PREVALENCE OF ENTERIC FEVER- HIV/AIDS CO-INFECTIONS IN AKWA IBOM STATE, NIGERIA.

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ABSTRACT: A total of 1,355 stool and blood specimens collected separately from HIV/AIDS patients in Akwa Ibom State were screened for enteric fever – HIV/AIDS co- infections using standard cultural and serological techniques. Out of the 1,355 stool and blood samples, 88 (22.4%) of salmonellae were isolated from children (1-15 years old), 172 (43.9%) from active age range of 16-35 years old, and 130 (33.2%) from adults (36 years old and above). There were 392 (28.9%) *Salmonellae* isolates with a mortality rate of 1.0% (14 patients) during the study period of 18 months. Nine hundred and sixty three stools samples yielded non *Salmonella* spp. However differences between the total number of positive and negative cases were not significant ($P< 0.05$). There was significant difference ($P<0.05$) in the inter-community endemicity of enteric fever – HIV/AIDS co-infections. Analysis of sex-specific distribution, revealed that females had the highest prevalence of 258 (19.0%). Analysis also revealed significant difference ($p <0.05$) in the level of co-infections among various occupational groups of patients. The clinical features of salmonellae infection were not different from other diarrhoeagenic agents and included diarrhea, fever, abdominal pains and vomiting. Assay of the antibiotics susceptibility patterns in relation to the ABO blood grouping system showed that patients with blood group O were more susceptible to enteric fever followed by blood group AB and A. The high prevalence of co-infections (43.9%) and (33.2%) among the active age group of 16-35years and 36 years and above is a cause for concern and calls for enforcement of control measures to avert imminent out- break in the study area.

INTRODUCTION

Typhoid fever is a bacterial illness caused by *Salmonella typhi* , a Gram negative rod found only in humans. This illness is characterized by persistent fever, abdominal pain, severe anorexia, constipation or mild diarrhea, and delirium (Ackers *et al*., 2000). Similar paratyphoid and enteric fevers are caused by other species of *Salmonella*, which infect domestic animals as well as humans (Abbott and Janda, 1992). Infection with typhoid fever is transmitted by consumption of contaminated food or water. Occasionally direct faecal-oral transmission may occur. Shellfishes taken from sewage polluted beds are an important source of infection. Infection also occurs through eating fruit and vegetables fertilized by biosolid and eaten raw, and milk and milk products that have been contaminated by those in contact with them. Flies may transfer infection to foods, resulting in contamination that may be sufficient to cause human infection (Evangs, 1989). Pollution of water sources may produce epidemics of typhoid fever, when large numbers of people use the same source of drinking water.

Typhoid fever is a systemic disease of varying severity. Severe cases are characterized by gradual onset of fever, headache, malaise, anorexia, and insomnia (Huckstep, 1990). Constipation is more common than diarrhea in adults and older children. Without treatment, the disease progresses with sustained fever, bradycardia, hepatosplenomegaly, abdominal symptoms and, in some cases, pneumonia. In white-skinned patients, pink spots (papules),

which fade on pressure, appear on the skin of the trunk in up to 50% of cases. In the third week, untreated cases develop additional gastrointestinal and other complications, which may prove fatal (Duesberg, 1992). It is reported that about 2-5% of those who contract typhoid fever become chronic carriers, as bacteria persist in the biliary tract after symptoms have resolved (Parry *et al*., 2002). The disease occurs most commonly in association with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking. Generally low risk for travelers, except in parts of North and West Africa, in South Asia and in Peru. Elsewhere, travelers are usually at risk only when exposed to low standards of hygiene with respect to food handling, control of drinking water quality, and sewage disposal (Lee *et al.*, 1989).

In developed countries such as Britain and United States, about 500 cases are reported annually. More than two thirds of those cases are contracted during travel abroad. Most cases acquired in the United States are as the result of local outbreaks or occurs in patients with underlying medical diseases. For example the rate among patients with HIV is 60 times greater than the general population (Jong, 1999). In tropical African countries like Nigeria and other developing countries, typhoid fever is most prevalent where sanitary water and sewage systems are lacking. As a result, prevention is mainly through improved hygienic standards, better sanitation, and availability of potable water and effective disposal of wastes (WHO, 2003). However there is paucity of information on Salmonellae infection as co-infections in HIV/AIDS patients in Akwa Ibom State. The increasing importance of *Salmonella* species in human diseases coupled with socio-economic problems of the people in Akwa Ibom State necessitates the present study on the prevalence of enteric fever- HIV/AIDS co-infections in Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

