Concomitant bacteremia in adults with severe falciparum malaria

Nguyen Hoan Phu[1,2], Nicholas PJ Day[2,3,4], Phung Quoc Tuan[1], Nguyen Thi Hoang Mai[1], Tran Thi Hong Chau[1], Ly Van Chuong[1], Ha Vinh[1], Pham Phu Loc[1], Dinh Xuan Sinh[1], Nguyen Thi Tuyet Hoa[1], Deborah J Waller[2], John Wain[2,5], Atthanee Jeyapant[3], James A Watson[3,4], Jeremy J Farrar[2], Tran Tinh Hien[1,2], Christopher M Parry[2,6,7,8], Nicholas J White[2,3,4]

1. Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam
2. Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam
3. Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
4. Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, United Kingdom.
5. Quadram Institute Bioscience, Norwich, UK
6. Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
7. Institute of Infection and Global Health, University of Liverpool, UK
8. School of Tropical Medicine and Global Health, Nagasaki University, Japan.

Correspondence: Professor NJ White: Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Rd, Bangkok, 10400, Thailand. Tel: +662 203 6301 Email: nickw@tropmedres.ac.

***Brief summary*:** Blood cultures were positive in 9 of 845 sequentially studied Vietnamese adults with severe falciparum malaria. In contrast to children, concomitant bacteremia in adults with severe malaria is uncommon, and does not warrant use of empirical antibiotics in all patients.

Abstract

Background: Approximately 6% of children hospitalised with severe falciparum malaria in Africa are also bacteremic. It is therefore recommended that all children with severe malaria should receive broad spectrum antibiotics in addition to parenteral artesunate. Empirical antibiotics are not recommended currently for adults with severe malaria.

Methods: Blood cultures were performed on sequential prospectively studied adult patients with strictly defined severe falciparum malaria admitted to a single referral centre in Vietnam between 1991 and 2003.

Results: In 845 Vietnamese adults with severe falciparum malaria admission blood cultures were positive in 9 (1.07%: 95%CI 0.37 to 1.76%); *S. aureus* 2*, S. pyogenes* 1*, S. Typhi* 3*,* Non-typhoid *Salmonella* 1*, K. pneumoniae* 1*, H. influenzae* type b 1. Bacteremic patients presented usually with a combination of jaundice, acute renal failure and high malaria parasitemia. Four bacteremic patients died compared with 108 (12.9%) of 836 non-bacteremic severe malaria patients; risk ratio 3.44 (95%CI 1.62 to 7.29). In patients with >20% parasitemia the prevalence of concomitant bacteremia was 5.2% (4/76: 95%CI 0.2 to 10.3%) compared with 0.65% (5/769: 0.08 to 1.2%) in patients with <20% parasitemia, a risk ratio of 8.1 (2.2 to 29.5).

Conclusions: In contrast to children, the prevalence of concomitant bacteremia in adults with severe malaria is low. Administration of empirical antibiotics, in addition to artesunate, is warranted in the small subgroup of patients with very high parasitemias, emphasising the importance of quantitative blood smear microscopy assessment, but it is not indicated in the majority of adults with severe falciparum malaria.

