ORIGINAL ARTICLE

Salmonella serovars associated with bacteraemia infection in persons infected with human immunodeficiency virus with low CD4$^+$ cell counts in Akwa Ibom State, Nigeria

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ABSTRACT

Background: Certain serovars of *Salmonella* show a much higher predilection for causing bacteraemia as opportunistic infections in human immunodeficiency virus (HIV) infected persons.

Objectives: The study was undertaken to characterize serovars of *Salmonella* isolated from bacteraemia infections in HIV/AIDS patients in Akwa Ibom State and to determine the relationship of the infection with the low CD4$^+$ cell counts of the subjects.

Materials and Methods: A cross-sectional descriptive epidemiological study was conducted among 300 HIV/AIDS patients and 105 non-HIV subjects. Blood culture samples were collected and cultured on *Salmonella-Shigella* agar (SSA) and Deoxycholate citrate agar (DCA) for recovery of *Salmonellae.* Isolates were identified using conventional tests and Microgen^{TM} GN ID System (Microgen Bioproducts). Serotyping was done using *Salmonella* antisera (Statens Serum Institut, Denmark). Estimation of CD4$^+$ cell counts was by Partec flow cytometry.

Results: *Salmonella species* and *Salmonella enterica arizonae* were identified. Seven serovars were obtained; *S. typhi, S. paratyphi* A, *S. paratyphi* B*, S. paratyphi* C, *S. typhimurium, S. cholerasius* and *S. enteritidis*. The prevalence of *Salmonella* associated bactereamia was significantly higher ($p<0.05$) in HIV persons with 13.7% in contrast to 3.8% non-HIV subjects. Regardless of the HIV/AIDS sero-status, subjects with CD4$^+$ counts below 200µL had the highest rates of *Salmonella* associated bacteraemia.

Conclusion: *Salmonella typhi, S. paratyphi* A, *S. paratyphi* B*, S. paratyphi* C, *S. typhimurium, S. cholerasius* and *S. enteritidis* are identified as serovars of *Salmonella* which are endemic in the study area and associated with bacteraemia infection with significantly higher occurrence in subjects with low CD4$^+$ counts compared to HIV seronegative subjects.

Keywords: *Salmonella*, Bacteraemia, HIV infection, CD4$^+$ counts.

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INTRODUCTION

Bacteraemia is defined as the presence of bacteria in the bloodstream (1). Bacteria can enter the bloodstream through severe complications of infections, dental procedures, catheterization, other foreign bodies entering the arteries and veins and during surgery involving the mucous membranes (2). Individuals with human immunodeficiency virus (HIV) infections are at greater risk of bacteraemia because of their defective immune status (3-5).

The rates of *Salmonella* invasive infections and mortality are higher in infants, elderly and people living with HIV and hemoglobinopathies (6). *Salmonella* species have emerged as a major public health problem in developing countries, particularly sub-Saharan Africa, including Nigeria (7,8). The increasing prevalence of people living with HIV/AIDS has increased the occurrence of both the typhoidal and non-typhoidal salmonellae in bacteraemia in sub-Saharan Africa (9, 10).

There are two species of *Salmonella* complex namely *Salmonella bongori* and *Salmonella enterica* (11). The species of *Salmonella enterica* has six subspecies with more than 2579 serovars currently identified (12). Certain serovars of *Salmonella* show a much higher predilection for causing bacteraemia and these serovars differ with geographic location (13, 14).

Infection with HIV is associated with a progressive decrease of CD4$^+$ T-Cells in the immune system, resulting in an increase in viral load. The normal CD4$^+$ T-Cell count for an adult is over 1,000 cells/µl (15). In HIV pathogenesis, the primary target is the cells bearing CD4$^+$ markers on their surfaces (16-18). Thus, CD4$^+$ cell count determination is the best validated predictor of the likelihood of developing series of opportunistic infections as well as an important parameter to initiate prophylaxis that helps to reduce the risk and severity which would have resulted in death of the patients (19, 20). Moreover, screening for opportunistic infections is an aspect of care and support to these immunosuppressed persons (18).

Therefore, this study aimed at screening and identifying *Salmonella* serovars causing bacteraemia in HIV/AIDS patients in the study area and to determine the relationship between CD4$^+$cell counts and the distribution of *Salmonella* infections in HIV-infected persons.