The study was conducted between May 2005 and December, 2006. A total of 1,355 stool and blood specimens used for this study were separately obtained from confirmed HIV/AIDS patients, attending the UUTH (University of Uyo Teaching Hospital, Uyo), UUHC, (University of Uyo Health Centre), SLHA (St. Luke Hospital Anua), IDHIE (Infectious Disease Hospital Ikot Ekpene), IHE (Immanuel Hospital Eket) GHO (General Hospital Oron), MHA (Mercy Hospital Abak), GHI (General Hospital Ikono), GHIN (General Hospital Ikpe Ikot Nkon), MSSHI (Mary Slessor Specialist Hospital Itu), GHUA (General Hospital Urua Akpan), GHIA (General Hospital Ikot Abasi), GHIM (General Hospital Ituk Mbang) and GHUO (General hospital Urue Offong/Oruko) all located in Akwa Ibom State. The sampled population was zoned for proper collection of specimens. Ethical approval was received from ethical committee Akwa Ibom State Ministry of Health. A standard questionnaire indicating the state, zone, patient card number, age, sex, clinical details including symptoms and signs of illness such as fever, diarrhoea, onset/duration, antibiotics treatment and other socioeconomic activities of patients were completed by HIV/AIDS patients from whom specimens were collected. Stool specimens of HIV/AIDS cases were bacteriolocally analyzed on weekly basis for the presence of *Salmonella* species. Plasma specimens (acute and convalescent whole blood) were also collected from all HIV/AIDS confirmed patients for widal (serology) and blood grouping tests. Using standard procedures, stool, blood and rectal swab were collected using clean, dry, disinfectant-free containers and EDTA Vacutainers which were appropriately labeled indicating sex, age, and place of residence, nature of specimen, date and time of collection. The specimens were transported to the laboratory with minimum delay to avoid death of enteric pathogens especially in the stools. (Baker *et al.*, 2001).

Bacteriological Analysis of Stool and Rectal Specimens

Stool and rectal samples were plated out on desoxycholate citrate agar, DCA. The media were prepared as recommended by the manunfacturer (Oxoid, UK). Selected portions of faeces with pus, bloody or mucus were added to about 10ml of selenite F broth (BBL, USA), in screw-capped bottles and incubated at 37^{o}C for 2-3 days. Subcultures were then made into plates of

desoxycholate citrate agar, and incubated for another 24 hours. Colonies with black centers were sub-cultured onto nutrient agar and incubated for another 24 hours. The cultures on nutrient agar plates were subjected to Gram-staining, motility, urease production, and hydrogen sulfide production and citrate utilization tests. All Gram-negative, rod-shaped, motile, urease-negative isolates that produced acid on triple sugar iron agar slants and able to utilize citrate as sole carbon source were identified as species of the genus *Salmonella*. Control experiments (positive and negative) were set-up to monitor the efficiency of the media, reagents and different biochemical and serological tests performed.

Statistical Analysis

Data obtained from this study were statistically analyzed using the Analysis of Variance (ANOVA) test was also used to determine statistical differences between data obtained using the method of Essien (2003).

