Tanzania Journal of Health Research
Volume 19, Number 4, October 2017
Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

Possible causes of fever among patients with blood smear negative for malaria parasites at Bombo Regional Referral Hospital in Tanga, Tanzania

MERCY CHIDUO[1*], MATHIAS KAMUGISHA[1], ATHANAS MHINA[1], FILBERT FRANCIS[1], JACKSON MCHOMVU[2], JUMA KAYANDA[2], EZEKIEL MALECELA[1], JOHARI SADI[1], JOSEPH KASEKA[1], HAMISI MSANGENI[1] and MARTHA LEMNGE[1]
[1]*National Institute for Medical Research, Tanga Research Centre, P.O. Box 5004, Tanga, Tanzania*
[2]*Bombo Regional Referral Hospital P. O. Box 452 Tanga, Tanzania*

Abstract
Background: Due to its diverse and non-specific clinical presentations, malaria has been associated with most infections causing febrile illnesses. Despite being non-specific, clinical diagnosis is still the main method of malaria diagnosis in most health facilities in sub-Saharan Africa. This study aimed to establish the probable diagnoses among fever cases admitted at Bombo Hospital in north-eastern Tanzania.
Methods: This study involved patients admitted in Medical and Paediatric wards with a clinical diagnosis of severe malaria but having negative blood smears (BS) for malaria parasites. Finger prick blood specimens were collected for blood smear microscopy and rapid diagnostic test. Blood and urine cultures were done for all specimens collected. Some patients were also screened for HIV infection.
Results: A total of 227 patients were recruited and the majority (62.1%) were under-five children. Out of the 227 blood specimens cultured, 25 (11.0%) grew different bacteria species. *Staphylococcus aureus* was the most frequent pathogen (68.0%), followed by *S. pneumoniae* (24.0%), *Salmonella* species (4.0%) and *Streptococcus pyogenes* (4.0%). Only 7 (3.2%) out of 219 urine specimens cultured showed growth of *Escherichia coli, Pseudomonas aeruginosa, S. aureus* and *Klebsiella pneumoniae*. Of the 215 patients screened for HIV, 17 (7.9%) had positive reaction.
Conclusion: The findings indicate that *S. aureus* and *S. pneumoniae* as the commonest bacteria isolates from blood and *P. aeruginosa, S. aureus* and *K. pneumoniae* from urine cultures. These bacteria and HIV should be considered as important contributors to febrile illness cases among patients found with negative BS for malaria parasites.

Keywords: febrile illness, malaria, bacteria, HIV, Tanzania

Introduction

Malaria has been traditionally, incriminated as the principal cause of febrile illnesses in the malaria endemic regions to an extent that any case of fever has been considered as malaria until proved otherwise (WHO, 2015). Malaria symptoms are not unique, and thus, can be confused with other febrile illnesses (Bouyou-Akotet *et al*., 2009), and often it may be difficult to distinguish it from viral infections such as HIV, influenza, parainfluenza adenovirus, rhinoviruses and arthropod borne viruses like Chikungunya and Dengue (Schacker *et al.*, 1996; Perlmutter *et al.*, 1999; Bell & Rosenberg, 2009). Unfortunately, despite being non-specific, clinical diagnosis is still the main method of diagnosis in most health facilities in malaria endemic areas, especially in sub-Saharan Africa where regrettably diagnostic facilities are inadequate (Mmbando *et al.*, 2010).

Despite the prevalence and incidence of febrile illnesses remaining high in the malaria endemic regions, recent reports indicate that malaria is declining, particularly in sub-Saharan Africa (D'Acremont *et al*., 2009; Bouyou-Akotet *et al*., 2009; Ceesay *et al*., 2010; Mmbando *et al.*, 2010; Mboera *et al.*, 2013). Results from a study conducted at Bombo regional hospital in Tanga, north-eastern Tanzania have shown that over a quarter of the patients reported to have severe malaria had no parasitaemia (Msangeni *et al.*, 2011), giving evidence that non-malaria conditions play a big role in the incidence of febrile illness in this area. Studies elsewhere in Tanzania and Uganda have shown that a

* Correspondence: Email: mercy_chiduo@yahoo.com

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

substantial proportion of patients presenting with clinical symptoms mimicking malaria did not have parasitaemia (Reyburn *et al.*, 2004; Njama-Meya *et al*., 2007; Kamugisha *et al.*, 2008; Mwanziva *et al.*, 2011).

