disease, and elderly and newborn patients (Feasey*et al.*, 2012).

The incidence of NTS is highest during and after the rainy season in tropical climates and during the warmer months in temperate climates. Adults in their third or fourth decade are at greatest risk (Feasey *et al.,* 2012). The prevalence rate of NTS bacteraemia among HIV infected patients is 31.9% in Thailand (Kiratisin, 2008). A previous systematic review and meta-analysis indicated a high prevalence (19%) of -invasive non-typhoid *Salmonella* infection among the community-acquired bloodstream infections in Africa (Reddy *et al.*, 2010).

MATERIALS AND METHODS

Study Area

Aminu Kano Teaching Hospital, Kano is a tertiary hospital in Tarauni Local Government Area (L.G.A) of Kano State. It is a 500 bed-capacity hospital with 17 departments including Professor SS Wali Virology Center. The department runs outpatient services daily and the clinic starts from 9 a.m to 4 p.m from Mondays to Fridays. The patients cut across all socio-economic, age, and sex. The average daily patients' attendance at the SS Wali virology center is 250.

Study Population

The study consists of HIV patients attending Professor S.S Wali Clinic of AKTH during all clinic sessions.

Inclusion and Exclusion Criteria

All registered and consenting HIV patients diagnosed by clinicians of having pyrexia (temperature ≥37.4 ^{o}C) for up to five days with any of the following symptoms: vomiting, headache, diarrhoea, loss of appetite, abdominal pain, and nausea. Non-consenting and patients without the above clinical presentations were excluded from the study.

Study Design

The study was a cross sectional descriptive and hospital-based.

Ethical Consideration

Ethical approval was obtained from the medical research ethics committee of Aminu Kano Teaching Hospital, (AKTH) Kano before the commencement of the study.

Sample Collection

Blood sample was collected aseptically at the time of study at Aminu Kano Teaching Hospital (AKTH). The venipuncture site was disinfected with 70% alcohol before collecting approximately 5ml of blood from the patient (Solayide*et al.*, 2017), which was dispensed into appropriately labelled screw capped containers.

Laboratory Analysis of Samples

All samples collected from patients were analyzed for blood culture, and $CD4^{+}$cell count as described below.

Processing and Culture of Blood Samples

Approximately 3ml of blood sample from the patient was inoculated aseptically into blood culture bottle containing approximately 7ml of Brain Heart Infusion (BHI) Medium. The blood and the BHI were mixed at once to prevent clotting. The bottle containing the blood and the BHI was labelled appropriately and incubated at 37^{0} C examined daily for 7days until the appearance of turbidity, haemolysis, gas bubble production or clot formation, which signifies a positive result for bacterial growth (Cheesbrough, 2000).

Both aliquots of positive and non-positive bottles were Gram stained and subcultured on Blood agar, MacConkey agar, Chocolate agar and Salmonella Shigella agar (SSA) after collection under aseptic condition. All the inoculated plates were incubated

aerobically, both at 37^0C for 18-26hrs as described by Clinical and Laboratory Standard Institute (CLSI, 2012).

Identification and Characterization of Isolated Bacteria

All inoculated plates that shows homogeneous colony appearance were further identified and characterize. Gram staining techniques was performed on heat-fixed smears prepared from the colonies growing on culture plates following standard bacteriological protocol. *Salmonella* species were further identified as Gram negative short motile with colonial appearance as grey white on the blood agar and as a nonlactose fermenters, transparent colorless colonies, with or without black centers on SSA.

The *Salmonella* isolates initially identified as above were subjected to biochemical reactions using Enterosystem 18R test kits by (Liofilchem Company (US) according to manufacturer's instructions. The isolates were identified by the pattern of biochemical reactions such as catalase, coagulase, indole, urease, citrate utilization, and oxidase tests using standard procedures (Tille and Forbes, 2014). Members of the family Enterobacteriaceae and other Gram-negative bacteria were identified by indole, urease, citrate utilization, and oxidase tests while for the detection of Gram-positive bacteria, the following tests were used: catalase and coagulase.

Gram's Staining was carried out according to standard procedure

Biochemical Tests

The biochemical tests were carried out to further identify the isolated organisms include:

Catalase test, Slide Coagulase test Indole test, Citrate Utilization test, Oxidase test and Urease test respectively.