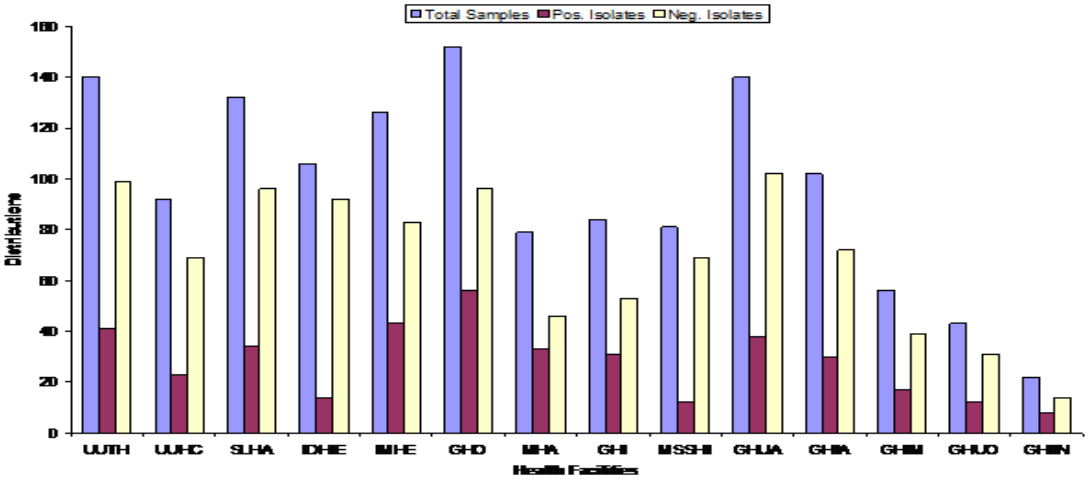

Fig. 1: Inter community distribution of Enteric fever HIV/AIDS co-infections in Akwa Ibom State

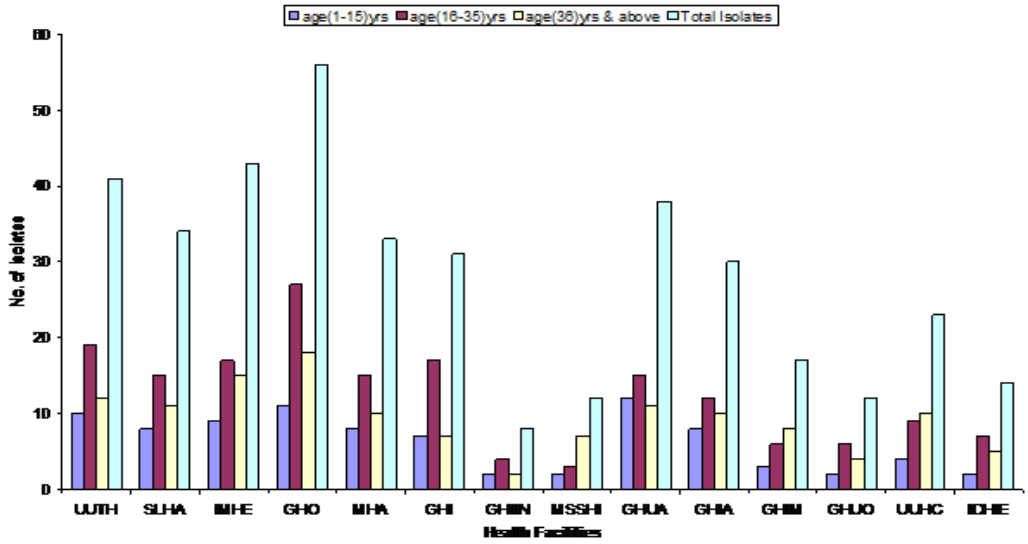

Fig. 2: Age specific distribution of Enteric fever HIV/AIDS patients in Akwa Ibom State

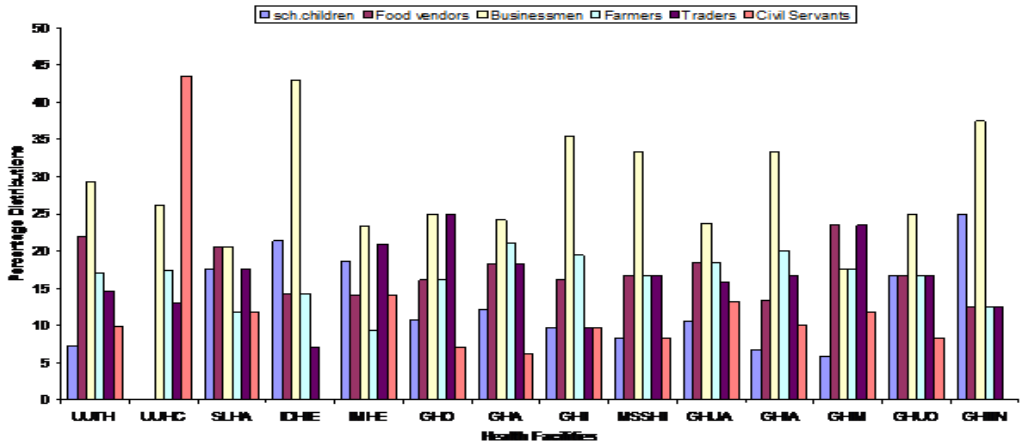

Fig. 3: Percentage distribution of Enteric fever HIV/AIDS co-infections of diverse occupational status in Akwa Ibom State