Keywords: *Plasmodium falciparum*, malaria, severe malaria, bacteremia

Introduction

Malaria is associated with bacterial infection but the relationship is complex (1). In endemic areas, where the majority of symptomatic malaria occurs in children (i.e. areas of moderate or high transmission), malaria is associated with an increased risk of bacteremia (2-9). This risk is greatest in severe falciparum malaria. In a meta-analysis of 7,208 children with severe malaria, included in 25 studies across 11 African countries, the mean prevalence of invasive bacterial infections was estimated to be 6.4% (95% confidence interval 5.81 to 6.98%) (8). In these malaria endemic areas it is now generally accepted that children presenting with severe malaria should receive broad spectrum antibiotics in addition to parenteral artesunate (1, 10), as concomitant bacteremia cannot be excluded, and it is difficult to distinguish clinically between severe malaria and sepsis (11, 12). A severely ill febrile child with a low parasitemia could have severe malaria, or sepsis with incidental parasitemia. In contrast in prospective studies of severe malaria conducted in low transmission settings, where the majority of patients are adults, concomitant malaria and sepsis have been reported rarely – although there are few incidence data (13,14). Currently empirical antibiotic treatment is not recommended in adults with severe malaria (1, 10). However, two recent overlapping studies from Myanmar have challenged this recommendation. These studies found that 13 of 87 (15%) adult patients hospitalised with a diagnosis of malaria were bacteremic (i.e. a substantially higher proportion than in African children with severe malaria) (15, 16). Their report concluded that "*clinicians should have a lower threshold for commencing empirical antibacterial therapy in adults diagnosed with falciparum malaria in these locations than is presently recommended".* Clearly this is an important issue. We report a very large prospectively studied series of Vietnamese adults

with strictly defined severe falciparum malaria in whom blood cultures were taken routinely in all patients on admission to the specialist treatment ward.

Methods

This investigation took place in the severe malaria ward of the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam during two sequential studies of adult patients admitted with strictly defined severe falciparum malaria. The first, conducted between 1991 and 1996, was a double-blind comparison of intramuscular quinine and intramuscular artemether (17), and the second, conducted between 1996 and 2003, was a double-blind comparison of intramuscular artesunate and intramuscular artemether (18). These two studies, reported previously in detail, were contiguous and all eligible patients were enrolled. Both studies were approved by the Ethical and Scientific Committee of the Hospital for Tropical Diseases, Ho Chi Minh City.

Entry criteria

Patients were included in the studies if they (or an accompanying relative) gave informed consent, they had asexual forms of *P. falciparum* on a peripheral-blood smear, were older than 14 years, were not in the first trimester of pregnancy, were not intravenous drug users, had received less than 3g of quinine or two doses of artemisinin or a derivative in the previous 48 hours, were not allergic to the study drugs, and had one or more of: Glasgow Coma Scale score <11 (indicating cerebral malaria); anemia (hematocrit, <20%) with a parasite density >100,000/uL; jaundice (serum total bilirubin, >2.5 mg/dL [50 µmol/L]) with a parasite density >100,000/uL; acute kidney injury (urine output, <400 ml/24 hours and serum creatinine, >3 mg/dL [250 µmol/L]); hypoglycemia (blood glucose, <40 mg/dL [2.2 mmol/L]); >10% parasitemia; and systolic blood pressure <80 mm Hg with cool extremities

(indicating shock). These criteria are similar to the WHO endorsed definition (1) except that the anemia criterion is more stringent.

Clinical Management and Procedures

On enrolment patients were examined, weighed, and baseline blood samples were taken for full blood count, clotting studies, biochemistry, arterial pH and blood gases, blood cultures and thin and thick film malaria parasite counts. A full history was taken from the patient or attendant relatives and a full clinical examination performed including a detailed neurologic assessment. A urinary catheter was inserted. Patients were managed by a dedicated team according to standard recommendations (1). Antimalarial treatment was started immediately with either artesunate, artemether or quinine according to randomization as described previously (17, 18). All patients were given isotonic saline initially, and fluid balance was then maintained with 0.9% saline or 5% dextrose in water. When necessary, a central venous catheter was inserted and the central venous pressure maintained at 5 cm of water. Blood was transfused if the hematocrit fell below 20%. Hypoglycemia was corrected with injection of 50 ml of 30% dextrose and a subsequent maintenance infusion of 5% to 10% dextrose in water. Detailed clinical and nursing observations were recorded a minimum of every 4 hours for the first 24 hours. A diagnostic lumbar puncture was performed if the Glasgow Coma Scale score was below 14. Hemofiltration was started in patients with established renal failure. Patients with respiratory failure were ventilated. Acetaminophen was given for high fever (>39°C), and intravenous diazepam, intramuscular phenobarbital, and if necessary, intravenous phenytoin were given for convulsions. Antibiotics with no clinical antimalarial activity (i.e., usually cefotaxime 2mg/kg 6 hourly or ceftriaxone 2g daily but not tetracyclines, macrolides, trimethoprim–sulfamethoxazole, or chloramphenicol) were given only if indicated clinically or cultures were positive, and confirmed enteric fever was treated with ofloxacin, but antibiotics were not started routinely.