MATERIALS AND METHODS

Study Design

This research work is a cross-sectional descriptive study undertaken to isolate and characterize *Salmonella* serovars associated with bacteraemia infection in persons infected with HIV/AIDS patients with CD4$^+$ cell counts of less than 1,000 cells /µL (15), in Akwa Ibom State, Nigeria.

Ethical approval

Approval for the work was obtained from the Ethical Committee of the University of Uyo Teaching Hospital (UUTH), Akwa Ibom State, Nigeria. Consents were also sought from the subjects who were willing to volunteer. Consents for minors (below 18 years) were obtained from the parents and guardians.

Collection of samples

Fresh blood samples for culture were collected from a total of 300 confirmed HIV/AIDS infected persons and 105 HIV negative people (control subjects) control who consented to be enrolled into the study.

Inoculation of Samples

Five millilitres (5mL) of blood sample was collected intravenously from each patient and 1mL of the blood was dispensed into blood cultured bottles containing 5mL tryptone soy broth medium and was incubated at 37^{o}C for 48 hours in a standard incubator (DNP-9052 Laboratory incubator, TCA, England). Subculture was made on *Salmonella-Shigella* agar (SSA) plates, while the remaining 4mL was dispensed into blood sample bottles containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant for HIV test and for CD4$^+$ cell estimation.

Identification of isolates

All purified colonies were identified using the different biochemical tests (15,21,22) and Microgen^{TM} GN ID System (Microgen Bioproducts, USA), an identification system for all currently recognized Enterobactereriaceae were used to confirm the species of *Salmonella* isolates obtained in the study.

Serotyping of *Salmonella*

Serotyping of *Salmonella* isolates was carried out using *Salmonella* antisera and monoclonal antibodies for serological confirmation. Polyvalent "H" (poly A –E + Vi) antisera and "O" (O:2, O:4, O:7, O:9) antisera (Statens Serum Institut, Denmark) were used.

Estimation of $CD4^+$ cell counts

Estimation of $CD4^+$ cell counts was conducted using Partec flow cytometry method. The analysis involves the use of cytoflow beads with a Partec flow cytometry counter machine. Flow cytometry uses laser to excite fluorescent antibody, probes specifically for $CD4^+$ and other cell surface markers, to distinguish one type of lymphocyte from another.

The method involves measuring 20µL of fresh whole EDTA blood (0.02mL) and this quantity of blood was added to 20µL of $CD4^+$/MAb (0.02mL of counting beads) in a test tube and incubated in the dark at room temperature of 37°C for 15 minutes after which, eight hundred microlitres (800µL/0.8mL) of phosphate buffered saline was added to the blood sample and run on the cytometry machine. Thereafter, the machine automatically generated the report of the quantity of the red blood cells present and the number of $CD4^+$ in the cells.

Statistical analysis

Percentages were employed to express the prevalence and the frequency of occurrences of the infection. Analysis of Variance (ANOVA) was used to determine the significant level of *Salmonella* associated infections in HIV sero-positive subjects and to determine the role between the infection and $CD4^+$cell counts of the subjects studied.

RESULTS

Prevalence of *Salmonella b*acteraemia among HIV/AIDS and the controls

Prevalence of *Salmonella b*acteraemia among HIV/AIDS and the control subjects is presented on Figure 1. The results revealed that of the 300 HIV/AIDS patients studied, a total of 41 (13.7%) had *Salmonella* associated bacteraemia while 4 (3.8%) of the blood samples of the 105 control subjects yielded *Salmonella* species. The prevalence in the test group differed significantly from that in the controls ($p<0.05$). The results of biochemical characterizations and Microgen™ GN ID System identification of the isolates are presented on Table 1. The isolates were identified as *Salmonella enterica* species and *Salmonella enterica Arizonae* respectively. The biochemical reaction results obtained from the biochemical tests carried out on the test isolates are shown in Table 2.

Table 3 shows serotyping results of *Salmonella* isolates obtained in the study. *Salmonella* isolates agglutinated the polyvalent H antisera used as genus specific antisera. Further, Results from agglutination of O2, O4, O7, O9 and Vi antisera further identified 7 different serovars including *S. typhi, S. paratyphi* A, *S. paratyphi* B, *S.* p*aratyphi* C, *S. typhimurium, S. choleraesius,* and *S. enteritidis*.