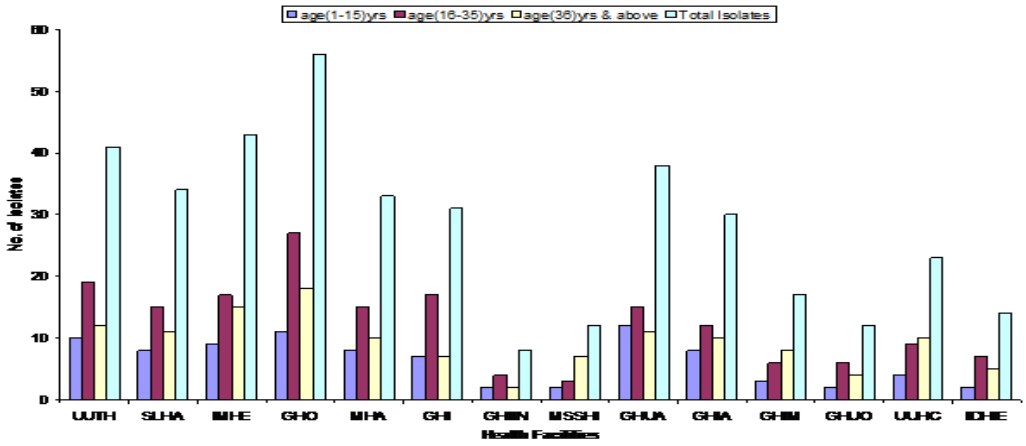

Fig. 4: Sex specific distribution of Enteric fever HIV/AIDS co-infections in Akwa Ibom State

RESULTS

The inter-community distributions of positive and negative cases of enteric fever in relation to Health facilities in Akwa Ibom State are presented in Table 1.

Table 1: Inter-community distribution of enteric fever- HIV/AIDS co-infections in Akwa Ibom State.

Health Facilities/ Communities	Total No Of Samples	No: Of Positive *Salmonella* Isolated (%)	No: Of Negative *Salmonella* Isolated (%)
UUTH	140	41(10.5)	99(10.3)
UUHC	92	23(5.9)	69(7.2)
SLHA	132	34(8.7)	96(10.0)
IDHIE	106	14(3.6)	92(9.6)
IMHE	126	43(11.0)	83(8.6)
GHO	152	56(14.3)	96(10.0)
MHA	79	33(8.4)	46(4.8)
GHI	84	31(7.9)	53(5.5)
MSSHI	81	12(3.1)	69(7.2)
GHUA	140	38(9.7)	102(10.6)
GHIA	102	30(7.7)	72(7.5)
GHIM	56	17(4.3)	39(4.0)
GHUO	43	12(3.1)	31(3.2)
GHIIN	22	8(2.0)	14(1.5)
Total	1355	392(28.9)	963(71.1)

Key: UUTH (University of Uyo Teaching Hospital) SLHA (St. Luke Hospital Anua) IMHE (Immanuel Hospital, Eket). GHO (General Hospital Oron) MHA (Mercy Hospital Abak), GHI (General Hospital Ikono) GHIN (General Hospital Ikpe Ikot Nkon), MSSHI, (Mary Slessor Specialist Hospital, Itu), GHUA, (General Hospital Urua Akpan) GHIA, (General Hospital Ikot Abasi), GHIM, (General Hospital Ituk Mbang), GHUO (General Hospital Urueoffong Oruko), UUHC, (University of Uyo Health Centre), IDHIE (Infectious Disease Hospital Ikot Ekpene)

Out of the 14 health facilities monitored, General Hospital Oron (GHO) had the highest number of salmonellae positive cases (14.3%) out of 152 stool samples analyzed, while General Hospital Ini Community had the least number (2.0%) out of 22 patients screened. However the total number of positive cases as compared with the negative subjects (Fig. 1) were statistically significant ($P< 0.05$). Results presented in Table 2 show that, out of the 1,355 stool and blood samples analyzed 133 (33.9%) cases of *Salmonella* were recorded for subjects 1-15 years old, 129 (32.9%) for active age range of 16-35 years old and 130 (33.2%) for adults of 36 years old and above. 392 (28.9%) salmonellae related cases were obtained with a mortality rate of 1.0% (14 patients) during the study period (18 months). Eight (0.59%) of the deaths were recorded in children 1-15 years old, 2 (0.15%) in active ages of 16-35 years old, and 4 (0.2%) were in adults above 36 years old. Nine hundred and sixty- three (71.1%) stools samples yielded non *Salmonella* spp. The highest number of *Salmonella* spp related cases recorded for HIV/AIDS patients of active age range 15-35 years old with 43.9% incidence rate, followed by adults, 36 years and above with incidence rate of 33.2% (Table 2)