Various studies have shown that bacterial infections have been responsible for high rate of malaria-related morbidities and mortalities in children in sub-Saharan Africa (Ayoola *et al*., 2002; Evans *et al.*, 2004; Adedoyin *et al*., 2013) and, the commonest sites of infection being the urinary and the respiratory tract systems causing urinary tract infection (UTI) and pneumonia, respectively (Hoberman & Wald, 1997; Shaw *et al*., 1998, 2010). As UTI is one of the commonest causes of fever in young children (Shaw *et al*., 1998; Foxman, 2002) and to a certain degree in the older age groups (Mbata, 2007), urine culture and sensitivity tests could be very helpful in the management of febrile illnesses. Likewise, carrying out blood culture and screening for HIV could reveal some blood-borne infections in those who would have been otherwise considered as cases of clinical malaria but without actually having malaria parasites.

The problem of malaria being over-diagnosed lies mainly on the standard case definition. For a definitive diagnosis to be made though, laboratory tests must demonstrate the malaria parasites or their components. The objective of this study was to determine other probable diagnoses among patients with fever who have negative blood smears for malaria parasites.

Materials and Methods

Study area and design

This study was conducted at the Bombo Regional Referral Hospital in Tanga City, north-eastern Tanzania. The city lies at about 5.17°, 5.33°S and 38.17°, 38.33°E along the Indian Ocean seashore. The area receives two seasons of rainfall; short rains during the months of October – December and long rains in March – June, with a humidity of about 100%. Temperature ranges between 27°C and 32°C. Tanga city covers an area of about 600km^2 and has an estimated human population of 305,715 (URT, 2013). Bombo with a bed capacity of 412 is the referral hospital for Tanga region, serving eight districts. It has a general laboratory for both in-patient and out-patient services. This cross-sectional study was conducted during the period of May to November 2009 and November 2011 to October 2012. The study involved in-patients from paediatric and medical wards admitted as malaria cases but having negative blood smears for malaria parasites. Patients with history of taking antibiotics seven days before admission as well as positive blood smears were excluded from the study.

Laboratory tests

Finger prick blood specimens were collected for preparation of blood smears and malaria rapid diagnostic test (mRDT). Thick and thin smears were prepared and stained with 10% Giemsa solution for 30 minutes after the thin smear being fixed with methanol. The blood smears were carefully examined by skilled microscopists and were considered negative if there were no evidence of any parasites observed. As for the mRDT (Paracheck®, Orchid, Biomedical system, Verna, Goa, India) blood samples were tested following manufacturer's instructions.

Midstream urine (MSU) was collected in sterile, dry, leak-proof containers from adults and sterile urine collection bags were used for children. About 20 ml urine was collected from each study participant. The collected specimens were eventually transferred into universal bottles and sent to the laboratory. In the laboratory, both blood agar and MacConkey agar (Oxoid LTD, Basingstoke, Hampshire, England) were used as culture media. A loopful portion of urine sample was then inoculated in the media incubated aerobically at 35-37°C overnight. An additional portion of urine measuring about 10 ml was centrifuged for 5-10 minutes. After discarding the supernatant fluid, the deposit was re-suspended to get a uniform mixture by shaking it well. The whole sediment was then

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

transferred to a container of selenite enrichment broth and, incubated aerobically at 35-37°C overnight. From the second day onwards, examination for growth of bacteria on blood agar and MacConkey agar plates was done, and report given on the bacterial numbers meanwhile subculture of the selenite broth was done on xylose lysine deoxycholate (XLD) agar for the isolation of *Salmonella* species.

About 10 ml of venous blood was collected and divided in two portions, each with 5 ml. One portion was mixed in Tryptone soya diphasic medium and the other part was mixed in Thioglycollate broth medium. The blood was then gently mixed with the culture medium (or broth). On day 2 onwards, both specimens on tryptone soya medium and thioglycollate broth were sub-cultured on blood agar, MacConkey agar and chocolate agar. The blood agar and chocolate agar plates were incubated for about 48 hours, the former anaerobically and the latter in a carbon dioxide atmosphere. The MacConkey agar plate was incubated aerobically overnight. All plates were incubated at a temperature of 35-37°C (Wilson *et al*., 2007). The test was considered positive when there was a bacterial growth of a single type of bacteria at high colony counts (greater than 10,000 colony forming units (CFU)/ml). In case a culture grew several different types of bacteria, the growth was considered as due to contamination. Gram stain examination was performed in urine and blood specimens that showed any bacterial growth. HIV-1 testing was performed following the Tanzanian HIV testing algorithm (MoHSW, 2012). Patients with positive results were counselled and were referred to care and treatment clinic (CTC) for proper care and management.