The recovery rates of different serovars of *Salmonella* isolated from blood cultures of HIV/AIDS subjects and the control subjects are presented on Table 4. A total of 58 *Salmonella* isolates agglutinated the polyvalent H antisera used as genus specific antisera. These isolates were obtained from 41 HIV /AIDS patients and 4 control subjects. Some subjects had more than one serovar of *Salmonella. Salmonella typhi* was the most commonly observed *Salmonella* serovars identified from the samples in our study, making up 32% (17/53) of the *Salmonella* isolates in HIV/AIDS patients studied, and 40% (2/5) from the control subjects.

It was observed that irrespective of the HIV/AIDS status, subjects with low $CD4^+$ cell counts of ≤ 200µL had higher percentages *Salmonella* associated bacteremia (Table 5). Data analysis showed a significant relationship ($p< 0.05$) between $CD4^+$ cell counts of HIV/AIDS subjects and the prevalence of *Salmonella* bacteraemia.

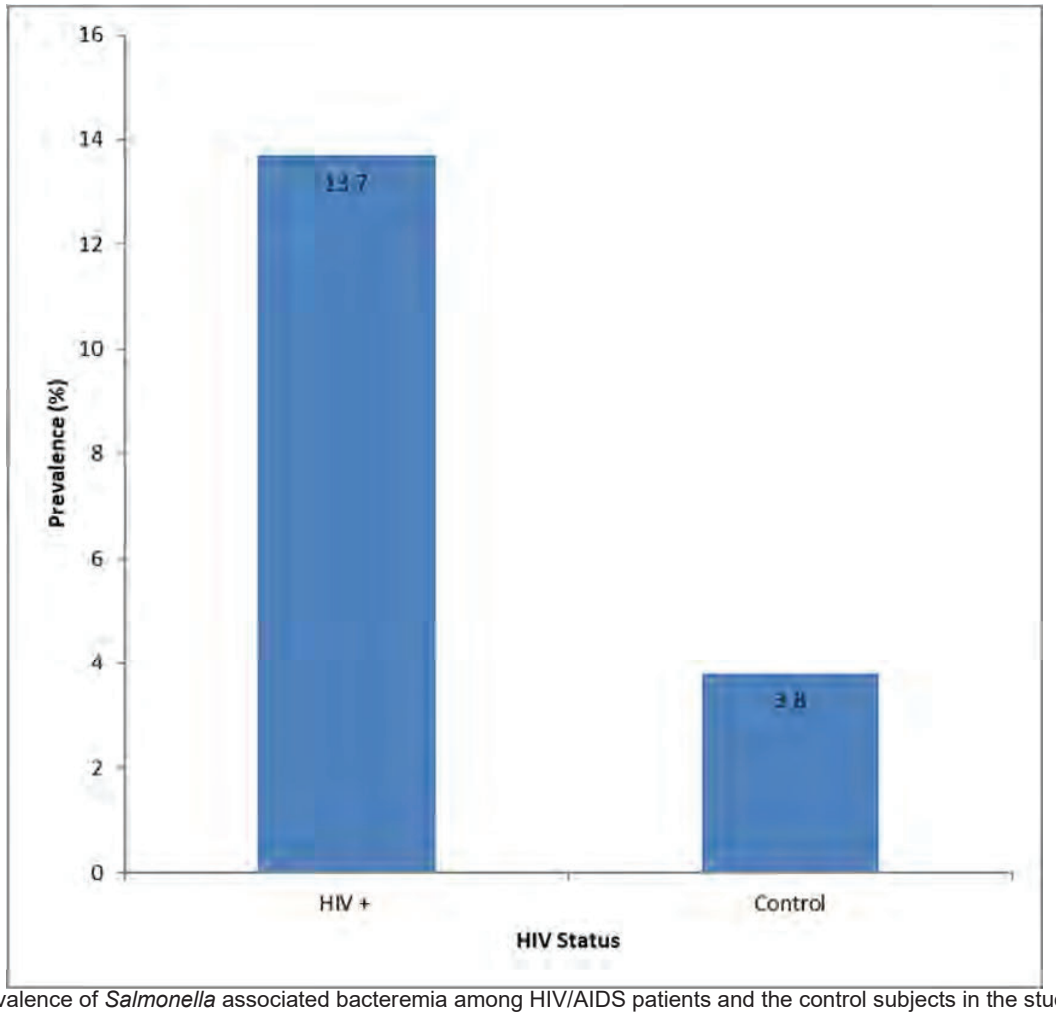

Figure 1: Prevalence of *Salmonella* associated bacteremia among HIV/AIDS patients and the control subjects in the study