The results of distribution of salmonellae among HIV/AIDS patients based on their various occupational status (Table 3) revealed that businessmen were more affected with 27.0% incidence rate, followed closely by traders with incidence rate of 17.3%, the least infected were children and civil servant with 12.5% incidence rate (Fig. 3). The percentage occurrences for other occupational status were 15.3% for food vendors and 15.8% for farmers.

The differences among diverse occupational status were statistically significant at $P<0.05$ (ANOVA). Studies on the sex-specific distribution (Table 4) revealed that females were more prone to salmonellosis than males. Of the 1355 clinical samples screened for enteric fever 820 (60.5%) were collected from female HIV/AIDS patients, while 535 (39.5%) were obtained from male patients. The salmonellae occurrence were 19.0% for female and 9.9% for male. Statistical analysis has revealed no significant difference ($P<0.05$) between sex of patient and incidence of enteric fever (Fig. 4).

Table 2: Age specific distribution of enteric fever - HIV/AIDS patients in Akwa Ibom State.

Hospitals	Total Nos. of Samples Collected	Children (1-15) Years. N0. (%)	Active Age (16-35) Years N0. (%)	Adults 36-Years & Above N0. (%)	Total Positive Cases	Total Negative Cases
UUTH	140	10	19	12	41	99
SLHA	132	08	15	11	34	96
IMHE	126	09	17	15	43	83
GHO	152	11	27	18	56	96
MHA	79	08	15	10	33	46
GHI	84	07	17	07	31	53
GHIIN	22	02	04	02	08	14
MSSHI	81	02	03	07	12	59
GHUA	140	12	15	11	38	102
GHIA	102	08	12	10	30	72
GHIM	56	03	06	08	17	39
GHUO	43	02	06	04	12	31
UUHC	92	04	09	10	23	69
IDHIE	106	02	07	05	14	92
Total	1355	88 (22.4%)	172 (43.9%)	130(33.2%)	392(28.9)	963(71.1)

Table 3: Distribution of enteric fever - HIV/aids co-infections of diverse occupational status in Akwa Ibom State.

Endemic Comm.	Total Sal Isolated (%)	Occupational Status					
		School Children (%)	Food Vendors (%)	Businessmen (%)	Farmers (%)	Traders (%)	Civil Servant (%)
UUTH	41(10.5)	03(7.3)	09(22.0)	12(29.3)	07(17.1)	06(14.6)	04 (9.8)
UUHC	23(5.9)	00 (0.00)	00 (0.00)	06(26.1)	04(17.4)	03(13.0)	10(43.5)
SLHA	34(8.7)	06(17.6)	07(20.6)	07(20.6)	04(11.8)	06(17.6)	04(11.8_
IDHIE	14(3.6)	03(21.4)	02(14.3)	06(42.9)	02(14.3)	01(7.1)	00(00.0)
IMHE	43(11.0)	08(18.6)	06(14.0)	10(23.3)	04(9.3)	09(20.9)	06(14.0)
GHO	56(14.3)	06(10.7)	09(16.1)	14(25.0)	09(16.1)	14(25.0)	04(7.1)
GHA	33(8.4)	04(12.1)	06(18.2)	08(24.2)	07(21.1)	06(18.2)	02(6.1)
GHI	31(7.9)	03(9.7)	05(16.1)	11(35.5)	06(19.4)	03(9.7)	03(9.7)
MSSHI	12(3.1)	01(8.3)	02(16.7)	04(33.3)	02(16.7)	02(16.7)	01(8.3)
GHUA	38(9.7)	04(10.5)	07(18.4)	09(23.7)	07(18.4)	06(15.8)	05(13.2)
GHIA	30(7.7)	02(6.7)	04(13.3)	10(33.3)	06(20.0)	05(16.7)	03(10.0)
GHIM	17(4.3)	01(5.9)	04(23.5)	03(17.6)	03(17.6)	04(23.5)	02(11.8)
GHUO	12(3.1)	02(16.7)	02(16.7)	03(25.0)	02(16.7)	02(16.7)	01(8.3)
GHIIN	8(2.0)	02(25.0)	01(12.5)	03(37.5)	01(12.5)	01(12.5)	00 (00.0)
Total	392(28.9)	45(12.5%	64(15.3%)	106(27.0%)	62(15.8%)	68(17.3%)	45(12.5%)

