OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Co- infection of Methicillin Resistant *Staphylococcus aureus* Bacteremia and Malaria in Children Attending Cottage Hospital Ogidi Ilorin Kwara State, Nigeria

Muritala Issa Bale[1*], Shola Kola Babatunde[2] and Sunday Awe[1]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University Malete, Nigeria
[2]Department of Natural and Environmental Sciences, Crown Hill University Eiyekorin, Nigeria

DOI: 10.36348/sjpm.2021.v06i09.006 | **Received:** 02.08.2021 | **Accepted:** 04.09.2021 | **Published:** 20.09.2021

***Corresponding author:** Muritala Issa Bale

Abstract

Staphylococus aureus is a major agent of bacteremia in children in African where malaria is endemic and always presented with the same symptoms. The main objective of this study is to determine the co-infection of malaria and *Staphylococcus aureus* bacteremia in children less than 5 years using standard procedures from a major secondary health center in Ilorin, Kwara State and determine antibiotics susceptibility of *S. aureus*. A total number of 235samples blood from febrile children suspected of having bacteremia were screened, 138 of the children were females and 97 were males. A total of 39 (16.6%) with positive blood culture was recorded in this study and *Staphylococcus aureus* bacteremia (SAB) detected is 20.5%. Age distribution of bacteremia were 14.3, 17.4, 27.3, 13.5 and 14.3 % for children in the age group ≤1, 1-2, 2-3, 3-4 and 4-5 years respectively while SAB distribution were 24.4, 12.5, 22.2, and 66.6 for children in the age group 1 month-1, 1-2, and 4-5 years above respectively. Also, a total number of 148 (63%) have a positive malaria parasite test, co-infection of malaria and bacteremia detected in 18 children with 6 (33.3%) having SAB and malaria co-infection. Age distribution of co-infection of malaria and SAB were 33.3, 50, and 25% with no co-infection recorded in age group 3-5years. A total of 2 (25%) methicillin resistant *Staphylococcus aureus* were isolated. Antibiotic sensitivity profile shows that 62.5% of *S. aureus* were resistance to cefuroxime, 80% to cefepime and 100% sensitivity to augmentin, linezolid, teicoplanin, ofloxacin, gentamicin and vancomycin. Age is an important risk for development of SAB and prompt treatment with a suitable antibiotics is required to reduce mortality associated with bacteremia.

Key words: Co-infection, *Staphylococcus aureus*, bacteremia, malaria , MRSA.

INTRODUCTION

Bacteremia is a one of the major and serious infections caused by *Staphylococcus aureus* and *Staphylococcus aureus* bacteremia (SAB) infections are one of the most difficult infection with significantly higher morbidity and mortality compared with bacteremia caused by other microorganisms (Naber, 2009).

Entrance of *S. aureus* into the blood stream can result in its dissemination, resulting in metastatic and serious infection of almost all the internal organs of the body leading to many complicatons such as infective endocarditis (Tong *et al.*, 2015). The increase incidence of SAB complications are mainly due to higher number of immune-compromised patients, increase frequency of invasive surgeries and use of intravascular devices (Rasmussen *et al.*, 2011). Invasive bacterial diseases was responsible for about 1.4 million worldwide and Nigeria as the largest country in Sub Saharan African contributes large percentage to the global mortality rate of 100-250 per 1000 in under 5 years children (WHO, 2019). Also research has shown that mortality as a result of non typhoidal *Salmonella*- bloodstream infection in children below 5 years exceeds that of malaria globally (Takem *et al.*, 2014). Studies have also shown that more than 50% of SAB patients lack clinical symptoms consistent with staphylococcal infection making drug prescription difficult thus resulting in high mortality (Ahman and Zupan, 2007).

Despite the well documentation of severity and frequency of *S aureus* bacteraemia, the choice of antibiotics is not well documented most especially in the developing world. This study thus aimed at

determining the determining co-infection of *Staphylococcus aureus* bacteremia and malaria in a secondary health centre in Ilorin, Nigeria.

Ethical Considerations

Ethical approval was approved by Kwara State Ministry of Heath, Kwara State University Center for Community development while all the parents/ guardians of the participants gave a written consent.

Study Area

The study was conducted between October, 2017 and December, 2020 at Cottage Hospital Ogidi a Seconadary Health Center in Ilorin, the Capital City of Kwara State, Nigeria.

Blood Sample collection

The physician that is well informed on the study protocol collects the venous blood aseptically by venipuncture from children into a sterile syringe using a butterfly attachment.