Table 1. Biochemical characterizations and Microgen identification of the isolates obtained in the study

Isolates	Biochemical Tests														
	TDA	Cit	Mot	Oxi	Glu	Lys	Orni	Man	H2S	Xyl	ONPG	IT	UT	VP	Nit
S. spp	+	+	+	-	+	+	-	+	±	-	-	±	-	-	+
S. ariz	±	±	+	-	+	±	±	+	±	+	±	±	-	-	+

***S spp :** *Salmonella enterica* species, **S ariz:** *Salmonella enterica Arizonae*, **TDA:** Tryptophan deaminase test, **Cit:** Citrate Utilization test, **Mot**: Motility test, **Oxi**: Oxidase test, **Glu:** Glucose fermentation test, **Lys:** Lysine decarboxylase test, **Orth**: Orthinine decarboxylase test, **Man:** mannitol fermentation test, **H2S**: Hydrogen sulphide production test, **Xyl**: Xylose oxidation test, **ONPG:** beta -galactosidase test, **IT:** Indole production test, **UT:** Urease test **VP**: Voges Prokauer Test, **NT**: Nitrate reduction test. + = positive reaction, - = negative reaction, ± = variable.

Table 2. Biochemical reactions obtained from the serovars of *Salmonella* isolates in the study.

Salmonella serovars	Biochemical Tests and Their Reactions								
	KIA	Cit	Lys	Lac	Orth	ONPG	IT	H2S	Mot
S. typhi	**A**	-	+	-	-	-	-	+	+
S. paratyphi A	**AG**	-	-	-	-	+	-	-	+
S. paratyphi B	**AG**	+	-	-	+	+	-	+	+
S. paratyphi C	**AG**	+	+	-	-	-	+	+	+
S. typhimurium	**AG**	+	+	-	+	+	+	+	+
S. cholerasius	**AG**	+	+	-	-	+	+	-	+
S. enteritidis	**A**	+	-	-	-	+	-	+	+

***KIA=** Kligler iron agar test, **Cit:** Citrate Utilization test, **Lys:** Lysine decarboxylase test, **Lac**: Lactose fermentation, **Orth**: Orthinine decarboxylase test, **ONPG:** betagalactosidase, **IT:** Indole production test, **H2S**: Hydrogen sulphide production test, **Mot**: Motility test, **+ :** Positive reaction, **- :** Negative reaction.

Table 3. Serotyping results of Salmonella isolates obtained from blood cultures

Antisera used	Serotypes of *Salmonella* species	'O' & Vi antigens possessed by the isolates	'H' antigens possessed by the isolates
O2	*S. paratyphi* A	1, 2, 12	a
O4	*S. paratyphi* B	1, 4, 5, 12	b
	S. typhimurium	1, 4, 5, 12	i
O7	*S. paratyphi* C	6, 7 (Vi)	c
	S. choleraesius	6, 7	y
O9	*S. typhi*	9, 12(Vi)	d
	S. enteritidis	4, 9, 12	g

Table 4: Recovery rate of *Salmonella* serovars from blood of HIV/AIDS subjects and the control subjects

Salmonella species	No. (%) of isolates from HIV/AIDS (n =53)	No. (%) of isolates from control subjects (n = 5)
S. typhi	17 (32.1)	2 (40.0)
S. paratyphi A	7 (13.2)	0 (0.00)
S. paratyphi B	9 (17.0)	1 (20.0)
S. paratyphi C	5 (9.4)	1 (20.0)
S. typhimurium	4 (7.6)	0 (0.00)
S. cholaerasius	6 (11.3)	0 (0.00)
S. enteritidis	5 (9.4)	1 (20.0)

Table 5: Relationship between $CD4^+$ cell counts and the distribution of *Salmonella* bacteraemia in HIV/AIDS patients.