Antimicrobial susceptibility testing (AST) of Bacterial Isolates

Antimicrobial susceptibilities were determined using Kirby-Bauer modified disk diffusion technique on Mueller Hinton agar with antibiotic impregnated disks. The antibiotics tested on Gram-positive cocci included ampicillin (10µg), vancomycin (30µg), amikacin (30µg), chloramphenicol (30µg), cefoxitin (10µg), erythromycin (10µg), and ciprofloxacin (5µg). The antibiotics tested on Gram-negative include ceftriaxone (30µg**),** ceftazidime (10µg), cefuroxime (30µg), meropenem (10µg), amikacin (10µg), ciprofloxacin (5µg), ampicillin (10µg), chloramphenicol (30µg) and trimethoprim/sulphamethoxazole (25µg), piperacillin (100µg), and colistin (10µg). Ampicillin, trimethoprim/sulfamethoxazole, cefuroxime, ceftazidime, ciprofloxacin and amikacin were defined as clinically important antimicrobials (Varma *et al.,* 2005). Multi-drug resistance (MDR) was defined as resistance to three or more classes of antimicrobials (Magiorakos *et al.,* 2012). Inocula of bacteria were prepared with a turbidity equivalent to McFarland 0.5 standard (1.5×10^8 CFU/ml) and tested against all the aforementioned antibiotic discs. A sterile cotton swabs stick was dipped into the bacteria suspension and the excess suspension was removed by gentle rotation of the swab against the surface of the tube, the swab was then used to streak the MH agar after which the antibiotic disc was placed on the surface of MH agar plate with the help of sterile forceps and incubated at 37^0C for 24 hours. A quality control check of each of the antibiotic disk was perform on the American Type Culture Collection (ATCC) strain of each of the isolates. Antimicrobial activity was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter using a calibrated scale, which was compared with the standard value of zone of inhibition of each antibiotic. The diameter of the inhibition zone was interpreted as resistant, intermediate or susceptible according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012) (Appendix VIII).

$CD4^+$ T Cell Count Test

The CD4 cell measurement was carried out using the flow cytometry technique. Each reagent tube was labelled with patient's identification number. The tubes were then vortexed upside down for six seconds and upright for six seconds. Each reagent tube was opened, the EDTA tube was inverted about seven to ten times to ensure that the whole was adequately mixed, 2ml of whole blood was pipetted into each reagent tube labelled with the corresponding patient identification number. The tubes were capped and vortexed upright for six seconds after which they were incubated at room temperature for 1 hour in a dark place to protect the reagents from light.

After the period of incubation, each sample tube was uncapped and 2ml of fixative (5% formaldehyde in phosphate buffered saline [PBS]) was added into each tube. The tubes were recapped and each tube vortexed upright for six seconds. The patient's identification number was entered into the machine, the sample tubes were placed in the machine and the RUN button was pressed. A software message

indicated when each analysis was complete. The result was displayed on the screen.

Data Analysis

Statistical Package for Social Sciences SPSS version 26 (2018, IBM corp) was used for data analysis. The prevalence of non-tyhoidal salmonella and other aerobic bacteria was expressed in simple proportions and percentages for the study groups. Chi-square was used to determine the relationship between prevalence of NTS and demographic characteristics including CD4 cell counts and to compare the percentage of multi-drug resistant isolates in a given serotype. A *P*-value <0.05 will be considered as statistically significant.

RESULTS

Thirty five (13.5%) of the entire study population (260) show positive cultures for bacteria NTS showed prevalence rate of 2 (5.7%) out of the total samples that are positive for bacteraemia, of which both were *Salmonella choleraesuis*. Other isolates were *Staphylococcus aureus,* 19(54.1%), *Klebsiella spp.*, 6(17.1%), *Escherichia coli,* 3 (8.6%), *Pseudomonas aeruginosa,* 3(8.6%), and *Salmonella typhi,* 2 (5.7%). Altogether, 35 patients; 30(85.7%) females and 5(14.3%) males—had bloodstream bacterial infections

Seventy-seven 77(29.6%) of the study population were males while 183 (70.4%) were females, out of which 5(1.9%) males and 30 (11.5%) females were cultured positive for bacterial infection.

The prevalence of the NTS, aerobic bacterial isolates and the gender of the patients screened showed no statistical significant at (P>0.05; P=0.0622; df=5, x^2=10.5) The NTS is recorded in the age range of 41-50 years and prevalence of 2(5.7%).