Ethical approval

Ethical approval was obtained from the Medical Research Coordinating Committee (MRCC) of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/772). Oral and written consents were sought from patients or guardians/caretakers of children with blood smear negative results for malaria parasites and, respective specimens were collected from consenting participants.

Data analysis

Data was managed by using Microsoft Access programme. It was double entered and then validated for inconsistence between the first and second entry. The analysis was performed using STATA version 11. Descriptive statistics that included, frequencies and cross tabulations were used to summarize categorical variables while means and ranges were used to summarize continuous variables.

Results

A total of 227 patients admitted in paediatric and medical wards with febrile conditions but having blood smears negative for malaria parasites were recruited. Majority of them 141 (62.1%) were under five children and over 60% of the patients were males. Overall, body temperature ranged from 36.3°C to 40.7°C and fever prevalence was 83.2% (188/227). Children in the age group of 1 to 4.9 years had the highest fever rate of 29.5% (Table 1).

Table 1: Demographic characteristics of patients

Variable	Age group (years)				
	Overall	<1	1-4.9	5-14.9	≥15
Number enrolled	227	59 (26.0%)	82 (36.1%)	36 (15.9%)	45 (19.8%)
Sex ratio (male: female)	1:9	2:0	1:8	2:6	1:6
Mean age	2.7	0.7 months	2.1 years	7.4 years	35 years
Mean axillary temp [range]	38.6°C [36.3-40.7°C]	38.3°C [36.5-40.0°C]	38.6°C [36.3-40.1°C]	38.7°C [36.6-40.7°C]	38.7°C [36.9-40.0°C]
Prevalence of fever (≥37.5°C)	188 (83.2%)	46(20.3%)	67(29.5%)	31(13.6%)	39(17.2%)

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

*****5 Participants were excluded due to missing information

Out of the 227 blood specimens cultured, 25 (11.0%) grew different bacteria species. The highest positivity rate was observed among children < 1 year (50.0%), followed by those in the age group of 1-4.9 years (23.1%). Only 7 (3.2%) out of the total 219 urine specimens cultured showed growth of different bacteria species, majority (85.7%) recorded in children aged 1-4.9 years. Out of the 215 patients screened for HIV, 17 (7.9%) had a positive reaction (Table 2).

Table 2: Blood and urine culture, and HIV status of patients by age group

Test	No. examined	No. positive	< 1 year	1-4.9 year	5-14.9 years	15+ years
Blood culture	227	26 (11.5%)	13 (50.0%)	6 (23.1%)	4 (15.4%)	3(11.5%)
Urine culture	219	7 (3.4%)	0	6 (85.7%)	1(14.35)	0
HIV screening	215	17 (7.9%)	2 (11.8%)	4 (23.5%)	3 (17.6%)	7(41.2%)

Four different bacteria, namely *Staphylococcus aureus, S. pyogenes, S. pneumoniae* and *Salmonella* species were isolated from blood among the 25 blood specimens that grew bacteria. *S. aureus* was the most frequent bacterium isolated in 17 (68.0%) specimens, followed by *S. pneumonia* in 6 (24.0%). *Salmonella* species and *S. pyogenes* were each isolated in one specimen. The micro-organisms identified in urine specimens were *Pseudomonas aeruginosa, S. aureus, Klebsiella* species and *Escherichia coli* (Table 3).

Table 3: Proportion of microorganisms isolated in blood and urine specimens

Test	Isolate	Number of isolates (%)
Blood culture (N=25)	*Staphylococcus aureus*	17(68.0)
	Streptococcus pneumoniae	6(24.0)
	Streptococcus pyogenes	1(4.0)
	Salmonella spp	1(4.0)
Urine culture (N=7)	*Pseudomonas aeruginosa*	2 (28.6)
	Staphylococcus aureus	2 (28.6)
	Klebsiella pneumoniae	2 (28.6)
	Escherichia coli	1 (14.3)

Table 4: Distribution of bacterial isolates from blood and urine samples.

