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Full Length Article

Staphylococcal bacteraemia among human immunodeficiency virus positive patients at a screening center in Lagos, Nigeria

Solayide A. Adesida [a,*], Olusegun A. Abioye [b], Babajide S. Bamiro [c], Kehinde O. Amisu [d], Sikiru O. Badaru [e], Akitoye O. Coker [f]

[a] Department of Microbiology, Faculty of Science, University of Lagos, Nigeria
[b] Leicester School of Pharmacy, Faculty of Health and Life Sciences, DeMontfort University, The Gateway, Leicester LE1 9BH, United Kingdom
[c] Microbiology and Endocrinology Research Laboratory, Department of Obstetrics and Gynaecology, College of Medicine, University of Lagos, Nigeria
[d] Department of Microbiology, Lagos State University, Apapa – Badagary Expressway, Ojo, Lagos, Nigeria
[e] Epidemiology Division, Nigeria Centre for Disease Control, Abuja, Nigeria
[f] Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria

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ABSTRACT

Bacteraemia due to *Staphylococcus aureus* in Human immunodeficiency virus (HIV) – positive patients is associated with increased mortality rate. The present study aimed at determining the species distribution and occurrence of staphylococcal bacteraemia in HIV – positive patients in Lagos, Nigeria. Staphylococcal blood stream infection in febrile HIV patients was investigated by culture technique. The antibiotic resistance pattern was investigated using the disk diffusion and methicillin resistance was confirmed by the salt agar methods. The genetic relatedness of *S. aureus* was determined using Pulsed Field Gel Electrophoresis (PFGE). Eighty-six patients comprising 47 (55%) female and 39 (45%) male, median aged 34 years took part in the study. Staphylococci were identified in 16 (18.6%) patients; 13 (15.1%) and 3 (3.5%) with single and dual *Staphylococcus* species respectively. The isolates consisted of *S. aureus* (7 patients), followed by *S. haemolyticus* (4 patients). Of the thirteen (13) antibiotics tested, isolates were resistant to ampicillin (AMP; 89.5%), tetracycline (TET; 68.4%), cloxacillin (CXC; 89.5%), oxacillin (OXA; 68.4%); chloramphenicol (CHL; 57.9%) and trimethoprim-sulphamethoxazole (SXT; 63.1%). The overall percentage of all the isolates resistant to gentamicin, erythromycin and amoxicillin-clavulanic acid was less than 50%. All the isolates were susceptible to ciprofloxacin and vancomycin and none was positive for methicillin resistance except a strain of *S. haemolyticus*. Significant genetic diversity was observed among the *S. aureus* isolates with a predominant pulsotype A. The two isolates with pulsotype A had identical resistotype (AMP, ERY, TET, CXC, SXT). Other PFGE patterns were represented by single isolates except pulsotype C which had a subtype. In these patients, the most frequent *Staphylococcus* species isolated was *S. aureus* and the results revealed that clonal dissemination of a virulent pulsotype of *S. aureus* among this population is plausible and should be a cause for concern.

1. Introduction

Human immunodeficiency virus (HIV)-infected individuals are often at risk of various opportunistic bacterial infections (Moges and Kassa, 2014). This subject has special implication in developing countries where occurrence of bacterial infections remains high due to poverty and poor hygiene. Staphylococci are particularly considered as important pathogens in HIV-infected patients (Varma et al., 2010; Furuno et al., 2011; Haddy et al., 2012) and are responsible for substantial cases of bacteraemia or blood stream infection (BSI) which may be followed by infective endocarditis with considerable morbidity and mortality (del Rio et al., 2009). Though, the epidemiology of the bacteraemia varies according to demographic and geographical factors (Huson et al., 2014; Taramasso et al., 2016), in the United States and Europe, BSIs in HIV-infected patients have been studied extensively (Tumbarello et al., 2002; Ortega et al., 2008; Furuno et al., 2011). Senthilkumar et al. (2001) established that the frequency of staphylococcal bacteraemia among hospitalized HIV-positive

* Corresponding author.
E-mail addresses: sadesidashittu@gmail.com, sadesida@Unilag.edu.ng (S.A. Adesida), olusegunabioye@yahoo.com (O.A. Abioye), jidebamiro@yahoo.co.uk (B.S. Bamiro), koamisu@yahoo.com (K.O. Amisu), badaru@yahoo.com (S.O. Badaru), aocoker@hotmail.com (A.O. Coker).

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patients was 16.5-fold greater than the frequency of such episodes among HIV-negative patients.

In the past few decades, the prevalence of antibiotic resistant *Staphylococcus* is presumed to increase in HIV-infected patients. At a general teaching hospital in Sao Paulo, Brazil, Conterno et al. (1998) recorded 39% mortality rate among 136 HIV-infected patients within 14 days of developing bacteraemia and methicillin resistant *S. aureus* (MRSA) was responsible for 66% of the cases (90/136). MRSA bacteraemia is associated with significantly higher mortality than methicillin-susceptible *S. aureus* bacteraemia (Cosgrove et al., 2003). Coagulase negative staphylococci (CoNS) have also emerged as important causes of BSI (NNIS, 2001; Rahman et al., 2013), though, they may not induce sufficient inflammatory response because of their low virulence. Yet, most patients with coagulase negative staphylococcal BSI may have atypical clinical manifestations and laboratory indices of infection (Hirakata et al., 1996). Thus, it is crucial to recognise that CoNS can represent true bacteraemia with devastating consequences, particularly if untreated due to misinterpretation as contaminants. For HIV patients, CoNS bacteraemia is being increasingly reported (Bonadio et al., 1998; Adeleye et al., 2010).