Statistical analysis showed that there was no significant association (P>0.05; P=0.4052; df=20, x^2 =20.864) between the age of the patients and prevalence of NTS and other aerobic bacterial isolates (Table 5).

The result showed that, among the bacteria isolated, both the patients with NTS infection were on ART (anti-retroviral therapy). Statistically, there was no significant relationship (P>0.05; P=0.4189; df=5, x^2=4.9755) between ART status of the patients screened and the prevalence of NTS and other aerobic bacteria isolated. One of the patient's with NTS had a $CD4^+$T cell counts of 420 cells/µl and the other patient had a $CD4^+$T cell counts of 815 cells/µl. There was no significant relationship (P>0.05; P=0.6615; df=25, x^2=21.551) between the prevalence of NTS and other aerobic bacteria isolates and $CD4^+$T cell counts of the patients (Table 5). The result of this study showed that the ages of the participants ranged from 22–75 with the mean age of 40 ± 2 years. Approximately, 36.5% of the patients were between the ages 31 and 40 years. The distribution of NTS shows the preponderance (2, 0.8%)in the age group 41-50 years. There was no significant relationship (P> 0.05; P=0.568, df=5, X^2=3.872) between the age of patients and prevalence of NTS isolates. The distribution of NTS isolates according to gender showed that NTS was isolated in both sexes, having equal prevalence 1(0.4%). Statistically, there was no significant relationship (P> 0.05; P=0.523, df=1, X^2=0.411) between the distribution of NTS isolates and gender of the patients.

Most of the patients (182, 70%) were married followed by widows (44, 16.9%). Nineteen (7.3%) were single and (15, 5.8%) were divorcee. Non-typhoidal Salmonella was distributed among the married (1, 0.4%) and divorced patients (1, 0.4%). Statistical analysis showed that there was no significant relationship (P> 0.05; P=0.0592, df=3, X^2=7.438) between marital status and distribution of NTS isolates. A total of (126, 48.5%) of the Study population were traders making up the largest in the occupational status.

The prevalence of NTS among different occupations showed (2, 0.8%) of which both were traders. Seventeen (65%) of 36 patients positive for bloodstream infections belonged to this group. When statistically analyzed, there was no significant relationship (P> 0.05; P=0.709, df=4, X^2=2.143) between occupational status and distribution of NTS isolates. Eight three (40.1%) of the patients had tertiary education, (109, 41.9%) had secondary school education, (32, 12.3%) had primary school education while (36, 13.9%) had no formal education. Each of the NTS isolates was isolated from the blood sample of female with informal and male with tertiary education. There was no significant relationship (P> 0.05; P=0.362, df=3, X^2=3.202) between educational status and distribution of NTS isolate (Table 4).

Nineteen (19, 7.3%) patients had a $CD4^+$T cell count <200 cells/µl of blood while fifteen of the patient with $CD4^+$T cell count >1000 cells/µl were all females. Two of the patients with NTS had a $CD4^+$T cell counts within the range of 401-600 and 801-1000 cells/µl respectively. Statistically, there was no significant relationship (P> 0.05; P=0.745, df=5, X^2=2.711) between the distribution of NTS isolates and $CD4^+$T cell counts of the patients.

Among the (10, 3.9%) patients that were not on ART (also ART – nai've), four patients had a $CD4^+$T cell count <200 cells/µl with one female patient having $CD4^+$T cell count >1000 cells/µl. Both of the patients with NTS were on ART. Based on statistical analysis, there was no significant relationship (P> 0.05; P=0.777, df=1, X^2=0.081) between the distribution of NTS isolates and ART therapy of the patients.

One of the patient with NTS presented with GIT symptoms (vomiting and diarrhoea) while the other patient had only headache, both with the same temperature of 39.5^{o}C. Statistical analysis showed that, there was no significant relationship (P> 0.05; P=0.637, df=1, X^2=0.223) between the distribution of NTS isolates and GIT symptoms of the patients.

Of the 260 patients, (168, (68.6%) were on antibiotics. Clinical data from the two cases who had NTS (n=2) revealed that the patient's $CD4^+$T count was >400 cells/µl, and were not on any antibiotics at the time of sample collection. There was no significant relationship (P> 0.05; P=0.055, df=1, X^2=3.681) between the distribution of NTS isolates and antibiotic therapy of the patients (Table 5).