Microbiology

Between 5 and 15 mL blood was taken for blood cultures (target 10mL). Between 1991 and 1997 a manual blood culture system was used (18). Each 5mL venous blood aliquot was inoculated into 50mL of brain heart infusion broth (Tissue Culture Services, UK) with 0.05% sodium polyanethol sulfonate (Sigma, St Louis, MO, USA). Blood culture bottles were vented and incubated at 35-37^{o}C for 7 days. Blind sub-culture was performed at 24 and 48 h and at 7 days or whenever physical growth was observed in the bottles. In September 1997 a BACTEC® culture system was introduced. Aliquots of blood (5 to 8mL) were inoculated into BACTEC plus aerobic bottles (Becton-Dickenson, USA) and then incubated for five days in a BACTEC 9050® automated analyser. Bottles that gave a positive signal were sub-cultured.

Sub-cultures were onto fresh sheep blood agar, and heated blood (chocolate) agar if *Haemophilus influenzae* or *Neisseria meningitidis* was suspected, and onto Sabouraud's agar if a yeast or mould was suspected (all media supplied by Oxoid Unipath, Basingstoke, UK). Plates were incubated at 37^{o}C in air (blood agar) or 5% CO_2 (chocolate agar) for 48 h or 30^{o}C in air (Sabouraud's agar) for 5 days. Organisms were identified by standard methods including API® identification kits (Bio-Mérieux, Basingstoke, UK) when necessary. Specific antisera were used to identify *Salmonella* serogroups, including Vi for *Salmonella enterica* serovar Typhi (*S.* Typhi). *Staphylococcus epidermidis* or other skin commensals were considered contaminants.

Statistical methods

Proportions were compared using Fisher's exact test using Epiinfo®.

Results

Blood culture results were available for 845 adult patients admitted with severe falciparum malaria. Of these nine were positive for pathogens, a prevalence of 1.07% (95%CI 0.37 to 1.76%). The organisms cultured were *Salmonella* Typhi (3), non-typhoid *Salmonella* (1)*, Staphylococcus aureus* (2), Group A *streptococcus* (1)*, Haemophilus influenzae* type b (1) and *Klebsiella pneumoniae* (1) (Table 1). An additional patient's blood grew *B. cepacia* but this was regarded as a contaminant, and the patient recovered uneventfully without receiving antibacterial treatment. The usual clinical presentation in these bacteremic patients was with the hepatorenal syndrome of fever, jaundice and acute kidney injury accompanied by high parasitemia. One patient was unconscious (cerebral malaria).

Fatal cases

Four of the nine patients with severe malaria and concomitant bacteremia died;

1. A 47 year old male farmer admitted with 20.1% parasitemia, jaundice and shock died 2.5 hours after admission. Blood cultures subsequently grew *Staphylococcus aureus*.
2. A 24 year old male farmer with a seven day history of fever had generalized convulsions followed by coma on the day of admission. His parasitemia was 0.9%. He had clinical signs of pneumonia, for which he was given ceftriaxone but he died 29 hours later without regaining consciousness. Blood cultures subsequently grew *Salmonella Typhi*.

3. A 28 year old male soldier with a five day history of fever was admitted with 24.9% parasitemia, jaundice and acute renal failure. He died 16 hours later. Blood cultures subsequently grew *Haemophilus influenzae* type b.

