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Non-Salmonella Bacteremia Among Seropositive Hiv Patients Attending Three Tertiary Hospital In Nasarawa State, Nigeria.

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Abstract

Background: Bacterial blood stream infections constitute a significant public-health problem and it is an important cause of morbidity and mortality in HIV infected patients. Little is known in developing countries regarding salmonella bacteraemia among HIV patients. The purpose of this study was to determine the bacterial pathogens causing blood stream infection among febrile adults attending three medical centers in Nasarawa State. **Methods:** A prospective cross-sectional study involving 346 consecutive, febrile adult patients admitted at three medical centers in Nasarawa State, was conducted. Demographic and other data were collected using standardized questionnaires. Blood culture was done followed by susceptibility testing using disc diffusion method. HIV testing was also performed as per Nigeria national algorithm and total white blood cell counts and CD4+ counts determined. **Results:** Of 346 febrile adult patients 33 (9.5%) had blood stream infections. The common isolates were *Salmonella spp* 13(39.4%), *Escherichia coIi* 8 (24.2%), *Streptococcus pneumonia* 5(15.2%), *Staphylococcus aureus* 4(12.1%), *Citrobacter spp* 1(3%), *Streptococcus pyogenes* 1(3%) and *Kiebsiella pneumonia* **1(3%).** A total of 156 (45.1%) patients were HIV infected; of whom 12/1 56 (7.6%) were infected by non-typhoid *Salmonella spp* compared to 1/190 (0.5%) of non-HIV infected patients (RRR 11.2, p=0.029) infected with *Salmonella typhi.* HIV infected patients with bacteremia had significantly lower CD4+ count than those without bacteremia (median 28 vs. 88 cells/mI, p=0.01). Patients with salmonella bacteremia had significantly lower median of WBC than those with non-salmonella as well as those without bacteremia (median, 3.6 vs. 17.5 vs. 9.8x109, p0.0001). All *Salmonella spp* were sensitive to ceftriaxone and imipenem, while being 84%, 69.2%, 38% and 8% resistant to chloramphenicol, ampicillin, sulphamethaxazole/trimethoprim and ciprofloxacin respectively. Predictors of mortality were HIV infection (OR 2.3, p=0.005), Glasgow coma score of less than 15 (OR **3.4,** p=0.0001) and night sweats (OR 2.4, p=0.014). **Conclusion:** Non-typhoid *Salmonella spp* that are highly resistant to common antibiotics are predominant cause of bacterial blood stream infection among HIV patients attending Three tertiary medical centers in Nasarawa State. Continuous surveillance and intervention strategies should be put in place to monitor and manage cases of bloodstream infections in HIV-positive patients in Nasarawa state.

Background

Globally burden of sepsis is substantial; in the United State of America (USA), severe sepsis claims 210,000 lives per year [1]. The exact burden of bacteremia in the low and middle income countries remains unknown. The high incidence of bacterial, parasitic, and HIV infection suggests that bacteremia might be a substantial problem in developing countries [2]. A mortality rate of up to 38% among patients with bacteremia has been reported, with a strong correlation between HIV infection and bacteremia [3]. In Gambia, patients with positive blood cultures were more likely to die than those without bacteremia In many studies majority of bacteraemia are due to *Staphylococcus aureus* (including methicillin-resistant), *Streptococcus pneumoniae, Pseudomonas* spp, *Salmone lla enterica, Escherjchja coli* and disseminated tuberculosis. The pattern can differ between developed and low in-income countries [6-121. The use of blood culture to assess seriously ill patients infected with HIV has led to a growing understanding of their increased risk of a range of invasive bacterial and fungal disease including *Streptococcus pneumoniae,* disseminated tuberculosis, cryptococcosis, and *Salmonella* bacteremia [13,14]. Non —typhoid salmonella bacteremia has been identified as AIDS defining illness according to CDC and WHO [15]; while for *Salmonella typhi* infection HIV was found to be protective as demonstrated by Crump et al. [10]. The diagnosis of these infections can be confirmed by blood culture, which is routinely available in few hospitals in developing countries [2,16-18]. Factors thought to contribute to poor outcomes of critically ill patients in these settings include treatment cost, deficiency of diagnostic facilities and poor health seeking behavior [19,20]. Since bloodstream infections are common and associated with high mortality, improved clinical and microbiology services as well as reassessment of empirical treatment guidelines might contribute to better outcomes. In resource-constrained environment like ours, simple measures like timely provision of intravenous us fluid resuscitation coupled with prompt empirical antibiotics administration may improve outcomes. Identification of common organisms isolated in blood cultures and antimicrobial susceptibility testing

with regular data updates should promote appropriate antimicrobial prescribing and improve outcome of patients with bacteremia.