The two patients with NTS bacteraemia were found to be among the (250, 96.2%) patients that were on ART (also ART-experienced) and had a $CD4^+$T cell counts of 420 cells/µl and 815 cells/µl respectively. There was no any bacteria isolated from patients that were not ART-na'ive. There was no significant difference (P> 0.05; P=0.735, df=5, X^2=2.771) between the distribution of NTS isolates among ART patients and their $CD4^+$T Cell counts (Table 5).

Antimicrobial Sensitivity Pattern of NTS and Aerobic Bacterial Isolates from HIV Seropositive Patients

The result of antimicrobial sensitivity pattern of NTS and other aerobic bacteria isolates commonly used antimicrobial agents show that *Salmonella Typhi and* NTS demonstrated a good sensitivity (100%) to ceftriaxone, ceftazidime, cefuroxime, meropenem, amikacin, and ciprofloxacin. *S. Typhi* was fully sensitive to chloramphenicol. *Escherichia coli, Klebseila spp* and *Pseudomonas aeruginosa* were highly susceptible to meropenem, amikacin, chloramphenicol and ciprofloxacin. *Staphylococcus aureus also* demonstrated 100% susceptibility rates to amikacin, chloramphenicol, ciprofloxacin, and vancomycin (Table 6).

Antimicrobial Resistance Pattern of NTS and Aerobic Bacterial Isolates from HIV Seropositive Patients

Salmonella Typhi isolates were 100% resistant to only ampicillin, and trimethoprim/sulphamethoxazole (SXT) while NTS isolates shows 100% resistance to ampicillin, chloramphenicol and trimethoprim/sulphamethoxazole (SXT). *Escherichia coli* shows resistance rates of 33% to both ampicillin and ceftriaxone while *Klebseila spp s*hows a resistance rates of 100%, 33%, and 17% to ampicillin, ceftriaxone and chloramphenicol respectively and comparatively resistant to ampicillin (53%) and erythromycin (32%). *Pseudomonas aeruginosa* were 100% resistance ceftazidime and colistin (Table 7).

Table 1. Bacterial Isolates from Blood Culture of HIV Patients (n=260) Screened

Isolates	Number(%)of Isolates	Overall prevalence
Staphylococcus aureus	19 (54.1)	7.3
Escherichia coli	3 (8.6)	1.2
Klebsiellaspp	6 (17.1)	2.3
Pseudomonas aeruginosa	3 (8.6)	1.2
Salmonella Typhi	2 (5.7)	0.8
Salmonella Choleraesuis	2 (5.7)	0.8
Total	**35(100)**	**14**

Table 2. Prevalence of Aerobic Bacteria among HIV Seropositive Patients

Demographic Characteristics	***Escherichia coli* (n=3)**	***Klebsiella spp* (n=6)**	***Pseudomonas aeruginosa* (n=3)**	***Staphylococcus aureus* (n=19)**	***Salmonella typhi* (n=2)**	**P –value**
Age-group (years)						
21-30	1 (2.9)	2 (5.7)	0 (0)	4 (11.4)	0 (0)	
31-40	1 (2.9)	2 (5.7)	2 (5.7)	8 (22.6)	2 (5.7)	
41-50	0 (0)	2 (5.7)	1 (2.9)	6 (17.1)	0 (0)	
51-60	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	0.4544
61-70	0 (0)	0 (0)	0 (0)	1 (2.9)	0 (0)	
Total	**3 (8.6)**	**6 (17.1)**	**3 (8.6)**	**19 (54.1)**	**2 (5.7)**	
Sex						
Male	1 (2.9)	2 (5.7)	0 (0)	0 (0)	1 (2.9)	
Female	2 (5.7)	4 (11.4)	3 (8.6)	19 (54.1)	1 (2.9)	0.491
Total	**3 (8.6)**	**6 (17.1)**	**3 (8.6)**	**19 (54.1)**	**2 (5.7)**	
$CD4^+$(Cells/µL)						
<200	0 (0)	0 (0)	0 (0)	1 (2.9)	0 (0)	
201-400	2 (5.7)	4 (11.4)	1 (2.9)	5 (14.3)	0 (0)	
401-600	1 (2.9)	1 (2.9)	1 (2.9)	3 (8.6)	0 (0)	
601-800	0 (0)	1 (2.9)	1 (2.9)	5 (14.3)	1 (2.9)	0.00
801-1000	0 (0)	0 (0)	0 (0)	4 (11.4)	0 (0)	
>1000	0 (0)	0 (0)	0 (0)	1 (2.9)	1 (2.9)	
Total	**3 (8.6)**	**6 (17.1)**	**3 (8.6)**	**19 (54.1)**	**2 (5.7)**	
ART Status						
ART Nai’ve	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.00
ART Experienced	3 (8.6)	6 (17.1)	3 (8.6)	19 (54.1)	2 (5.7)	
Total	3 (8.6)	6 (17.1)	3 (8.6)	2 (5.7)	19 (54.1)	