4. A 48 year old male builder with a six day history of fever presented with 24.6% parasitemia, hyperlactatemia, acute oliguric kidney injury, jaundice, pulmonary edema and upper gastrointestinal bleeding. Hemofiltration was started immediately. His parasitemia rose to 54% within 8 hours of admission. He received artemether and, because sepsis was suspected clinically, he was given ceftriaxone. On day 2 blood cultures grew *Klebsiella pneumoniae,* and amikacin was added. After a protracted course he developed nosocomial pneumonia and he died 24 days later.

The overall mortality of patients with severe malaria but no concomitant bacteremia was significantly lower; 12.9% (108/836). The risk ratio for death in patients with concomitant bacteremia was 3.44 (95%CI 1.62 to 7.29), p=0.022.

Risk factors

The nine patients admitted with severe malaria and concomitant bacteremia were slightly older (median 47 years, range 17 to 60 years) than the other 836 patients (median 31 years, range 15 to 79 years). They were also more likely to be hyperparasitemic; the median (range) parasite count was 501,144/uL (39,564 to 1,765,936/uL) compared to 81,766/uL (12,811 to 316,512)/uL in the non-bacteremic severe malaria patients. Four of the 9 bacteremic patients had >20% parasitemia compared with 72 of 836 non-bacteremic patients; risk ratio 5.16 (95%CI 2.41 to 11.07); p =0.0054. Thus the prevalence of concomitant bacteremia in patients with >20% parasitemia was 5.2% (4/76: 95%CI 0.2 to

10.3%) compared with 0.65% (5/769: 0.08 to 1.2%) in patients with <20% parasitemia, a risk ratio of 8.1 (95%CI 2.2 to 29.5). Mortality in non-bacteremic patients with >20% parasitemia was 18% (13 of 72). Leukocytosis, which may also occur in very severe malaria infections, and other hematological or biochemical indices were not useful as indicators of concomitant bacteremia. Only one of the eight bacteremic patients with a differential white count performed on admission had a neutrophilia.

Community acquired bacteremias

Between 1991 and 2000, during which 90% of the patients in this series were recruited, *Salmonella* Typhi was the predominant pathogen recovered from blood cultures taken in the hospital (18) comprising 41% (91/219) of isolates in 1991 and 25% (85/334) in 2000. Corresponding proportions for *Staphylococcus aureus* were 14% (31/219) in 1991 and 10% (33/334) in 2000.

Discussion

In this large prospective study of Vietnamese adults admitted to hospital with strictly defined severe falciparum malaria the rate of concomitant bacteremia was low. This contrasts with large studies in African children with severe malaria in whom concomitant bacteremia is sufficiently common (more than five times more common than in adults in this series) (8), and the clinical distinction between severe malaria and sepsis is sufficiently difficult (11, 12) to warrant administration of antibiotics on admission to all children with a diagnosis of severe malaria (1, 10). This low rate of concomitant bacteremia in adults supports current recommendations that empiric antibiotics should not be given on admission to adults with severe malaria, unless there is clear evidence of a bacterial

infection (1, 10). The important exception is patients with very high parasite densities (>20% parasitemia) who were 5.2 (95% CI 2.4 to 11.1) times more likely to be bacteremic. These high parasite counts in bacteremic patients, particularly the fatal cases, suggest that disease severity resulted primarily from malaria illness. The increased risk of bacteremia with very high parasitemias may reflect more intense parasitized sequestration (e.g. in the gut) and vital organ dysfunction (1) or more specifically host-phagocytic dysfunction resulting from the massive intravascular release of parasite cellular components and malaria pigment. Salmonella infections (particularly non-typhoid Salmonella) have been associated specifically with falciparum malaria infections in African children (2, 3, 5, 8, 20). In this study one third of the bacteremias were with *Salmonella* Typhi and it is noteworthy that *S.* Typhi was also the most common cause of community acquired bacteremia identified in Ho Chi Minh City during this period (19, 21). Although empirical antibiotics are not indicated on admission in adults with severe malaria unless they have very high parasite counts, antibiotic treatment may well be needed subsequently in patients who deteriorate (1), as nosocomial bacterial infections are relatively common following admission in severely ill patients.