This is done after cleaning thoroughly the patient's skin with alcohol and allowing the skin to dry before the blood samples were collected. About 2 mL to 3mL of blood was cultured in Brain heart infusion broth unless these volumes could not be collected, in such case the maximum volume that was collected was cultured. Samples were transported to the laboratory immediately for appropriate analysis. Two drops of blood were each place on separate spots of a slide. A spot was spread to make thick blood film while the second was spread to make thin blood film.

Patients

All the febrile children suspected of bacteremia were screened for *S. aureus* bacteremia as well as malaria parasite.

Malaria Screening

Malaria parasite screening was done by microscopy of thick and thin blood films. The thin film formed was fixed thoroughly with methanol, after which the slide was stained with a 1:10 dilution of freshly prepared Giemsa stain for 10 minutes. The stain is later washed off with buffer water and allow to air-dried. The stain was subsequently examined under the light microscopy at ×1000 magnification.

Bacterial isolation and identification

The blood cultures were incubated at 37°C for seven consecutive days. After 24hours incubation, a sterile loopfuls of broth were sub-cultured on to nutrient agar and blood agar and incubated aerobically for 18-24 hours at 37°C.

On a daily basis haemolysis, broth coagulation and gas production (air bubbles presence) were observed for the presence of positive growth of micro-organisms. Subcultures were done on the 3rd, 5th and 7th day on selective and enriched media such as Blood agar and mannitol salt agar.

The agar plates were subsequently incubated and colonies on solid agar plates were later identified based on characteristic morphology and biochemical tests.

Isolation and identification of *Staphylococcus aureus* was done through the exploitation of its biochemical activities and culture characteristics using appropriate standard microbiological techniques. These include usage of selective culture media, catalase production, Gram reaction, slide and tube coagulase test.

Antibiotic susceptibility testing

Resistance and susceptibility to antimicrobial agents used in the treatment of blood stream infections was done by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2018).The antibiotic discs to be used include Linezolid (30 µg), Teicoplanin (30 µg), augmentin (30 µg), gentamicin (10 µg), ofloxacin (5 µg),cefuroxime (30 µg), vancomycin (30 µg) and cefepime (30 µg) All from Oxoid UK. The discs was placed on to the surface of inoculated Mueller- Hinton agar plates. After overnight/ 24hours incubation, the inhibition zone diameters was measured to the nearest millimeter. Antimicrobial susceptibility testing was performed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). *S. aureus* ATCC 25923 was used as controls. Phenotypic identification of MRSA was done by determination of resistance to cefoxitin (30 µg).

RESULTS

Table-1: Age distribution of children with *Staphylococcua aureus* bacteremia and *Plasmodium falciparium* co-infection

Age	Total Sample	Positive bacteria growth	SAB	*P. falciparum*	*P. falciparum* and bacteremia	*P. falciparum* and SAB
0≥1	98	14	3	53	6	2
1≥2	46	8	1	27	6	3
2≥3	33	9	2	21	4	1
3≥4	37	5	-	29	2	-
4≥5	21	3	2	18	-	-

percentage

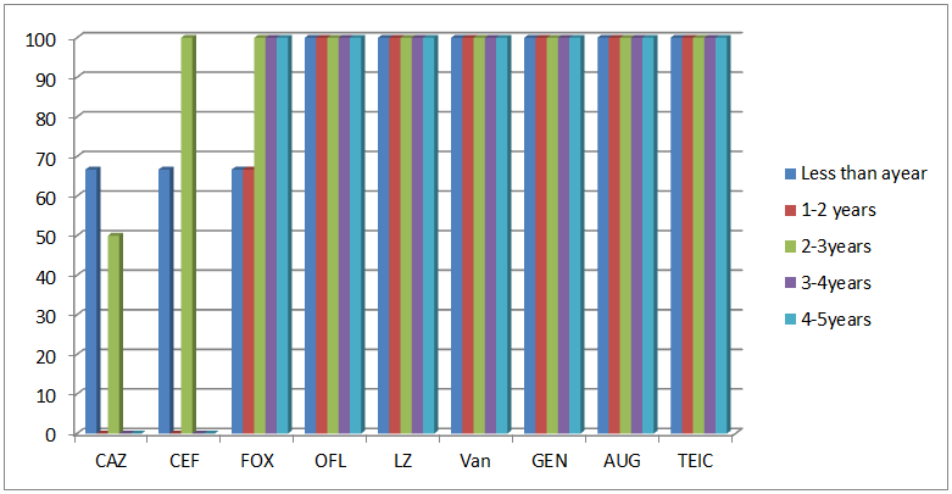

Fig-1: Sensitivity profile against *S.aureus* isolated fromblood of children attending Ogidi Cottage Hospital
Key: Caz= cefuroxime, CEP= cefepime, Fox= cefoxitin, OFL =Linezolid, Van=Vancomycin, Gen=Gentamicin, Aug=augmentin Teic= teicoplanin