Table 3. Prevalence of NTS among HIV Seropositive Patients

Demographic Characteristics	***S. choleraesuis* (n=2)**	**P-value**
Age-group (years)		
21-30	0 (0)	
31-40	0 (0)	
41-50	2 (5.7)	0.000
51-60	0 (0)	
61-70	0 (0)	
Total	**2 (5.7)**	
Sex		
Male	1 (2.9)	
Female	1 (2.9)	0.000
Total	**2 (5.7)**	
CD4^{+}(Cells/µL) <200		
201-400	0 (0)	
401-600	0 (0)	
601-800	1 (2.9)	
801-1000	0 (0)	0.000
>1000	1 (2.9)	
Total	**2 (5.7)**	
ART Status		
ART Nai’ve	0 (0)	
ART Experienced	2 (5.7)	0.000
Total	**2 (5.7)**	

Table 4. Demographic Characteristics and Distribution of NTS among HIV Patients Screened

Demographic Characteristics	Number (%) Tested	Number (%) Positive	P-value
Age-group (years)			
21-30	49 (18.9)	0 (0)	
31-40	95 (36.5)	0 (0)	
41-50	89 (34.2)	2 (0.8)	
51-60	16 (6.2)	0 (0)	0.568
61-70	8 (3.1)	0 (0)	
71-80	3 (1.2)	0 (0)	
Total	**260 (100)**	**2 (0.8)**	
Sex			
Male	77 (29.6)	1 (0.4)	
Female	182 (70.4)	1 (0.4)	0.526
Total	**260 (100)**	**2 (0.8)**	
Marital Status			
Single	19 (7.3)	0 (0)	
Married	182 (70.0)	1 (0.4)	
Widow	44 (16.9)	0 (0)	0.059
Divorce	15 (5.8)	1 (0.4)	
Total	**260 (100)**	**2 (0.8)**	
Occupation			
Housewife	71 (27.3)	0 (0)	
Civil Servant	49 (18.9)	0 (0)	
Student	10 (3.9)	0 (0)	
Trader	126 (48.5)	2 (0.8)	0.709
Others	4 (1.5)	0 (0)	
Total	**260 (100)**	**2 (0.8)**	
Educational Status			
Informal	36 (13.9)	1 (0.4)	
Primary	32 (12.3)	0 (0)	
Secondary	109 (41.9)	0 (0)	0.362
Tertiary	83 (40.0)	1 (0.4)	
Total	260 (100)	2 (0.8)	

Table 5. Clinical Characteristics and Distribution of NTS Isolates among HIV Patients Screened

Characteristics	Number Positive	(%)	Number Negative	(%)	Number Tested	(%)	P-value
GIT Symptoms							
Yes	1 (0.4)		170 (65.4)		171 (65.8)		
No	1 (0.4)		88 (33.8)		89 (34.2)		0.637
Total	**2 (0.8)**		**258 (99.2)**		**260 (100)**		
Use of Antibiotics							
Yes	0 (0)		168 (64.6)		168 (64.6)		
No	2 (0.8)		90 (34.6)		92 (35.4)		0.055
Total	**2 (0.8)**		**258 (99.2)**		**260 (100)**		
ART Status							
ART Nai’ve	0 (0)		10 (3.9)		10 (3.9)		