These results contrast markedly with a recent study from Myanmar in which 15% (13 of 87) of adults hospitalised with a primary diagnosis of malaria were bacteremic (16). Malaria transmission in both countries is generally low and seasonal. However the Myanmar patient characteristics were very different to those of the Vietnamese adults with strictly defined severe falciparum malaria. Only some of the Myanmar patients may have had severe malaria, semi-quantitative malaria parasite counts were generally low (and were significantly lower in bacteremic than non-bacteremic patients), and many had neutrophil leucocytosis, all suggesting a primarily bacterial illness. The prevalence of bacteremia in the Myanmar adult patients was 23 times higher than in the Vietnamese adults with strictly defined severe falciparum malaria and <20% parasitemia (15% versus 0.65%).

Even in the three Myanmar fatal cases parasite counts were low (recorded as 1+ in two, and 2+ in one), whereas all the parasite counts of the Vietnam bacteremic patients would have scored 4+ in the semi-quantitative system - and with quantitative counts four had >20% parasitemia (of whom 3 died). The low overall mortality in the Myanmar series 3.4% (3/87) was attributed to early use of antibiotics, yet most of the Vietnamese patients did not receive antibiotics. It is therefore very unlikely that a high proportion of them had covert bacterial septicemia. The simplest and most probable explanation for the marked difference between the two studies relates to the primary diagnosis. The Vietnam patients undoubtedly had severe falciparum malaria as their primary condition, with bacteremia occurring late in the course of their illness, whereas it is likely that the bacteremic Myanmar patients had bacterial sepsis as their primary condition, and their malaria parasitemia was incidental (i.e. their fever and illness were caused by their bacterial infections and not malaria). This would explain the apparent high prevalence of bacteremia, their very low parasite counts and their neutrophil leukocytosis. Asymptomatic parasitemias are common in malaria endemic areas. It is understandable that an ill febrile patient with a positive malaria smear would be considered to have malaria if there was no obvious focus of bacterial infection. However severe falciparum malaria results from a current or previous large sequestered parasite burden (1), whereas incidental parasitemia is associated with parasite burdens which are many orders of magnitude lower (22). These two very different syndromes, requiring different management, can be distinguished by quantitative malaria parasite counts and by other parasite burden indicators such as the proportions of neutrophils containing malaria pigment, the stage of malaria parasite development, and plasma concentrations of *Pf*HRP2 or *P. falciparum* DNA (23-26). As parasite counting, staging and neutrophil pigment assessment can all be done rapidly on admission thin and thick film blood films, this emphasises the value of experienced microscopy in the assessment of patients hospitalised with malaria. From taking the blood smear to completing the thin-film parasite count can take as little as five minutes (27). Semi-quantitative counts using the old “plus” or “cross “ system are unreliable and are no longer recommended by the World Health Organisation (28). They

are particularly unsuited for patients hospitalised with malaria as the maximum semi-quantitative count of 4+ (>10 parasites in one thick blood film oil-immersion high power field) encompasses parasitemias ranging from <1% to 100%. Misdiagnosis also contributes significantly to the high rates of concomitant bacteremia and "severe" malaria reported in African children (25), and explains many of the apparent associations between both falciparum and vivax malaria and a variety of unrelated conditions. The high prevalence of asymptomatic parasitemia in endemic areas means that many patients admitted to hospital with other conditions will be labelled as having malaria. In a retrospective review of 400 adult patients with severe imported malaria admitted to 45 French intensive care units 9 (2.3%) were bacteremic and one had candidemia on admission (29). However the patients in France were substantially older (median age 45 years) than the Vietnamese adults in this series, 7.3% of patients had immune deficiencies, and 14.3% had one or more co-morbidities.