Methods

Study design

A cross-sectional prospective study was conducted. This study was conducted in the medical wards at Dalhatu Araft specialist Hospital Lafia,General Hospital Akwanga and Federal Medical Centre Keffi; all the medical center are tertiary referral center for the three senatorial Zone of the state, serving a total population of 2.6 million in Nasarawa state.

Sample size and sampling

Sample size was determined using Kish and Leslie formula for cross-sectional studies [21]. Prevalence of bacteremia estimated from previous studies of 16.4% [5] was used. A minimum sample size obtained was 210 but in the study 346 patients were enrolled. Patients with fever (defined as auxiliary temperature >37.5^{0}C) admitted to the medical wards of the three tertiary Hospital were consecutively enrolled into the study until the sample size was reached.

Data collection and laboratory procedures

After counseling and informed consent, a full history, physical examination and laboratory investigations were conducted and recorded in the data collection forms. Data collected included demographic characteristics, history of antibiotic/antimalaria use, presenting clinical signs/symptoms, physical examination and the current diagnosis. Two blood specimens were collected at the time of study enrolment before any antibiotic treatments were initiated at three medical centers to Innovative Biotech, Keffi, Nasarawa State. The venipuncture site was disinfected with 70% alcohol and 2% tincture of iodine before collecting approximately 10 ml of blood for culture, l-HV serology, and white blood cell counts. For blood culture 5m1 of blood was inoculated into 5Oml of Brain Heart Infusion broth (Oxoid UK). Subculture and identification of isolates were as described previously [22,23]. Two aerobic blood culture bottles were used for each patient and growth in both bottles was considered as significant. All isolates suspected to be *Salmonella spp* were confirmed using polyvalent salmonella Latex identification KIT (Oxoid UK) performed as described by vendor and further biochemical tests which included citrate utilization and amount of hydrogen sulphide produced were used to differentiate between typhi and non-typhi salmonella [24]. Antibiotic susceptibility was determined by disc diffusion on Mueller-Hinton agar and for *Streptococcus pneumoniae* Mueller-Hinton agar was supplement ed with 5% sheep blood. Interpretation was done according to the Clinical Laboratory Standard Institute (CLSI) guidelines and Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World [24]. Quality-control of antibiotic discs (Oxoid UK), media and incubation conditions was ensured using *Escherichia coli* ATCC 25922, *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25923 (23,24]. The laboratory is participating in a bacteriology external quality assurance coordinated by a reference laboratory in South Africa (WHO/NICD). HIV testing was done using a Nigeria National algorithm 1251 and total WBC determined using haematology analyzer (Beckman Coulter (UK) Ltd).

Data analysis

Data collected were entered into a computer using epidata version 3.1 (CDC, Atlanta, USA) and analyzed using STATA version 11 (College Station, Texas, USA). Data were summarized in form of proportions and frequent tables for categorical variables. Depending on variable distribution either means with standard deviation or medians with interquartile range were used to summarize continuo us data. For non-parametric continuous variables Kruskal-Wallis and Mann—Whitney tests were performed to test for the significance of the difference of the medians between and within groups respectively. To determine predictors of bacteremia type, the multinomial logistic regression analysis was performed, starting with univariate then followed by multivariate multinomial logistic regress ion analysis with the nominal outcome being the type of bacteremia namely as no bacteremia, bacteremia due to salmonella and bacteremia due to none-salmonella pathogens. Based on patients with no bacteremia as the reference group, relative risk ratio with 95% confidence interval was calculated to test for strength of association between predictor variables and bacteremia type. On the other hand, to determine the predictors of mortality univariate followed by multivariate multinomial logistic regression were performed. Odds ratio with 95% confidence interval was calculated to test for strength of association between predictor variables and mortality. On both hands analyses, predictors with p-value less than 0.2 on univariate analysis were then fitted into the multivariate multinomial logistic regression and predictors with a p-value of less than 0.05 were considered to have significant strength of association.

Ethics statement

Ethical clearance was obtained from Nasarawa State ministry of Health.

Results

Demographic and other characteristics of the study population

A total of 346 patients were recruited of whom 170 (51.7%) were male and 167 (48.3%) were female The median age was 35 years (interquartile range (IQR) 25—48years).The median temperature was 38.5C (IQR 38-39'C). Of 346 patients 156 (45.1%) were HIV positive; of these, 112 (71.8%) were WHO clinical stage four, Only 48/156 (30.8%) of patients with HIV were receiving antiretroviral therapy (ART). See Table 1. A total of 307 (88.7%) received antibiotic treatment from other hospitals before they were referred to BMC; 33% used penicillin group (amoxicillin, ampicillin and cloxacillin), 27% used ceftriaxone, 20% used ciprofloxacin, 18.2% used cot rimoxazole, 10.6% used gentamicin, 8.7% used augment in and 7% used chloramphenicol.