DISCUSSION

This study examined a total number of 235 blood from children with febrile condition suspected of having bacteremia or malaria infection who are under 5 years old at a major Secondary Health Center in Ilorin (Cottage Hospital Ogidi), 138 of the children are females while the remaining 97 were males. A total of 39 (16.6%) positive blood culture was recorded in this study. This is higher than 10.8% recorded by Obaro *et al*. (2011) at Abuja but lower than 22.4% reported by Arowosegbea *et al.* (2017). Also in this study the prevalence of *Staphylococcus aureus* bacteremia (SAB) detected is 20.5%. Obaro *et al*. (2011) reported that *Staphylococcus aureus* is the number causative agent of bacteremia in Nigeria. The total children with *Plasmodium falciparium* parasitemia were 148 (63%) and Co- infection of malaria and bacteremia is seen in 7.7% with co-infection of malaria and SAB been 33.3%. In most Africa countries including Nigeria where malaria is endemic, majority of febrile presentation are always presumed as malaria, however this study shows that bacteremia is alaway a major factors that could responsible as reported in many other studies (Chowdhary *et al.*, 2006; Fortuin-de *et al.*, 2015; Carpenter *et al.*, 2016; McMullan *et al.*, 2016; Arrieta *et al.*, 2018). Age distribution shows that 0-1 the highest bacteremia (27.3%) compare to other age groups while the highest SAB (66.6%) were detected in the children between 4years to 5 years old. Children between the age group 3 years to 4 years have the highest *Plasmodium falciparium* parasitemia with 78.4% while the lowest were seen in children less than 1 year old. Also the the highest co infection of malaria and bacteremia were seen in age group 1 year to 2 years old with 13% with the lowest seen in age group 3- 5 years old. The lowest co-infection of SAB and plasmodium falciparium were seen in children between the age group 3 years to 5 years while the highest was recorded in children between the age group 1year to 2 years. One of the major risk factors for SAB is young age, infants of I year of age have been reported in some studies to have higher incidence of SAB in comparison to children with older age (CDC, 2016).

In a study by Vanderkooi *et al.* (2011), 7/ 100, 000 popualtion bacteremia prevalence were recorded in infants and 124.8/ 100,000 recorded in the neonates. Also Shane *et al.* (2012) reported that younger age and lower birth weight is proportional to the frequency of SAB while Kempley *et al.* (2021) repored that theses factors is associated with poorer outcome in Neonatal intensive care unit.

Also, incidence of SAB in children have been shown to correlate with household crowding, geographic factors and or socioeconomic status (Tong *et al.*, 2012). In the United State of America, African-American ethnicity has been shown to be associated with a higher incidence of invasive methicillin-resistant *S aureus* (MRSA) infection compare to white (Kempker *et al.*, 2010), however in another study by Shane *et al.* (2012) in NICU, no significant difference was reported regarding ethnicity. One of the major factors responsible for development of SAB in this study as reported else where could be malnutrition, previous hospitalization, presence of central venous catheter, HIV/AIDs infection, and residence in a long-term care facility (Liu *et al.*, 2011). Also *S aureus* frequently cause bacteremia in healthy children but Hamdy *et al.* (2017) reported 48% of children having MRSA bacteremia lacked any serious underlying medical conditions.

However, medical co-infection is a major factor in development of bacteremia. SAB can vary in severity from mild infection to severe form of infection, and apparently asymptomatic isolation from the blood stream as a result of contamination is rare (Roediger *et*

al., 2017) therefore, any positive culture with *S. aureus* is always considered to clinically significant and treated promptly. Antibiotic sensitivity against the isolated *Staphylococcus aureus* shows that 62.5% and 80% resistance was recorded against cefuroxime and cefepime respectively with 100% sensitivity recorded againt augmentin, linezolid, teicoplanin, ofloxacin, vancomycin and gentamicin. Age distribution shows that the highest resistance (50%) were recorded against cefuroxime in children in the age group 2 years to 3 years. A total of 2 (25%) methicillin resiatance *Staphylococcus aureus* (MRSA) was detected in this study with 100% resistance against cefuroxime which is one of the major antibiotics used in empirical treatment of SAB in children. This percentage is higher compare to the study by Oren *et al.* (2019) involving 427 episodes of SAB, where 284 SAB were hospital acquired and 15% MRSA detected with 2% Community acquired -MRSA.