On the other hand, correlation between salmonellosis and blood grouping of the patients, (Table 5) showed that, out of 392 (28.9%) salmonellae related cases in the state. Patients with blood group O had the highest incidence rate (40.1%) while Group B had the least (16.8%) followed by Group A (18.9%) and AB (24.2%). The values however varied with the different hospitals (Fig. 5) investigated. Statistical analysis also revealed significant difference among blood groups of HIV/AIDS patients with enteric fever ($P<0.05$).

Table 4.: Sex specific distribution of enteric fever – from stool of HIV/AIDS infected patients in AKS.

Health Facilities	Total No. Of Samples	No. Of Samples (%)		*Salmonellae* Isolated In Male (%)	*Salmonellae* Isolated In Female (%)
		MALE	FEMALE		
UUTH	140	58(41.4)	82 (58.6)	15(10.7)	26(18.6)
UUHC	92	36 (39.1)	56 (60.9)	09(9.8)	14(15.2)
SLHA	132	34 (25.8)	98 (74.2)	13(9.8)	21(15.9)
IDHIE	106	26 (24.5)	80 (75.5)	04(3.8)	10(9.4)
IHE	126	57 (45.2)	69 (54.8)	15(11.9)	28(22.2)
GHO	152	53 (34.9)	99 (65.1)	18(11.8)	38(25.0)
GHA	79	29 (36.7)	50 (63.3)	13(16.5)	20(25.3)
GHI	84	36 (42.9)	48 (57.1)	11(13.1)	20(23.8)
MSSHI	81	40 (49.4)	41 (50.6)	04(4.9)	08(9.9)
GHUA	140	68 (48.6)	72 (51.4)	12(8.6)	26(18.6)
GHIA	102	41 (40.2)	61 (59.8)	08(7.8)	22(21.6)
GHIM	56	29 (51.8)	27 (48.2)	05(8.9)	12(21.4)
GHUO	43	18 (41.9)	25 (58.9)	04(9.3)	08(18.6)
GHIIN	22	10 (45.5)	12 (54.5)	03(13.6)	05(22.7)
Total	1355	535 (39.5)	820 (60.5)	134(9.9)	258(19.0)

Table 5: Distribution of enteric fever – HIV/AIDS co- infections based on blood group of subjects in AKS.

Health Facilities	Total No. of Salmonellae Isolated	ABO grouping system (%)			
		A	B	AB	O
UUTH	41	07	06	10	18
UUHC	23	06	03	05	09
SLHA	34	08	06	08	12
IDHIE	14	03	01	02	08
IMHE	43	09	10	11	13
GHO	56	10	10	13	23
GHA	33	07	06	08	12
GHI	31	08	04	06	13
MSSHI	12	01	02	02	07
GHUA	38	06	09	11	12
GHIA	30	04	04	08	14
GHIM	17	02	02	06	07
GHUO	12	01	02	03	06
GHIIN	08	02	01	02	03
Total	392	74(18.9%)	66(16.8%	95(24.2%)	157(40.1%)

Table 6; In-vitro antimicrobial susceptibility pattern of *enteric fever* with their blood group in AKS

Blood Group	Total No of Isolates (%)	Total No. of Resistance To: (%)				
		AMP	TET	CHLOR	AMOX	CIPRO
A	74	74	72	07	03	00
B	66	66	60	05	01	01
AB	95	94	93	08	01	02
O	157	156	152	10	03	00
Total	392	390 (99.5%)	377 (96.2%)	30 (7.7%)	08 (2.0%)	03 (0.8%)

Key AMP (Ampicillin) TET (Tetracycline) CHLOR (Chloramphenicol) AMOX (Amoxicillin) CIPRO (Ciprofloxacin)