Specimen	Species of bacteria	Age in years			
		<1	1-4.9	5-14.9	≥15
Blood	*Salmonela* spp	0(0)	1(16.7)	0(0)	0(0)
	Staphylococcus aureus	11(84.6)	2(33.3)	2(50)	2(66.7)
	Streptococcus pyogenes	1(7.7)	0(0)	0(0)	0(0)
	Streptococcus pneumoniae	1(7.7)	2(33.3)	2(50)	1(33.3)
	Total	13	6	4	3
Urine	*Escherichia coli*	0(0)	1(16.7)	0(0)	0(0)
	Klebsiella pneumoniae	0(0)	1(16.7)	1(100)	0(0)
	Pseudomonas aeruginosa	0(0)	2(33.3)	0(0)	0(0)
	Staphylococcus aureus	0(0)	2(33.3)	0(0)	0(0)
	Total	0	6	1	0

Majority of the micro-organisms isolated in blood 13 (52.0%) were from children below 1 year with *S. aureus* being the most common 11 (84.6%) organism. However, isolates from urine were mostly 6(85.7%) seen in children between 1-4.9 years with Pseudomonas and Staphylococcus species with each isolated in 2 (33.3%) samples (Table 4).

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

Discussion

This study was done in order to establish causes of fever among patients admitted with the intention of treating for malaria and with negative blood smears for malaria parasites. Apart from malaria, the most commonly identifiable causes of fever in patients with negative blood smears for malaria parasites are bacteraemia from any source, urinary tract infection, other viral and HIV infection, particularly during its early stages of seroconversion (Chan, 1990; Okwara *et al.*, 2004; Obaro *et al.*, 2011). In this study we have found that among the admitted patients with negative blood smears for malaria parasites, very few of them had bacteraemia, urinary tract infection or HIV infection. These results are similar to those reported in a study designed to find out the aetiology of acute, non-malaria febrile illnesses in Indonesia. In some parts of Africa, however, the contribution of bacteraemia in the incidence of febrile illness is enormous. In Ghana, for instance, recorded mortality rate due to bacteraemia of 40% in children who had negative blood smears for malaria parasites has been reported (Evans *et al*., 2004).

Since the advent of HIV during the 1980s', the epidemiology of febrile illnesses in sub-Saharan Africa has changed very much. HIV/AIDS is now claimed to be the leading cause of mortality in adults in Tanzania (Michael *et al.*, 2014) and probably in most parts of sub-Saharan Africa (Adjuik *et al.*, 2006). Therefore, considering the small proportion HIV infection in this study, the HIV should be considered in febrile cases with negative blood smear for malaria parasites.

Our findings indicate that in addition to malaria, bacterial infections and HIV other studies in Tanzania have reported other aetiological causes for febrile illnesses in the malaria endemic regions. Hertz *et al.* (2012) in northern Tanzania found Chikungunya and dengue viruses among hospitalised febrile patients; while Vairo *et al.* (2016) reported that one-fifth of febrile patients seeking care from health facilities in Dar es Salaam had dengue infection. Elsewhere in Tanzania, D'Acremont *et al.* (2014) reported that about more than two thirds of outpatient febrile cases were of viral origin. Our study showed majority of the isolated micro-organism were found in blood from children under 1 year. This is in accord with other studies done in elsewhere Africa (Nielsen *et al.* 2012; Christopher *et al.*, 2013). This may be due to their immature immune system among children which make them prone to infections.

This facility-based study was able to contribute in case management of patients admitted with febrile illnesses following the culture results. The study, however, had some limitations; not investigating a wide range of bloodstream and non-blood stream infections such as bacterial zoonoses (rickettsioses, brucellosis, borreliosis, Q-fever, leptospirosis), arboviral infections (dengue and Chikungunya), nasopharyngeal infections and toxoplasmosis which might also be responsible for the febrile illness in non-malaria fevers cases.

In conclusion, the study observed *S. aureus. K. pneumoniae* and *P. aeruginosa* are the commonest isolates from blood and urine cultures among febrile patients. It is important that HIV/AIDS is considered in the management of febrile cases with negative blood smear for malaria parasites.

Acknowledgements

We would like to thank the participants for agreeing to participate in the study. Dr. Hamisi Msangeni is thanked for initiating the study. Tanga Regional Referral Hospital administration is thanked for their collaboration. This study received financial assistance from the Health Research Users Trust Fund. The manuscript is published with permission from the Director General, National Institute for Medical Research.

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

References

Adedoyin, O.T., Oyeyemi, B.O., Aiyedehin, (2003) Screening of febrile children on hospital admission for urinary tract infections (UTI). *African Journal of Clinical & Experimental Microbiology* 4: 56–62.

Adjuik, M., Smith, T., Clark, S., Todd, J., Garrib, A., Kinfu, Y., Kahn, K., Mola, M., Ashraf, A. & Masanja, H. (2006) Cause-specific mortality rates in sub-Saharan Africa and Bangladesh. *Bulletin of the World Health Organization* 84: 181–188.