Empirical pretreatment is a common in the study hospital and drug abuse by the patients before getting to hospital is a major factor that could contribute to antimicrobial resistance. Higher antimicrobial usage in the community could also be responsible for some patients with bacteremia to postpone seeking additional healthcare thus reducing pathogen recovery rate in those who do seek medical support. As noticed in this study beta lactam antibotics has been reported as first line drug in the treatment of MSSA-B with a superior outcome compare to glycopeptides. Furthermore, McMullan *et al.* (2016) reported increase in mortality in children treated with glycopetides in a study involving 1000 cases of SAB. Vancomycin is the first-line and major antibiotics for the MRSA bacteremia and beta lactam allergic patients at starting doses of 45 to 60mg/kg per day while linezolid an oxazolidinone antibiotic with a very high tissue penetration and bioavailability is also a drug of choice that could be used in the treatment of MRSA infections, howvever toxicity from linezolid usage may include peripheral neuropathy, optic and bone marrow suppression and this could occur after third week of treatment (Gould, 2011).

Gentamicin sensitivity in this study is 100% sensitivity however due to increased risk of nephron-toxicity gentamicin therapy is no longer indicated in the treatment of SAB (Selby *et al.*, 2009).

CONCLUSIONS

Staphylococcus aureus bacteremia is a major factors to be considered in febrile children reported to hospital as malaria may not be responsible and it is recommended that care must be taken in the choice of antibiotics in pretreatment of bacteremia. Also, setting up of microbiology laboratory in all the hospitals should be a priority and early screening of bacteremia using high technology equipment is also recommended for early diagnosis and prompt treatment to reduce mortality rate associated with bacteremia.

REFERENCES

- Ahman, E., Zupan, J. (2007). Neonatal and perinatal mortality: country, regional and global estimates 2004. Geneva: World Health Organization;.
- Ahman, E., Zupan, J. (2007). Neonatal and perinatal mortality: country, regional and global estimates 2004. Geneva: World Health Organization.
- Arowosegbea, A. O., David, A. O., Iyabode, O.D., Olufunke, B.S., & Olusola, A.A. (2017). Neonatal sepsis in a Nigerian Tertiary Hospital: Clinical features, clinical outcome, aetiology and antibiotic susceptibility pattern *Southern African Journal of Infectious Diseases*; *32*(4); 127–131 https://doi.org/10.1080/23120053.2017.1335962
- Arowosegbea, A. O., David, A.O., Iyabode, O.D., Olufunke, B.S., & Olusola, A.A. (2017). Neonatal sepsis in a Nigerian Tertiary Hospital: Clinical features, clinical outcome, aetiology and antibiotic susceptibility pattern *Southern African Journal of Infectious Diseases*; *32*(4):127–131 https://doi.org/10.1080/23120053.2017.1335962
- Arrieta, A.C., Bradley, J.S., Popejoy, M.W. (2018). Randomized multicenter study comparing safety and efficacy of daptomycin versus standard-of-care in pediatric patients with staphylococcal bacteremia. *Pediatr Infect Dis J, 37*(9); 893–900
- Carpenter, S.L., Goldman, J., Sherman, A.K. (2016). Clinical variables and *Staphylococcus aureus* virulence factors associated with venous thromboembolism in children. *Thromb Res*. 138:69–73
- Centers for Disease Control and Prevention. (2015). Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Methicillin-Resistant *Staphylococcus aureus*,. Atlanta, GA: Centers for Disease Control and Prevention;
- Chowdhary, G., Dutta, S., Narang, A. (2006). Randomized controlled trial of 7-day vs. 14-day antibiotics for neonatal sepsis. *J Trop Pediatr, 52*(6); 427–432
- Clinical and Laboratory Standards Institute. (aro201). Performance standards for antimicrobial susceptibility testing. Wayne, PA: CLSI,:258.
- Fortuin-de Smidt, M.C., Singh-Moodley, A., Badat, R. (2015). *Staphylococcus aureus* bacteraemia in Gauteng academic hospitals, South Africa. *Int J Infect Dis*, 30;41-48
- Gould, F.K. (2011). Linezolid: safety and efficacymin special populations. *J Antimicrob Chemother*, 66(suppl 4):iv3-I v6
- Hamdy, R.F., Hsu, A.J., Stockmann, C. (2017). Epidemiology of methicillin-resistant