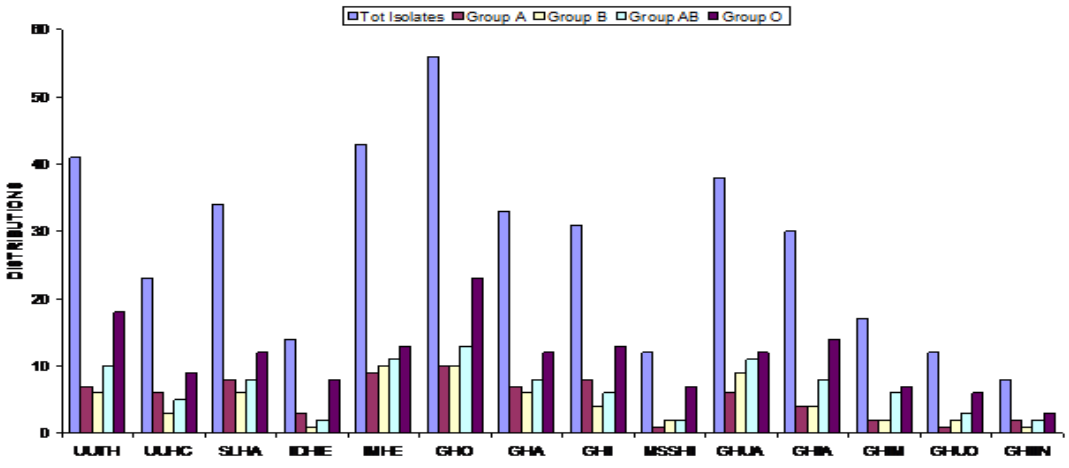

Fig. 5: Distribution of Enteric fever HIV/AIDS co-infections based on ABO blood grouping system in Akwa Ibom State

Results of antimicrobial sensitivity test of the test isolates (Fig. 6) revealed that, out of the 5 antimicrobial agents used, the isolates exhibited remarkable resistance to ampicillin 99.5% and

tetracycline (37.7%), but 0.8% resistance to ciprofloxacin followed closely by amoxicillin (2.0%), chloramphenicol (7.7%). The differences among the antimicrobial susceptibility test were significant at P< 0.05 (ANOVA).

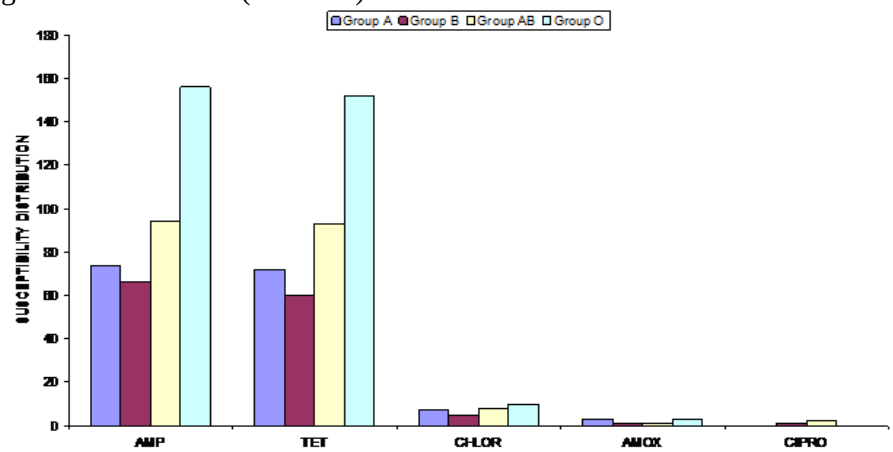

Fig. 6: In vitro antimicrobial susceptibility pattern of enteric fever based on ABO blood grouping system in Akwa Ibom state

DISCUSSION

Salmonellosis is prevalent in many developing countries and is still a common cause of fever and diarrhoea in these areas. It continues to exist as co-infection with HIV/AIDS patients in this part of the world. The present study revealed an overall prevalent rate of 28.9% of salmonellae as co-infections in HIV/AIDS patients. The high prevalence reported in the Akwa Ibom State could be due to a variety of factors. The densely populated status of the state is associated with high level of human activities, overcrowding and poor sanitation which probably increased the occurrence of *Salmonella* enteritis. There was statistically significant (p< 0.05) difference among the communities in the incidence of enteric fever- HIV/AIDS co-infections. The co-infection is predominant in the coastal communities of the state. Occupational distribution of its incidence in Akwa Ibom State were statistically significant at (P<0.05).