Although this study in Vietnamese adults was long, large and detailed, it has several limitations. Empirical use of antibiotics before admission to hospital is common in Asia (and was reported in 35% of the Myanmar series) and could have obscured some bacterial infections. However, prior antibiotic use was unusual in the low-income rural population at risk from malaria in Vietnam between 1991 and 2003, so is unlikely to be a significant confounder. Blood culture is intrinsically insensitive so bacteremia was probably underestimated. The target blood volume cultured (10mL), which was the same in both the Myanmar and Vietnam series, is not maximally sensitive (30, 31). It is also noteworthy that 6 of the 9 positive blood cultures were obtained following change to the automated blood culture system, so the earlier manual culture system may have been less sensitive. However, the Vietnamese patients with severe malaria were not given routine antibiotics after admission, yet 87% survived, and most deaths were clearly attributable to severe falciparum malaria. It seems very unlikely that these various differences could account for the marked discrepancy between this and the Myanmar series, particularly as many of the Myanmar patients did not have strictly defined severe malaria.

High parasitemia was clearly a risk factor for concomitant bacteremia. Of the 76 patients with >20% parasitemia (9% of the total), 4 (5.2%) were also bacteremic. This group has a high mortality, so giving broad spectrum antibiotics empirically to patients with very high parasitemias is justified. Thin blood smear assessment should be performed on all patients admitted with severe falciparum malaria. Overall however, in contrast to children in areas of higher malaria transmission, the incidence of concomitant bacteremia in adults with severe malaria is low and does not warrant use of empirical antibiotics in all patients.

Notes

Authors' contributions: NJW designed the study, NHP, NPJD, PQT, NTHM, TTHC, LVC, HV, PPL, DXS, DJW, JJF, and TTH conducted the clinical studies, NTTH and CMP conducted the laboratory studies, AJ, JW and NJW analysed the data. All authors contributed to the study implementation, interpretation and reporting

Acknowledgement: We are very grateful to the nurses and doctors of the malaria ward and the staff of the microbiology laboratory of the Hospital for Tropical Diseases, Ho Chi Minh City. Deidentified individual patient data from these two sequential studies are available on request to the OUCRU data access committee.

Funding: These studies were supported by the Wellcome Trust through serial grants to support the clinical research programme.

Declaration of Interests: There are no conflicts of interest.

References

1. World Health Organization, 2014. Severe malaria. Trop Med Int Health 19 (Suppl 1): 7–131
2. Mabey DC, Brown A, Greenwood BM. *Plasmodium falciparum* malaria and *Salmonella* infections in Gambian children. J Infect Dis 1987; 155 : 1319 – 21.
3. Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteremia complicating severe malaria in children. Trans R Soc Trop Med Hyg 1999; 93: 283–286.
4. Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B, Berkley JA. Salmonella bacteremia in Kenyan children. Pediatr Infect Dis J. 2006;25:230-6.
5. Bronzan RN, Taylor TE, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, Njobvu A, Kondowe W, Chalira C, Walsh AL, Phiri A, Wilson LK, Molyneux ME, Graham SM. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. J Infect Dis. 2007;195:895-904.
6. Bassat Q, Guinovart C, Sigaúque B, Mandomando I, Aide P, Sacarlal J, Nhampossa T, Bardají A, Morais L, Machevo S, Letang E, Macete E, Aponte JJ, Roca A, Menéndez C, Alonso PL. Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital. Trop Med Int Health. 2009;14:1011-9.
7. Were T, Davenport GC, Hittner JB, Ouma C, Vulule JM, Ong'echa JM, Perkins DJ. Bacteremia in Kenyan children presenting with malaria. J Clin Microbiol 2011; 49: 671-6.
8. Church J, Maitland K. Invasive bacterial co-infection in African children with *Plasmodium falciparum* malaria: a systematic review. BMC Med 2014; 12: 31.