Staphylococcus aureus bacteremia in children. *Pediatrics*, *139*(6): e20170183

- Hayk, M. (2019). Sepsis: mechanisms of bacterial injury to the patient *Minasyan Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine;* 27; 19
- Kempker, R.R., Farley, M.M., Ladson, J.L., Satola, S., Ray, S.M. (2010). Association of methicillinresistant *Staphylococcus aureus* (MRSA) USA300 genotype with mortality in MRSA bacteremia. *J Infect*, *61*(5); 372–381
- Kempley, S., Kapellou, O., McWilliams, A., Banerjee, J., McCorqodale, A., Millar, M. (2021). Antibiotic treatment duration and PEDIATRICS 146, number 3, September 2020 11 Downloaded from www.aappublications.org/news
- Liu, C., Bayer, A., Cosgrove, S.E. (2011). Infectious Diseases Society of America. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin resistant *Staphylococcus aureus* infections in adults and children *Clin Infect Dis.*
- McMullan, B.J., Bowen, A., Blyth, C.C. (2016). Epidemiology and mortality of *Staphylococcus aureus* bacteremia in Australian and New Zealand children. *JAMA Pediatr*, *170*(10); 979–986
- Naber, C. K. (2009). *Staphylococcus aureus* Bacteremia: Epidemiology, Pathophysiology, and Management Strategies *Clinical Infectious Diseases*, 48, S231–S237, https://doi.org/10.1086/598189
- Obaro, S., Lovett, L., Uduak, E., Khalid, I., Kevin, B., Adekunle, O., Denis, S., Patience Ahmed, Theresa, A., Michael Olugbile3, David Idiong3, Damola O.,Comfort O., Grace O., Walid K. and Richard A. (2011). Community Acquired Bacteremia in Young Children from Central Nigeria- A Pilot Study *BMC Infectious Diseases*, 11; 137 http://www.biomedcentral.com/1471-2334/11/137
- Oren, G., Cohen, M., Gross, I., Amit, S., Averbuch, D., Engelhard, D., Milstone, Aaron M., Moses, A. E. *Staphylococcus aureus* Bacteremia in Children: Antibiotic Resistance and Mortality *The Pediatric Infectious Disease Journal*: doi: 10.1097/INF.0000000000002202
- Perl, T.M., Golub, J.E. (1998). New Approaches to Reduce *Staphylococcus aureus* Nosocomial Infection Rates: Treating *S. aureus* Nasal Carriage. *In: Ann Pharmacother*, 32. 1998:S7–S16
- Popoola, O., Aderemi, K., Veronica, O., Oluwafemi, J. Trevor, T. (2017). Bacteremia Among Febrile Patients Attending Selected Healthcare Facilities in Ibadan, Nigeria *Clinical Infectious Diseases*
- Rasmussen, R. V., Fowler Jr, V. G., Skov, R., & Bruun, N. E. (2011). Future challenges and treatment of Staphylococcus aureus bacteremia with emphasis on MRSA. *Future microbiology*, *6*(1), 43-56.
- Roediger, J.C., Outhred, A.C., Shadbolt, B., Britton, P.N. Paediatric. (2017). *Staphylococcus aureus* bacteraemia: a single-centre retrospective cohort. *J Paediatr Child Health*, *53*(2); 180–186
- Shane, A.L., Hansen, N.I., Stoll, B.J. (2012). Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Methicillin-resistant and susceptible Staphylococcus aureus bacteremia and meningitis in preterm infants. *Pediatrics,129*(4). Available at: www.pediatrics.org/cgi/content/full/129/4/e914
- Selby, N. M., Shaw, S., Woodier, N., Fluck, R. J., & Kolhe, N. V. (2009). Gentamicin-associated acute kidney injury. *QJM: An International Journal of Medicine*, *102*(12), 873-880.
- Takem, E. N., Anna, R., & Aubrey, C. (2014).The association between malaria and nontyphoid Salmonella bacteraemia in children in sub-Saharan Africa: a literature review *Malaria Journal* 13:400 http://www.malariajournal.com/content/13/1/400.
- Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*, *28*(3), 603-661.
- Tong, S. Y., Van Hal, S. J., Einsiedel, L., Currie, B. J., & Turnidge, J. D. (2012). Impact of ethnicity and socio-economic status on Staphylococcus aureus bacteremia incidence and mortality: a heavy burden in indigenous Australians. *BMC infectious diseases*, *12*(1), 1-9.
- Van Hal, S. J., Jensen, S. O., Vaska, V. L., Espedido, B. A., Paterson, D. L., & Gosbell, I. B. (2012). Predictors of mortality in Staphylococcus aureus bacteremia. *Clinical microbiology reviews*, *25*(2), 362-386.
- Vanderkoo, O. G., Gregson, D. B., Kellner, J. D., & Laupland, K. B. (2011). Staphylococcus aureus bloodstream infections in children: a population-based assessment. *Paediatrics & child health*, *16*(5), 276-280.
- WHO. (2019). Malaria.