Rate of occurrence of enteric fever was higher within the very active ages of 16-35 years followed closely by adult ranging from 36 years and above. This is a very unhealthy development and may be associated with poor hygiene. The sex specific distribution revealed that females are more prone to salmonellosis than males, possibly because their physiological system exposes them more to HIV/AIDS infection and as such females are mostly found in hospitals facilities than their male counterparts, although statistically not significant (P<0.05).. Introduction of monthly environmental sanitation practice Akwa Ibom State may help to the reduction of incidence of salmonellosis. This is because the environmental sanitation practice reduces garbage and waste that may contribute to transmission of salmonellae infection through contamination of drinking water and foods.

In this study, 258 (19.0%) *Salmonella* isolates were obtained from females and 134 (9.9%) from males in Akwa Ibom State. 106 (27.0%) isolates were associated with businessmen, 68 (17.3%) with traders, 64 (16.3%), with vendors, 62 (15.8%), with farmers, 45 (12.5%), with civil servants and 45 (12.5%), with school children. The relatively low incidence in children may be attributed to the high level of immunity in this age group and inability for children to survive with HIV virus beyond 9-12 months.

This research has also revealed that patients with blood group O were more prevalent to enteric fever followed by group AB, B and A in the State. The significance of this relationship has not been fully identified. *In vitro* antimicrobial susceptibility test showed that *Salmonella* isolates were susceptible to five drugs tested. Most isolates exhibited a high degree of susceptibility to chloramphenicol, amoxicillin and ciprofloxacin. Some isolates were resistant to tetracycline

and ampicillin. Ciprofloxacin was however found to be the most effective with the lowest Minimum Inhibitory Concentration (MIC) of 2.0µml. This was followed by amoxicillin and chloramphenicol. These results are in conformity with earlier report by Baron (1996), that floquinolone are the best antimicrobial agent for the treatment of enteric fever. The high susceptibility of isolates to ciprofloxacin, amoxicillin and chloramphenicol indicates that these drugs may be recommended in cases of enteritis due to Salmonellae after isolation of an etiologic agent and sensitivity testing performed. Few isolates resistant to chloramphenicol may be due to abuse that causes bacteria mutation or possibly production of acetylating enzymes, acetyl transferase, which destroys or hydrolysis the drug making them inactive. Many Salmonellae isolates were resistant to Ampicillin and Tetracycline, this observation is not surprising in a community such as ours where anti-microbial agents like Ampicillin and tetracycline are freely hawked in the streets and taken by individuals without clinical prescription. Most Ampicillin resistant strains were beta-lactamase producers. This enzyme is capable of cleaving the beta-lactam ring of Penicillin to form an inactive penicilloic acid to which the organisms are not susceptible. There is evidence that resistance to these strains could also be chromosomal. Widal serology of 392 culturally-confirmed cases of Salmonellae infection showed that most of them had Salmonellae antibody titre $\geq$ 80 except in some few cases where the titre was 40. The elevation of O' antibodies titre is suggestive of current and active infection, while Hi antibodies suggest previous infection or immunization with typhoid vaccine (Baron 1996). However, the in-vitro Ciprofloxacin susceptibility pattern of isolates from patients with co-infections was statistically significant ($P<0.05$) when compared to patients with different blood groups ($P<0.05$)

CONCLUSION AND RECOMMENDATION

The overall prevalence rate of enteric fever in Akwa Ibom State was 28.9%. however, there was no statistically significant difference ($P<0.05$) in incidence between the male and female patients. This implies that enteric fever is not sex related. Active ages of 16years through 40years and above were more infected. In respect of occupational status of patients, the highest prevalence was among businessmen followed by traders, probably due to indiscriminate consumption of food and drinks by this class of patients in Akwa Ibom State.

The prevalence of Salmonellae infection among HIV/AIDS subjects emphasize the necessity for enlightenment campaign on the need for people to know their HIV status and more importantly the hygienic preparation and preservation of food, environmental sanitation, availability of portable water and effective disposal of wastes as these will prevent *Salmonellae*.. Early report of outbreaks, prompt investigation of cases, proper identification of isolates and treatment of cases are indispensable and must be improved as it will minimize transmission and spread of the *Salmonellae* disease. The use of widal serology in this study has shown that titres of > 80 in single (acute) serum from bacteriologically proven cases are presumptively diagnostic. *In-vitro* antibiotics susceptibility pattern indicated that Chloramphenicol, Amoxicillin and Ciprofloxacin are effective and may be recommended in suspected typhoid cases.

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