Bacteremia and susceptibility pattern

Among the 346 febrile adults in our study, 33/346(9.5%) had positive blood stream bacterial infections. Among the 33 patients with positive blood cultures, 16/33 (48.5%) were HIV positive. Common bacterial isolates were *Salmonella spp* 13/33 (39.4%), *Escherichia coli* 8/33 (24.2%), *Streptococcus pneumonia* 5/33 (15.2%), *Staphylococcus aureus* 4/33 (12.1%), *Citrobacter spp* 1/33 (3%), *Streptococcus pyogenes* 1/33 (3%) and *Kiebsiella pneumonia* 1/33 (3%). Of *Salmonella spp* 12/13 (92.3%) were from HIV patients and all were identified as non-typhoid salmonella. *Salmonella spp* were 84.5%, 69.2%, 38.5%, 23% and 7.6% resistant to chloramphenicol, ampicillin, cotrimoxazole, gentamicin and ciprofloxacin respectively. All *Salmonella spp* isolates were sensitive to ceftriaxone and imipenem. *Escherichia colt* demonstrated multiple resistances to many commonly used antibiotics including:

ampicillin 6/8(75%), cotrimoxazole 6/8(75%), amoxicillin in/clavulanate 5/8 (62.5%), gentamicin 5/8 (62.5%), and ciprofloxacin 2/8 (25%).Three isolates (37.5%) were found to be ESBL producers and exhibited resistance to ceftazidime, cefotaxime and ceftriaxone.

Gram positive bacteria were 30% and 40% resistant to ampicillin and cotrimoxazole and they were all sensitive to vancomycin and imipenem.

Table 1 Baseline Demographic and clinical characteristic of 346 febrile adult patients

Admitted to 3 medical centers in Nasarawa State, Jan-Mar 2013.

CHARACTERISTIC	PROPORTION (%) OR MEDIAN (IQR)
SEX	
MALE	179(51.7%)
FEMALE	167(48.3%)
AGE in years	35(25-48)
MARITAL STATUS	
Never married	100 (28.9%)
Married	160(46.2%)
Divorced	41(11.8%)
Widow	45(13.1%)
HIV Status	
Positive	156(45.1%)
Negative	190 (54.9%)
ART Use among 156 HIV-Positive	
Yes	48 (30.8%)
No	108 (69.2%)

Table 2 Multivariate logistic regression analysis for predictors mortality for 346 febrile adult patient admitted To 3 medical center in Nasarawa State, Jan-Mar 2013.

PPREDICTIVE FACTOR YES **(n=86) (%)**	**MULTIVARIATE OR (95% cl)**	
		P-Value
Age (yrs.)		
< 50	21 (31.8)	1
>50	65(23.2)	1.7 (0.9-3.2) 0.119
Fever		
<40 ‘C	7	6(23.8) 1
>40 “C	10 (37.0)	1.5 (0.6-3.7) 0.437
Vomiting		
No	77 (26.2)	1
Yes	9 (17.3)	0.7 (0.4-1.7) 0.483
Neck Stiffness		
No	68 (22.0)	1
Yes	18 (48.7)	1.6 (0.7-3.7) 0.266
HIV		
No	32 (16.8)	1
Yes	54 (34.6)	2.3 (1.3-3.8) 0.006

Of HIV infected patients 12 (7.7%) were found to have salmonella bacteremia compared to 1(0.5%) of HIV negative patients. By univariate analysis, HIV-infection was found to predict bacteremia in both salmonella and non-salmonella bacteremia (RRR 14.8, 95%CI, 19—115, p=0.01). WBC count was found to predict both salmonella and non-salmonella bacterial infection, patients with salmonella infection had lower median of WBC when compared to patients with non-salmonella infection and those without bacterial infection (median, 3.6 vs. 17.5 vs. 9.8x109, p=0.0001). The risk of getting non-salmonella bacteremia increases as WBC increases (p=O.00l). On univariate as WBC increases by 1*109 there 1.2 times risk of having non-salmonella bacteremia compared with patients without bacterial infection; while as WBC increases the risk of salmonella bacteremia decreases (RRR= 0.9, p=O.O37). By multivariate analysis HIV positive (RRR 11.2, 95% CI, 1.3-98.7, p=O.029) and low ‘WBC were found to predict salmonella bacteremia. 0100 (28.9%) High WBC was found to predict non-salmonella bacteremia. 60 (462%) HIV patients with bacteremia had lower CD4+ 41(11.8%) counts than HIV patients without bacteremia (27.5 vs. 88 cells/ml, p=O.O1). A total of 54 (34.8%) of HIV infected patients die compared to 32 (14.8%) of HIV negative patients (p=O.001, OR 2.6 (1.6-4.3)). On univariate analysis these findings were found to be statistically significant. Other factors found to be predict mortality on univariate analysis were night sweats (p=O.OO3, OR 2.6 (95%CI 1.4-5.0), neck stiffness (p=O.001, OR 3.4 (1.7-6.8)) and anemia (p=O.O01, OR 3.3 (1.7-6.2)). anemia and HIV positive were confirmed to predict mortality on multivariate logistic regression analysis (Table 2).