Table 6. Distribution of NTS among ART Patients Based on $CD4^{+}$T Cell Counts

CD4 Counts (Cell/µl)	No(%) of ART-Experienced patients	No (%) of ART-Na'ive	Number (%) Positive	P-value
<200	15 (5.8)	4 (1.5)	0 (0)	
201-400	60 (23.1)	1 (0.4)	0 (0)	
401-600	77 (29.6)	1 (0.4)	1 (0.4)	0.735
601-800	42 (16.1)	2 (0.8)	0 (0)	
801-1000	40 (15.4)	1 (0.4)	1 (0.4)	
>1000	16 (6.2)	1 (0.4)	0 (0)	
Total	**250 (96.2)**	**10 (3.9)**	**2 (0.8)**	

Table 7. Antimicrobial Sensitivity Patterns of Bacteria Isolated from HIV Seropositive Patients

Antibiotic (µg)	***Escherichia coli* (n=3)**	***Klebsiella spp* (n=6)**	***Pseudomonas aeruginosa* (n=3)**	***Salmonella typhi* (n=2)**	***S. choleraesuis* (n=2)**	***Staphylococcus aureus* (n=19)**
AMP (10)	2 (67)	0 (0)	_	0 (0)	0 (0)	9 (47)
CTR (30)	2 (67)	4 (67)	_	2(100)	2(100)	_
CAZ (10)	_	_	0 (0)	2(100)	2(100)	_
CRX (30)	_	_	_	2(100)	2(100)	_
MEM (10)	3 (100)	6 (100)	3 (100)	2(100)	1 (50)	_
AK (10)	3 (100)	6 (100)	3 (100)	2(100)	2(100)	19 (100)
SXT (25)	_	_	_	0 (0)	0 (0)	_
CIP (5)	3 (100)	6 (100)	_	2(100)	2(100)	19 (100)
PRL (100)	_	_	3 (100)	_	_	_
CT (10)	_	_	0 (0)	_	_	_
C (30)	3 (100)	5 (83)	3 (100)	2(100)	0 (0)	19 (100)
E (15)	_	_	_	_	_	13 (68)
FOX (30)	_	_	_	_	_	3 (16)
VA (30)	_	_	_	_	_	19 (100)

AMP, Ampicillin; AK, Amikacin; CTR, Ceftriaxone; CAZ, Ceftazidime; CRX, Cefuroxime; C, Chloramphenicol; CIP, Ciprofloxacin; SXT, Trimethoprim/ Sulfamethoxazole MEM, Meropenem; PRL, Piperacillin; CT, Colistin; VA, Vancomycin; E, Erythromycin; FOX, Cefoxitin.

Table 8. Antimicrobial Resistance Patterns of Bacteria Isolated from HIV Seropositive Patients

Antibiotic(µg)	***Escherichia coli* (n=3)**	**Klebseila spp (n=6)**	***Pseudomonas aeruginosa* (n=3)**	***Salmonella typhi* (n=2)**	***S. choleraesuis* (n=2)**	***Staphylococcus aureus* (n=19)**
AMP (10)	1 (33)	6 (100)	_	2 (100)	2 (100)	10 (53)
CTR (30)	1 (33)	2 (33)	_	0 (0)	0 (0)	_
CAZ (10)	_	_	3 (100)	0 (0)	0 (0)	_
CRX (30)	_	_	_	0 (0)	0 (0)	_
MEM (10)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	_
AK (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SXT (25)	_	_	_	2 (100)	2 (100)	_
CIP (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PRL (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	_
CT (10)	_	_	3 (100)	_	_	_
C (30)	0 (0)	1 (17)	0 (0)	0 (0)	2 (100)	0 (0)
E (15)	_	_	_	_	_	6 (32)
FOX (30)	_	_	_	_	_	16 (84)
VA (30)	_	_	_	_	_	0 (0)

AMP, Ampicillin; AK, Amikacin; CTR, Ceftriaxone; CAZ, Ceftazidime; CRX, Cefuroxime; C, Chloramphenicol; CIP, Ciprofloxacin; SXT, Trimethoprim/ Sulfamethoxazole MEM, Meropenem; PRL, Piperacillin; CT, Colistin; VA, Vancomycin; E, Erythromycin; FOX, Cefoxitin

DISCUSSION

In this study, the prevalence of NTS bacteraemia among HIV/AIDS patients was 5.7% (2 out 0f 260) which implies that, NTS is not common among HIV/AIDS patients. This result is lower than the prevalence of 23% observed in Lagos in a study conducted by Adeyemi *et al.* (2010). Likewise, in the study conducted by Dhaona and Fatt (2009) and Kruger *et al.* (2004) respectively a prevalence of 20% was reported.