In Nigeria, many literature reports have emphasised the socio-economic impacts of the HIV infections (Odu and Akanle, 2008; Agaba et al., 2011). Although, septicaemia was a common indication for hospitalisation and contributed to 17.1% (21 out of 354) of recorded in-hospital deaths among HIV-infected Nigerians (Agaba et al., 2011), bacteraemia due to staphylococcal species among this group of patients remains to be elucidated. Consequently, this study examines the occurrence of staphylococcal bacteraemia in HIV-positive patients and describes the spectrum of staphylococcal species in bacteraemic cases.

2. Materials and methods

2.1. Study population

The study was conducted at an out-patient service center for screening HIV/AIDS patients over 4 months. The center provides diagnostic testing only and further methodical clinical assessment and treatment of patients are performed by the physicians and management teams in different hospitals where the patients are referred from. All patients included in the study were HIV-1 positive patients with febrile conditions indicated by an elevation of body temperature above the normal range of 36.5–37.0 °C. Staphylococcal bacteraemia was defined as isolation of the same *Staphylococcus* species in two independent blood cultures obtained from the same patients during the screening processes. To be included in the study, the patient was required to have had a temperature of approximately 38 °C when the blood sample was collected and were willing to give their informed consent. Body temperatures were taken with a digital thermometer. The exclusion criteria included patients below 18 years of age, patients who declined to participate and those who had blood culture isolates other than *Staphylococcus* species from their samples. Demographic data obtained from patients include age and sex. Permission to conduct the study was obtained from Lagos State Health Service Commission and written informed consent was obtained from every patient who participated in the study.

2.2. HIV testing

The HIV test was performed on a single blood sample using a rapid enzyme immunoassay (Genie II HIV-1/HIV-2: Sanofi Pasteur) and confirmed with NEW LAV BLOT (Biorad, France) according to manufacturers' recommendations. The technologists who participated in the phlebotomy and Western blot testing were trained personnel of the referral center.

2.3. Blood culture

Blood was aseptically collected from the patients using our in-house protocol. Briefly, venipuncture sites were cleaned with 70% ethyl alcohol, then 2% tincture of iodine and allowed to dry for 30–60 s. Carefully avoiding any visible lesion on the skin, 10 ml of blood was drawn from the patients and dispensed into Oxoid signal blood culture system (Oxoid, UK) as instructed by the manufacturer. Duplicate individual's blood culture was performed with blood obtained at a separate venipuncture and incubated at 37 °C for 48 h. The culture systems were examined for turbidity daily and then plated out on Mueller Hinton agar (Oxoid, UK), incubated for 18–24 h at 37 °C.

2.4. Bacterial identification

Bacterial colonies suspected to be staphylococci on the primary plates were Gram-stained and further investigated for mannitol fermentation, catalase and coagulase activities (Cowan and Steel, 1993). Species identification was accomplished with API staph kit (bioMérieux, Marcy l'Étoile, France).

2.5. Susceptibility testing

Using the disk diffusion technique, antimicrobial susceptibility testing of all isolates was carried out according to the guidelines of the Clinical and Laboratory Standards Institute (2006). The antimicrobials tested were Ampicillin (AMP; 10 μg), Gentamicin (GEN; 10 μg), Chloramphenicol (CHL; 30 μg), Amoxicillin-Clavulanic acid (AMX; 30 μg), Erythromycin (ERY; 15 μg), Tetracycline (TET; 30 μg), Cloxacillin (CXC; 5 μg), Vancomycin (VAN; 30 μg), Trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 μg), Oxacillin (OXA: 1 μg) and Ciprofloxacin (CIP; 5 μg). Quality control was achieved with *S. aureus* ATCC 2921 and *S. epidermidis* ATCC 14990 strains. Methicillin susceptibility was confirmed phenotypically by the salt agar method after 24 h incubation on Muller-Hinton agar containing 4% sodium chloride (Ribeiro et al., 2005). MRSA ATCC 43300 was included as positive control.

2.6. Pulsed Field Gel Electrophoresis (PFGE)

All *S. aureus* isolates were subjected to PFGE (Fowler Jr et al., 1999) after *Sma*I enzyme restriction digestion (Roche Molecular Biochemicals). Restriction fragments were resolved on a Chef DR-III (PFGE) apparatus (Bio-Rad). Gels were stained with ethidium bromide and PFGE images were photographed. Banding patterns were analyzed by visual inspection through counting the number of band differences and isolates were assigned to PFGE type based on established criteria (Tenover et al., 1995).

2.7. Data analysis

Data analysis was performed using Epi-Info version 6.4 software. The significance level was set at $P < 0.05$. Descriptive statistics such as means and frequencies were used to express the results.

3. Results

Eighty-six (86) blood samples from consented HIV-1 seropositive patients were analysed. The characteristics of the entire study group are shown in Table 1. The median age of the patients was

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