$CD4^+$ Counts/µL	(%) of HIV/AIDS subjects with *Salmonella* bacteraemia (n=41)	(%) of controls subjects with *Salmonella* bacteraemia (n=4)
≤ 200	21 (51.2)	2 (50.0)
201-300	11 (26.8)	1 (25.0)
301-400	3 (7.32)	1 (25. 0)
401-500	2 (4.88)	0 (0.00)
501-600	3 (7.32)	0 (0.00)
601- 700	1 (2.44)	0 (0.00)
≥ 701	0 (0.00)	0 (0.00)

DISCUSSION

In this study, the 13.7% prevalence of *Salmonella* isolation from blood of HIV/AIDS subjects is of public health concern. Immunocompromised persons are more susceptible to *Salmonella* infection especially from its life-threatening bacteraemia (23). According to Keddy *et al.*, (2009) and Sharma *et al., (*2010), *Salmonella* organisms especially *S. typhi,* infection in people living with HIV/AIDS may result in complications like septic shock, meningitis, and local abscess formation. The isolation of *S. typhi* and other serovars including the non- typhoidal ones in this study, corroborates the work of (25, 26) Taramasso et al., (2016), Adisa *et al.,* (2017). *Salmonella* is known to cause serious infections ranging from febrile illness, typhoid fever, gastroenteritis and could lead to death (27). Previous studies have reported more than 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths, and is described as a common systemic infection in tropical regions (28).

People living with HIV/AIDS are predisposed to bacteremia and their systemic spread is uninhibited due to their defective adaptive immune system (29).

The isolation of serovars *S. typhimurium* and *S. enteritidis* agrees with the works of Brent, *et al*., (2006) and Gordon et *al.,* (2010) who reported these organisms as the leading cause of community-acquired bloodstream and focal infection in Sub-Saharan Africa. Their study further highlighted that non-typhoidal *Salmonella (NTS)* serotypes were predominant among the HIV/AIDS infected persons and children with HIV, malaria, and malnutrition (30).

In this study, the majority of HIV/AIDS infected persons had low CD4+ cell counts with a higher recovery rate of *Salmonella* serovars. Other studies have reported that in HIV infection, the primary targets by the virus are the cells bearing $CD4^+$ markers on their surfaces, hence the gradual decrease in $CD4^+$ marker receptors (16-18). Fisk *et al*., (2005) noted that the dysfunction observed with HIV/AIDS infected patients is usually as a result of opportunistic infections and the vulnerability to *Salmonella* infections which correlates inversely with the $CD4^+$ lymphocyte count.

CONCLUSION

This study isolated two species of *Salmonella* and seven serovars from 13.7% of the HIV/AIDS infected persons in the study area, and their distribution largely depends on the level of $CD4^+$ cell counts of the subjects. Therefore, emphasis should not be laid only on retroviral drugs for the HIV/AIDS infected persons, but also regular screening of the subjects for bacteraemia as well as advising the subjects to consume a balanced diet including fruits to boost their immune systems, maintain personal hygiene and sanitation coupled with provision of available *Salmonella* vaccines by Government to HIV/AIDS infected individuals so to eliminate *Salmonella* associated infections among this group.

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REFERENCES

1. Woc-Colbur L, Bobak, DA. The expanding spectrum of disease due to *salmonella* an international perspective. *Curr Infect Dis Rep* 2009; 11(2): 120- 124.
2. Ron-Bin H., Yeou-Guang, T, Robert JC., Shu-Hsun, C. Risk factors for primary bacteraemia and endovascular infection in patients without acquired immunodeficiency syndrome who have non-typhoid salmonellosis. *Clin Infect Dis* 2003; 36: 829-834.
3. Karp CL, Auwaerter PG. Coinfection with HIV and tropical infectious diseases. I protozoal pathogens. *Clin Infect Dis.* 2007; 45(9):1 208-213
4. Srifuengfung S, Chokephaibulkit K., Yungyuen T, Tribuddharat C. (2005) Bacteraemia and antimicrobial susceptibilities in HIV-infected patients at Siriraj hospital. *Southeast Asian J Trop Med Pub Health* 2005; 36(2): 347-351
5. Dangana, A, Ajobiewe J, Nuhu A. Haematological changes associated with *salmonella typhi* and *salmonella paratyphi* in humans. *Inter J Biomed and Health Sci* 2010; 6(4): 219- 222
6. Dhanoa A, Fatt, QK. Non-typhoidal salmonella bacteraemia: epidemiology clinical characteristics and its association with severe immunosuppression. *Ann Clin Microbio Antimicrob* 2009; 8: 15.
7. NPD National population commission. Nigeria Demographics Profile NPC 2011.
8. Udoh DI, Onwuchekwa IS, Owoh UM. Isolations of bacteria and fungi responsible for secondary infections in people living with HIV/AIDS in Akwa Ibom State of Nigeria. *Inter J Biotech and Allied Sci* 2009; *4(1): 534-539.*