9. Maltha J, Guiraud I, Kaboré B, Lompo P, Ley B, Bottieau E, Van Geet C, Tinto H, Jacobs J. Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. PLoS One. 2014; 9 :e89103.
10. World Health Organisation. Guidelines for the Treatment of Malaria. 3rd edition. WHO Geneva, 2015.
11. Evans JA, Adusei A, Timmann C, May J, Mack D, Agbenyega T, Horstmann RD, Frimpong E. High mortality of infant bacteraemia clinically indistinguishable from severe malaria. QJM. 2004; 97: 591-7.
12. Nielsen MV, Amemasor S, Agyekum A, Loag W, Marks F, Sarpong N, Dekker D, Adu-Sarkodie Y, May J. Clinical indicators for bacterial co-infection in Ghanaian children with *P. falciparum* infection. PLoS One. 2015; 10: e0122139.
13. Pattanaik SS, Tripathy R, Panda AK, Sahu AN, Das BK. Bacteraemia in adult patients presenting with malaria in India. Acta Trop. 2012; 123: 136-8
14. Sandlund J, Naucler P, Dashti S, Shokri A, Eriksson S, Hjertqvist M, Karlsson L, Capraru T, Färnert A. Bacterial coinfections in travelers with malaria: rationale for antibiotic therapy. J Clin Microbiol. 2013; 51: 15-21.
15. Nyein PP, Aung NM, Kyi TT, Htet ZW, Anstey NM, Kyi MM, Hanson J. High Frequency of Clinically Significant Bacteraemia in Adults Hospitalized With Falciparum Malaria. Open Forum Infect Dis. 2016; 3: ofw028.
16. Aung NM, Nyein PP, Htut TY, Htet ZW, Kyi TT, Anstey NM, Kyi MM, Hanson J. Antibiotic Therapy in Adults with Malaria (ANTHEM): High Rate of Clinically Significant Bacteremia in Hospitalized Adults Diagnosed with Falciparum Malaria. Am J Trop Med Hyg. 2018; 99: 688-696.

17. Tran TH, Day NP, Nguyen HP, Nguyen TH, Tran TH, Pham PL, Dinh XS, Ly VC, Ha V, Waller D, Peto TE, White NJ. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N Engl J Med. 1996; 335:76-83.
18. Phu NH, Tuan PQ, Day N, Mai NT, Chau TT, Chuong LV, Sinh DX, White NJ, Farrar J, Hien TT. Randomized controlled trial of artesunate or artemether in Vietnamese adults with severe falciparum malaria. Malar J. 2010; 9: e97.
19. Hoa NTT, Diep TS, Wain J, Parry CM, Hien TT, Smith MD, Walsh AL, WhiteNJ. Community-acquired septicaemia in southern Vietnam; the importance of multi-drug resistant *Salmonella Typhi*. Trans Roy Soc Trop Med Hyg 1998; 92: 503-508
20. Takem EN, Roca A, Cunnington A. The association between malaria and non-typhoid Salmonella bacteraemia in children in sub-Saharan Africa: a literature review. Malar J. 2014; 13: e400.
21. Nga TV, Parry CM, Le T, Lan NP, Diep TS, Campbell JI, Hoang NV, Dung le T, Wain J, Dolecek C, Farrar JJ, Chau NV, Hien TT, Day JN, Baker S. The decline of typhoid and the rise of non-typhoid salmonellae and fungal infections in a changing HIV landscape: bloodstream infection trends over 15 years in southern Vietnam. Trans R Soc Trop Med Hyg. 2012; 106:26-34.
22. Imwong M, Stepniewska K, Tripura R, Peto TJ, Lwin KM, Vihokhern B, Wongsaen K, von Seidlein L, Dhorda M, Snounou G, Keereecharoen L, Singhasivanon P, Sirithiranont P, Chalk J, Nguon C, Day NP, Nosten F, Dondorp A, White NJ. Numerical Distributions of Parasite Densities During Asymptomatic Malaria. J Infect Dis. 2016; 213: 1322-9.
23. Nguyen PH, Day N, Pram TD, Ferguson DJ, White NJ. Intraleucocytic malaria pigment and prognosis in severe malaria. Trans R Soc Trop Med Hyg. 1995; 89: 200-4.

24. Silamut K, White NJ. Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. Trans R Soc Trop Med Hyg. 1993; 87: 436-43.