Other causes of fever

Of 346 patients; 97(28%) were found to have positive malaria blood slide; with 9 (2.6%) having both bacteremia and malaria. A total of 116 (33.5%) were diagnosed to have tuberculosis using microscopy for AFB and chest X-ray; of which 79(68.1%) and 37(31.9%) had pulmonary and extra-pulmonary tuberculosis respectively. A total of 16(4.6%) of adults were diagnosed to have bacteremia

Discussion

Bacteremia in developed countries accounts for a significant ant burden of disease and has been implicated as the 11(22.9%) leading cause of non-cardiac death amongst critically ill 5 (10.4%) patients [1,26]. The exact burden of bacteremia in low (16.7%) income countries remains unknown. In the present study 1 (2.1%) the prevalence of bacteremia among febrile adult patients 1 (21%) was 9.5%; similar findings have been reported in other 242% developing countries where routine blood culture is per formed [3, 13]. The finding may be lower than that 1 (2.1%) reported in developed countries a finding which could be explained by culture techniques used which does not favour growth of fastidious organisms. Higher incidences of bacteremia have been reported in immunocompromised patients. Several studies [2, 3,] from sub-Saharan Africa have revealed an association between HIV infection and an increased likelihood of bacteremia and

Mortality. This has been confirmed in the present study whereby the bacteremia was significantly higher in HIV positive patients than in HIV-negative patients. As in previous studies [3, 15] patients who were HIV positive had 11.2 times risk of having non typhoid salmonella bacteremia when compared to HIV-negative patients. Studies on bacteremia among febrile adults are few in East Africa. But in a prospective observation study [15] on sepsis in two Ugandan Hospitals, it was found that most patients with bacteremia were HIV infected with a median CD4+ count of 52 cells/mm3. Another factor which was found to predict bacteremia in the present study is WBC count; both HIV and non-HIV infected patients with non-salmonella bacteremia had higher count of total WBC compared to patients with non-typhoid salmonella bacteremia and patients without bacterial infection. On the other hand, patients with non-typhoid salmonella bacteremia had a lower WBC count than those without bacteremia. Also in this study it was noted HIV patients with bacteremia had significantly lower CD4+ count than HIV patients without bacteremia, a finding that is similar to other studies [27,28].Results of antimicrobial susceptibility tests revealed that most *Salmonella spp* were susceptible to ceftriaxone and imipenem while being highly resistant to chloramphenicol, ampicillin and cotrimoxazole this has been reported before [32]. Resistance rate of 8% of salmonella isolates to ciprofloxacin is relatively high compared to a study in Pemba [12] which found resistance rate of 1.2% and in a recent study in Kenya [11] in which none of salmonella isolates were found to be resistant to ciprofloxacin m. High resistance rate to ciprofloxacin could be explained by the fact that there is overuse of ciprofloxacin m in the primary health facilities; this was confirmed in this study. This calls for a continuous surveillance of ciprofloxacin resistance salmonella isolates in our setting. Other Gram-negative bacteria also showed good sensitivity patterns to imipenem but they were multiply resistant to ampicillin, amoxicillin/clavulanate, gentamicin m and cotrimoxazole. Fifty percent of gram-negative enteric isolates were resistant to third-generation cephalosporin. All isolates resistant to third-generation cephalosporin were found to produce extended spectrum beta-lactamase. The multi-drug resistance patterns observed in this study are similar to those earlier reported [22]. Predictors of mortality were night sweats ($p = 0.014$), HIV status ($p = 0.006$) and Glasgow Coma score ($p < 0.001$). These findings are similar to a prospective observation study on sepsis in two Ugandan hospitals which showed that clinical predictors of in-hospital mortality included variables easily measurable in any setting such as morbidity assessment scales (i.e., KPS and GCS), vital signs (i.e., RR>30 breaths/mm), leukocytosis and thrombocytopenia[15]. The high mortality among HIV infected adults could be explained by the fact majority of them had other co-morbidities like tuberculosis [5].The present study, thus, established that non-typhoid Salmonella bloodstream infection is a common occurrence in HIV-positive patients attending three Medical Centre in Nasarawa state. The majority of the study patients with salmonella bloodstream infection had low WBC. The resistance rate to commonly used antibiotic is high. Therefore, a continuous surveillance and intervention strategy should be put in place to manage cases of bloodstream infections in HIV-positive patients in Nasarawa State, Nigeria.

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