CASE REPORT

False Positive Blood Culture From Automated Microbial Detection System in Severe Malaria – A Case Report

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ABSTRACT

Automated microbial detection system (AMDs) are design to detect early growth of bacterial and fungal. We herein report a rare case of false positive blood culture by AMDs in *Plasmodium falciparum* infection. A 41-year-old previously healthy lady, with recent history of travelling to Lagos, Nigeria had presented to the casualty with history of fever and lethargy for three days. There was no malaria prophylaxis taken prior to the travelling history. Peripheral blood smear confirmed the presence of young trophozoite of *Plasmodium falciparum* with parasitemia of 7%. Concurrent blood culture sent was positive, however all subcultures were negative for any growth. She was treated with intravenous artesunate however succumbed to death on the day of admission due to severe falciparum infection complicated with multiorgan failure and shock. The aim of this report is to highlight, the circumstances that can trigger the false positive AMDs detection and the possible underlying mechanism.

Keywords: Blood culture, *Plasmodium*, Automated microbial detection system

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INTRODUCTION

Several automated microbial detection system (AMDs) are available and widely used in the laboratory. This automated and continuous monitoring system allow a rapid detection of positive blood cultures based on the colorimetric or fluorescent sensor that detect the production of the carbon dioxide (CO2) by the growing bacteria and fungi (1-2,4). The false-positive rate for this system is reported to be about 1-10% and high parasitemia count due to *Plasmodium* infections are among the causes (1). This *Plasmodium* parasite will produced CO2 either by stimulating the erythrocyte to release the gas or by the anaerobic glycolysis process (1,3,5). Hence, this will trigger the AMDs, but no microorganism (bacteria and fungi) is detected from the gram stain or grow from the subculture medium.

CASE REPORT

A 41-year-old lady, no known co-morbid before presented with three days history of fever, vomiting and malaise. The fever was associated with chills and rigors. Apart from that, she also vomit frequently. She was very lethargy but able to ambulate at home until she developed shortness of breath and was brought to our casualty via an ambulance call. Prior to that, she visited two different general clinics and was treated as an upper respiratory infection. She was a frequent traveller and just returned two weeks from Lagos, Nigeria before she developed all the symptoms. She did not taking any malaria prophylaxis prior to her travelling to Lagos. She is single and denied high risk behaviour.

On physical examination, she was alert and conscious but very dehydrated with cold peripheries, mottling skin, weak peripheral pulses and capillary refill time more than three second. She was neither pale nor jaundice. She was in decompensated shock with blood pressure of 47/20 mm/Hg and heart rate of 150 beats per minute. Apart from that, she also very tachypneic, her respiratory rate was 50 breath per minute and oxygen saturation of only 67% in the room air. Her lungs were clear and per abdomen examination revealed no significant findings. Full blood count showed severe thrombocytopenia (Table I) with normal haemoglobin and total white cell counts. She also developed severe metabolic acidosis with hyperlactatemia and coagulopathy. Her renal profile was deranged and there was liver impairment as evidence by elevated liver enzyme and bilirubin level. She was hypoglycaemic with dextrostix of 3.3mmol/L upon arrival. Chest radiograph shows no consolidation and bedside echogram revealed collapsed of inferior vena cava vein. Dengue combo kit and dengue IgM were both negative.

Blood film malarial parasite was sent and revealed

Table I: Laboratory investigation of the patient.

Test	Parameter	Interpretation
Full blood count	Hb: 12 g/dL	
	WCC: 16x10^9/L	
	Platelet : 16x10^9/L	Thrombocytopenia
Arterial Blood Gas	pH: 6.958	
	PCO_2: 38.6	*Metabolic acidosis with
	HCO_3: 8.7	hyperlactaemia
	BE: -22	
	Lactate :18.6	
Coagulation Profile	PT: 18.2 second	Coagulopathy
	INR: 1.52	
	APTT : 47.1 second	
Renal Profile	Urea: 23.2 mmol/L	*Acute kidney
	Potassium: 4.8 mmol/L	injury
	Sodium 136 mmol/L	
	Creatinine: 526 umol/L	
Liver Enzyme	Alanine aminotransferase: 107 U/L	Acute hepatitis
	Alkaline phosphatase: 99 U/L	
	Aspartate aminotransferase: 213 U/L	
	Bilirubin 103 umol/L	
Random blood sugar	Dextrostix: 3.3mmol/L	*Hypoglycemia

Parameter with (*), indicate severe and complicated malaria

presence of *Plasmodium falciparum* with high parasitemia (Figure 1). Numerous young trophozoites were seen with the parasite count of 355000 for asexual/uL and 30 sexual/uL blood which result in 7% of parasitemia. This finding was confirmed by polymerase chain reaction which revealed the same organism. One set of blood culture was drawn to rule out bacteria infection as patient was in severe sepsis and was flagged positive after four hours of incubation via BacT/Alert blood culture system. However, gram stain revealed the presence of malaria parasite and no bacteria or fungal were seen. Culture from the blood bottle showed no growth after 48 hours of incubation.