25. Hendriksen IC, Mwanga-Amumpaire J, von Seidlein L, Mtove G, White LJ, Olaosebikan R, Lee SJ, Tshefu AK, Woodrow C, Amos B, Karema C, Saiwaew S, Maitland K, Gomes E, Pan-Ngum W, Gesase S, Silamut K, Reyburn H, Joseph S, Chotivanich K, Fanello CI, Day NP, White NJ, Dondorp AM. Diagnosing severe falciparum malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. PLoS Med. 2012; 9: e1001297.

26. Imwong M, Woodrow CJ, Hendriksen IC, Veenemans J, Verhoef H, Faiz MA, Mohanty S, Mishra S, Mtove G, Gesase S, Seni A, Chhaganlal KD, Day NP, Dondorp AM, White NJ. Plasma concentration of parasite DNA as a measure of disease severity in falciparum malaria. J Infect Dis. 2015; 211: 1128-33.

27. White NJ, Silamut K. Rapid diagnosis of malaria. Lancet 1989; **i**: 435.

28. World Health Organisation. Basic Malaria Microscopy. Part 1: Learner's guide. 2nd Edition. World Health Organisation, Geneva 2010.

29. Bruneel F, Tubach F, Corne P, Megarbane B, Mira JP, Peytel E, Camus C, Schortgen F, Azoulay E, Cohen Y, Georges H, Meybeck A, Hyvernat H, Trouillet JL, Frenoy E, Nicolet L, Roy C, Durand R, Le Bras J, Wolff M; Severe Imported Malaria in Adults (SIMA) Study Group. Severe imported falciparum malaria: a cohort study in 400 critically ill adults. PLoS One. 2010; 5: e13236.

30. Mermel LA, Maki DG. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann Intern Med. 1993; 119:270–272.

31. Gonsalves WI, Cornish N, Moore M, Chen A, Varman M. Effects of volume and site of blood draw on blood culture results. J Clin Microbiol. 2009; 47:3482–3485.

Table 1: Clinical and laboratory features on admission of Vietnamese adults with severe falciparum malaria and concomitant bacteremia .

	Age/ sex	Weight (kg)	Days of fever	GCS	T (°C)	Antimalarial drug	Antibiotic	Parasite count/ µL	Parasitemia (%)	PCV (%)	White blood count / µL (%N)	Plasma Creatinine (mg/dL)	Total Bilirubin (mg/dL)	Plasma lactate (mmol/L)	Organism isolated	Outcome
1	47M	45	3	14	37	Quinine	none	1,161,298	20.1	46	-	5.6	-	5.9	*S. aureus*	Died
2	24M	54	7	15	38.3	Artemether	Ofloxacin	224,322	4.7	38	7,800 (74)	2.6	2.4	-	*S. Typhi*	Survived
3	17F	39	4	15	38.2	Artemether	Ofloxacin	501,144	11.4	35	4,300 (67)	8.5	3.6	3.8	*S. Typhi*	Survived
4	24M	65	15	4	38.5	Artemether	Ceftriaxone	39,564	0.9	35	9,210 (60)	1	1.7	-	*S. Typhi*	Died
5	60M	54	5	15	37.0	Artemether	Ofloxacin	128,740	4.1	25	3,500 (75)	8.6	0.92	0.8	NTS	Survived
6	57M	60	6	14	37.5	Artesunate	Ceftriaxone + Oxacillin	38,685	0.7	44	8,400 (79)	2.1	9.8	1.5	*S. aureus*	Survived
7	25M	50	3	12	37.5	Artemether	none	906,958	24.9	29	14,850 (54)	5.6	-	7.0	*H. influenzae* B	Died
8	48M	76	6	14	38.0	Artemether	Ceftriaxone + Amikacin	1,235,904	24.6	40	6,860 (53)	3.1	8.7	11.6	*K. pneumoniae*	Died

T (°C): temperature, (%N): % neutrophils, PCV: hematocrit, NTS: non-typhoid Salmonella.