9. Afessa B, Moraels I, Weaver B. Bacteraemia in hospitalized patients with human immunodeficiency virus: a prospective cohort study. *BMC Infect Dis* 2001; 31: 1231 -1235.
10. Al-ageedi RF, Kamha A., Al-aani FK, Al-ani AA. Salmonella myocarditis in a young adult patient presenting with acute pulmonary edema, rhabdomyolysis and multi-organ failure. *J Cardiol* 2009*;* 54(3): 475-479.
11. Grimont PA., Weill FX. Antigenic formulae of the salmonella serovars. 9th Edition: WHO Collaborating Centre for Reference and Research on Salmonella. Paris, France: *Institut Pasteur* 2007.
12. Malorny B, Hauser E, Dieckmann R. New approaches in subspecies-level salmonella classification. *Salmonella: from genome to function*. Edited by Porwollik S. UK, *Caister Academic Press.* 2011; 1-23
13. Kiratisin P. Bacteraemia due to non-typhoidal *salmone*lla in Thailand: clinical and microbiological analysis. *Trans R Soc Trop Med Hyg 2008; 102(4): 384-388.*
14. Gordon MA, Graham SM, Walsh AL, et al. Epidemics of invasive *salmonella enterica* serovar enteritidis and *salmonella enterica* serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 2008; 46(7): 963–966.
15. Cheesbrough M. District laboratory practice in tropical countries (Part 2). UK, *Cambridge University Press,* 2000; 182-186, 253-266.
16. Fisk TL, Lundberg BE., Guest JL, et al. Invasive infection with multidrug-resistant *salmonella enterica* serotype *typhimurium* definitive type 104 among HIV-infected adults. *Clin Infect Dis*. 2005; 40(7): 1016-1022.
17. Grass lJ, Finlay, BB. Pathogenesis of enteric salmonella infections. *Curr Opin Gastroenterol* 2008*;* 24(1): 22-26.
18. Sharma S, Dhungana GP, Pokhrel BM, Rijal BP. Opportunistic infection in relation to CD4 level among HIV seropositive patients from central Nepal. *Nepal Med Coll J* 2010; 12(1): 1-4.
19. WHO. African region on AIDS in Nigeria. *Nig J Infect Dis* 2006; 201(7): 473-474.
20. World Health Organization: AIDS epidemic update – global summary. 2007; ISBN: 9789291736218
21. Bergey DH, Holt JG, Krieg NR, et al. Bergey's manual of determinative bacteriology. 9th Edition. *The Williams and Wilkins Company Baltimore, Maryland, U.S.A*. 1994; 660-980.
22. Tortora GS, Funke BR, Case CL. Microbiology: an introduction. 9th Edition. *Pearson Education Inc. Publishing as Benjamin Cummings, San Francisco* 2007; 457-458, 753-754; 762.
23. Keddy KH, Dwarike S, Crowther P, et al. Genotype and demographic characterization of invasive isolates of salmonella typhimurium in HIV co-infected patients in South Africa. *J Infect Dev Ctries* 2009; 3(8): 585- 592.
24. McGhie EJ, Brawn LC, Hume PJ. Salmonella takes control: effector-driven manipulation of the host. *Curr Opin Microbiol* 2009; 12(1): 117-124.
25. Taramassa L, TararelliP, Biagio AD. Bloodstream infections in HIV-infected patients*. Virulence* (2016); 7(3): 320-328.
26. Adisa AK, Hassan-Hanga F, Oyelami OA. Prevalence and pattern of bacteremia among HIV-infected under-five children in a tertiary hospital in Kano, Nigeria. *Nig. J Paediatr* 2017; 44(1): 26 -32.
27. Udoh DI. Prevalence of HIV/AIDS infection in parts of Akwa Ibom State, Nigeria. *World J Applied Sci Tech* 2015; 7(1): 58-64.
28. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bull World Health Organ*. 2008; 86(4): 260- 268.
29. Crump JA, Ramadhani HO, Morrissey AB, et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in Northern Tanzania. *Clin Infect Dis* 2011*;* 52(3): 341-348.
30. Brent AJ, Oundo JO, Mwangi I, et al. Salmonella bacteremia in Kenyan Children. *Pediatr Infect Dis J* 2006; 25: 230-236.