Meanwhile, the prevalence of 5.7% obtained in this study is higher than the prevalence rate of 4.0% in a study conducted by Bola and Oluyege (2015) at Federal Medical Centre (FMC), IdoEkiti, Ekiti State, Nigeria and 2% by Ayele *et al.* (2017). The prevalence

from this study is also similar to that of Adisa *et al.* (2016) who published the prevalence of 5.3% from their study. Also, the low prevalence rate of NTS (5.7%) bacteraemia observed in this study, is similar to the study conducted by Atek *et al.* (2017) with 5% prevalence. The prevalence of NTS bacteraemia decreased significantly among patients who achieved favourable immune-virological response after receiving ART, a finding that is similar to other studies (Hung *et al.*, 1998).

In this study, the only serotype of NTS isolated was *S. choleraesuis.* This is similar to the findings conducted in Taiwan, where *S. choleraesuis* exhibits the highest degree of invasiveness (Chen, 1999). Other studies have also reported similar findings (Cohen *et al.,* 1987; Chen, 1999: Kiratisin, 2008). Kuan-Yeh*et al.,* 2016 proposed that *S. choleraesuis* shows high predilection to cause bacteraemia. *Salmonella enterica* serovar Typhimurium, the most common serotype isolated in Malawi (Gordon *et al.*, 2008) was not detected in this study. Shu-Kee*et al*. (2015) also reported that almost all the serotypes of Salmonella can cause bacteraemia, while S. Dublin and S. Choleraesuis are two invasive NTS strains that are highly associated with the manifestations of bacteraemia.

Among other bacteria, *S. aureus* was the most prevalent among gram positive bacilli (GPB) isolated in this study area. This has also been reported by other studies conducted in different areas that *S. aureus* was the most frequently isolated bacteria in bloodstream infection (Popovich *et al.*, 2008: Wasihun*et al.,* 2015: Patil and Dalal, 2016). Data from a study by Kempkerr*et al.* (2010) suggest an association of *S. aureus* with advanced age, black ethnicity and AIDS. *Klebseila* spp were the predominant isolated GNB with prevalence rate of (17.1%). This finding was comparable to study from Addis Ababa, Ethiopia where isolation rate of *Klebsiella* spp. and *E. coli* were (9.7%) and (8.1%) respectively (Kitila *et al.,* 2018). By contrast, *E. coli* and *P. aeruginosa* were the dominant species among Gram-negative pathogens reported in other studies (Ortega *et al.,* 2008*;* Taramasso e*t al.,* 2016).

The variations observed in these results could also be explained by the fact that Zidovudine has been documented to have anti-Salmonella activity in vitro (Casdo *et al.,* 1999). Several studies reported on the impact of ART on the incidence of community acquired bloodstream infections in HIV patients (Huson *et al*., 2014). All studies documented a decreased incidence of bacterial bloodstream infections in HIV patients after the introduction of ART. Other studies elsewhere in Gambia, Kenya and Tanzania have also reported reduction in invasive NTS (Scott *et al.,* 2011). This implies that highly active anti-retroviral therapy appears to reduce the incidence of bacteraemia in HIV-infected patients.

In this study, NTS bacteraemia occurred mainly among the age-group 40-50 which is in line with several studies (Kolo *et al*., 2015: Chiu *et al*., 2004). However, gender, age, educational, marital and occupational status of these patients did not play any significant role ($p>0.05$) in incidence of NTS bacteraemia in this study when analyzed statistically. The findings in this present study showed the presence of NTS bacteraemia and absence of gastroenteritis among the patients screened. One of the patient with NTS bacteraemia was not presented with diarrhea, which was also similar to most previously reported cases of NTS bacteraemia in adults which were described as having symptoms of gastroenteritis not developed at presentation (Shimoni*et al.,* 1999). Notably, in the African setting, the majority of invasive NTS cases do not have gastroenteritis (Matheson *et al.*, 2010; Feasey *et al.*, 2012).