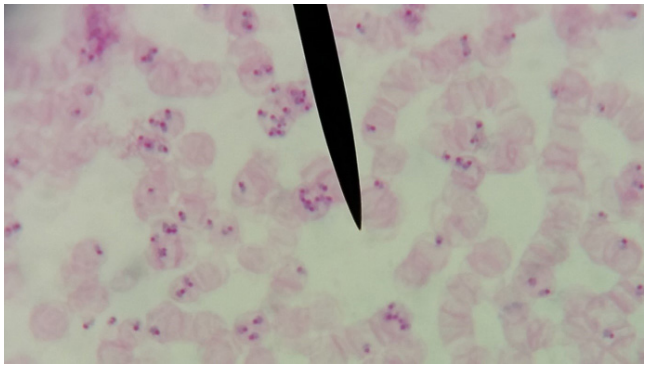

Figure 1: Plasmodium falciparum trophozoites seen in the giemsa stain

Diagnosis of severe *Plasmodium falciparum* infection with refractory shock and multiorgan failure was made. She was resuscitated and intubated in view of severe metabolic acidosis and for airway protection. Fluid and blood products were given and inotrope was initiated to maintain the circulation. Intravenous artesunate (2.4mg/kg) was started and she was transferred to intensive care unit for further management. However, her condition deteriorated with two episode of asystole and she succumbed after 8 hours of admission.

DISCUSSION

The differential diagnosis for a traveller returning from endemic area with fever should include malaria, hence the blood film malarial parasite (BFMP) smears should be performed to aid in the diagnosis (1). As for this patients, her initial investigation was BFMP which was sufficient to confirm the morphology of *Plasmodium falciparum* parasitemia (figure 1). Polymerase chain reaction was sent for molecular confirmation and revealed the same finding. In this patient, a single set (aerobe and anaerobe) of blood culture was sent on the day of admission to rule out the concurrent bacteraemia as illustrated in the several literatures regarding the cases of gram negative co-infection (1). The blood culture bottles were incubated inside the AMDs, which in this case using the BacT/Alert and aerobic blood culture was flagged positive after four hours of incubation. However microscopically revealed presence of intraeryhrocytic microorganism with thin and delicate ring forms and double infected of red blood cells which resembles *Plasmodium falciparum* trophozoite. The subcultures media were negative for any growth. The AMDs used the principle of colorimetric (BacT/Alert) or fluorescent (Bactec 9240) detection method. The microorganism (bacteria and fungal) utilized the specialized substrates that presence in the blood culture bottle and produced the carbon dioxide (CO_2) gas that detected by the AMDs (4). BacT/Alert bottle is imbedded with a colorimetric sensor that changes colour in the presence of CO_2 produced by growing bacteria and fungi (1-2,4). For, Bactec 9240, the dye at the bottom of the bottle will react with the CO2 and modulates the amount of light that absorbed by the fluorescent material in the sensor. These sensors are scanned every 10 minutes and voltage changes are digitized for analysis by the microcomputer which result in the growth curve (figure 2) (4). Even though, AMDs is design for detection of bacteremia and fungemia, a false positive result can occur in the case of high parasitemia level (1). As reported in the literatures *Plasmodium falciparum* did not utilize the substrate in the media broth, but produce CO_2 by the glycolysis process and second by the released of isoprenoid precursor (1,3,5).

Figure 2: Growth curve obtain from the positive blood culture bottle. The graph plotted as reflectance unit (carbon dioxide production) versus time

Plasmodium falciparum have a single mitochondria and completely lack of oxidative cycle, hence depending to the anaerobic glycolysis to support the rapid proliferation of the parasites (5). In anaerobic glycolysis, glucose will be split into the pyruvate, whereby later converted to the lactic acid. Carbon dioxide is the by-product produce from this metabolism process (1). Other than that, *Plasmodium falciparum* can produce an isoprenoid precursor that trigger the erythrocytes to release the CO_2. *In vivo* this mechanism is importance to attract the vector and feeding stimulation (3). Hence, increase CO_2 production from both mechanism can trigger the automated blood culture detection system. There were only 5 case reports published in the literature about the false positive AMDs due to *Plasmodium falciparum* infection (1). The period of incubation and percentage of the parasitemia in each report were variable ranging from 12-42 hours and 2%-10% respectively (1). As illustrated in the previous case report (Table II), there

Table II:"False"- positive blood culture cases caused by Plasmodium falciparum and the type of AMDs reported in literatures

	Plasmodium species	Para-sitemia (%)	Bottle	Duration of incu-bation (hr)	Blood cul-ture system	Reference
1	*P. falciparum*	2.3	Anaerobic	12	BacT/Alert	McCarthy et al,1999
2	*P. falciparum*	7.2	Aerobic	23	BacT/Alert	McCarthy et al,1999
3	*P. falciparum*	1.8	Aerobic	42	Bactec 9240	Richalet et al, 2002
4	*P. falciparum*	10	Anaerobic	24	BacT/Alert	Jutte et al,2007
5	*P. falciparum*	5	Aerobic	42	Bactec 9240	Scott et al,2014
6	*P. falciparum*	7	Aerobic	4	BacT/Alert	Present case

was no correlation between the parasitemia count and the time of detection. Both aerobe and anaerobe bottle can trigger the AMDs and as in this case, only aerobe was flagged positive. There are multiple type of AMDs but to date only BacT/Alert and Bactec 9240 automated system were reported in the literature capable to cause the false-positive detection (1). Other cause of false-positive blood culture is because of high leukocyte count (leukocyte count > 10.5 x 10^9/L) which usually occur in leukemic cases due to CO_2 production by metabolically active blast (1,2). White cell count was normal in this patient and was excluded as the cause of the false positive result.

CONCLUSION

Diagnosis of malaria should be included for fever travellers who is returning from endemic region. However, concurrent bacterial infection should be excluded even though the diagnosis of malaria have been established by doing the BFMP. High parasitemia load as illustrated by this case can trigger the automated blood culture system and cause the false positive result. Interpretation of the result should be done with correlation of subculture and BFMP.

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