Surprisingly, there was no clear relationship between CD4+ cell counts and the type of clinical presentation. In the present study, one of the patients had a high CD4+ cell counts (815 cells/µl) while the other had a low CD4+ cell counts (420 cells/µl). As such, patients with high or low CD4 + cell counts were present in each clinical group. The CD4 cell counts among HIV-infected patients shows no clear relationship between CD4+ cell counts and the NTS and this concurred with the findings of Michael *et al.* (2012). However, the majority of patients on ART had their CD4+ cell counts above 200 cells/µl threshold.

Results of antimicrobial susceptibility tests in this study, revealed that the NTS and *Salmonella* Typhi isolates were 100% susceptible to cephalosporin, ciprofloxacin and meropenem while being highly resistant to chloramphenicol, ampicillin and trimethoprim/sulphamethoxazole; this has been reported before (Scott *et al.*, 2011). In Malawi, epidemics of multidrug-resistant invasive non-typhoidal salmonella (defined as resistant to ampicillin, chloramphenicol, and co-trimoxazole) have been recorded (Gordon *et al.*, 2008: Reddy *et al.,* 2010). Other Gram-negative bacteria isolated in this study showed good sensitivity patterns to meropenem, ciprofloxacin, amikacin, and chloramphenicol, while showing a varying resistance to ampicillin, ceftriaxone, and colistin. The multi-drug resistance patterns observed in this study are similar to those earlier reported by Adeyemi *et al.* (2010).

Also, the antimicrobial susceptibility of the *Staphylococcus aureus* were susceptible to most antibiotics screened. Some of them were resistant to ampicillin, erythromycin and cefoxitin. This is in line with results of results of several studies (Shittu and Lin, 2006: Taiwo *et al.*, 2007). This is in contrast to results from a recent study in Nigeria which described nasal *S. aureus* strains from HIV-positive individuals that were resistant to methicillin (16%), chloramphenicol (47%), trimethoprim-sulfamethoxazole (90%) and ciprofloxacin (18%) (Olalekan *et al.,* 2012). Sixteen (84%) of the staphylococci aureus isolated in this study were methicillin resistant. Tumbarello *et al*. (2002) and Wood *et al.* (2009) in their study showed that almost half of the *S. aureus* isolates from the blood of HIV-infected patients were methicillin resistant. 2006). In contrast to these findings, no MRSA was identified in the study reported by Solayide*et al.* (2017).

CONCLUSION

The present study, thus, established that non-typhoidal Salmonella bloodstream infection is not a common occurrence in HIV-positive patients attending Aminu Kano Teaching Hospital as *S. choleraesuis* was the only serotype isolated. Other aerobic bacteria isolated were *Staphylococcus aureus, Klebsiella spp.*, *Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella Typhi.* The in vitro data of antimicrobial susceptibility showed that all the isolates were resistant to some readily available antimicrobials (including ampicillin, chloramphenicol, SXT) but sensitive to cephalosporins, penem and fluoroquinolones. The resistance rate to commonly used antibiotic was high. Patients' CD4 cell counts, and socio-demographic characteristics of the patients did not have any significant relationship with incidence of isolated bacteria in this study. This proves that the isolated bacteria can occur among different socio-demographic groups. The ART therapy of the patients has no any association with the incidence of bacteria isolated in this study. More so, most cases of NTS bacteraemia in adults did not have the presenting symptoms of gastroenteritis.

Based on the outcome of this study, there is need for continuous assessment of both in-patients and out-patients among HIV/AIDS patients for NTS infection to aid comprehensive knowledge about the prevalent serotypes with high invasive potential which will be of epidemiological and public health importance. Prospective studies that will focus on aggregate isolates from both stool and blood in a given population will be essential to fully understand the organism and to further test the hypothesis that nontyphoidal Salmonella is becoming adapted to human hosts in areas with high rates of HIV infection. The presence of high rate of MDR strains indicates the urgent need for continuous monitoring of antimicrobial resistance among NTS. This should be kept in mind when selecting empirical antibiotics for NTS in HIV-infected patients. The need for a continuous surveillance and intervention strategy should be put in place to manage cases and limit the spread of NTS infections in HIV-positive patients. Since there is no vaccine available for NTS infection in humans, public health education thus should remain the major approach for prevention of the disease. The importance of ART should be emphasized to all HIV positive cases including ART Nai've and ART experienced patients. HIV patients with low CD4+ cell counts should be educated on the need to adhere to treatment regimens and adequate nutritional supplements to boost their CD4 +T cell count.

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