Ayoola, O.O., Adeyemo, A.A. & Osinusi, K. (2002) Predictors of bacteraemia among febrile infants in Ibadan, Nigeria. *Journal of Health, Population and Nutrition* 20: 223–229.

Bouyou-Akotet, M. K., Mawili-Mboumba, D.P., Kendjo, E., Mabika-Mamfoumbi, M., Ngoungou, E. B., Dzeing-Ella, A., Pemba-Mihindou, M., Ibinga, E., Efame-Eya, E., Planche, T., Kremsner, P.G. & Kombila, M. (2009) Evidence of decline of malaria in the general hospital of Libreville, Gabon from 2000 to 2008. *Malaria Journal* 8: 300.

Ceesay, S.J., Casals-Pascual, C., Nwakanma, D.C., Walther, M., Gomez-Escobar, N., Fulford, A.J.C., Takem, E.N., Nogaro, S., Bojang, K.A., Corrah, T., Jaye, M.C., Taal, M.A., Sonko, A.A.J. & Conway, D. J. (2010) Continued decline of malaria in The Gambia with implications for elimination. *PLoS One* 5: e12242.

Chan, R.K. (1990) Early clinical manifestations of HIV infection. *Singapore Medical Journal* 31: 477–479.

Christopher, A., Mshana, S.E., Kidenya, B.R., Hokororo, A. & Morona, D. (2013) Bacteremia and resistant gram-negative pathogens among under-fives in Tanzania. *Italian Journal of Pediatrics* 39: 27.

D'Acremont, V., Kilowoko, M., Kyungu, E., Philipina, S., Sangu, W., Kahama-Maro, J., Lengeler, C., Cherpillod, P., Kaiser, L. & Genton, B. (2014) Beyond malaria — causes of fever in outpatient Tanzanian children. *New England Journal of Medicine* 370: 809–817.

D'Acremont, V., Lengeler, C., Mshinda, H., Mtasiwa, D., Tanner, M. & Genton, B. (2009) Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever. *PLOS Medicine* 6: e252.

Evans, J. A., A dusei, A., Timmann, C., May, J., Mack, D., Agbenyega, T., Horstmann, R.D. & Frimpong, E. (2004) High mortality of infant bacteraemia clinically indistinguishable from severe malaria. *QJM* 97: 591–597.

Foxman, B. (2002) Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *American Journal of Medicine* 113: 5–13.

Hertz, J.T., Munishi, O.M., Ooi, E.E., Howe, S., Lim, W.Y., Chow, A., Morrissey, A.B., Bartlett, J.A., Onyango, J.J. & Maro, V.P. (2012) Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. *American Journal of Tropical Medicine and Hygiene* 86: 171–177.

Hoberman, A. & Wald, E. R. (1997) Urinary tract infections in young febrile children. *The Pediatric infectious disease journal* 16: 11–17.

Kamugisha, M. L., Msangeni, H., Beale, E., Malecela, E.K., Akida, J., Ishengoma, D.R. & Lemnge, M.M. (2008) Paracheck Pf compared with microscopy for diagnosis of Plasmodium falciparum malaria among children in Tanga City, north-eastern Tanzania. *Tanzania Journal of Health Research* 10: 14–19.

Mbata, T.I. (2007) Prevalence and antibiogram of UTIs among prisons inmates in Nigeria. *International Journal of Microbiology* 3: 6–12.

Michael, D., Kanjala, C., Calvert, C., Pretorius, C., Wringe, A., Todd, J., Mtenga, B., Isingo, R., Zaba, B. & Urassa, M. (2014) Does the spectrum model accurately predict trends in adult mortality? Evaluation of model estimates using empirical data from a rural HIV community cohort study in north-western Tanzania. *Global Health Action* 7: incl Supplements.

MoHSW (2012) *National Guidelines for Management of HIV and AIDS*. Ministry of Health and Social

Doi: http://dx.doi.org/10.4314/thrb.v19i4.3

Welfare, Dar es Salaam, Tanzania

Mmbando, B.P., Vestergaard, L.S., Kitua, A.Y., Lemnge, M.M., Theander, T.G. & Lusingu, J.P.A. (2010) A progressive declining in the burden of malaria in north-eastern Tanzania. *Malaria Journal* 9: 1–9.

Msangeni, H.A., Kamugisha, M.L., Sembuche, S.H., Malecela, E.K., Akida, J.A., Temba, F.F., Mmbando, B.P. & Lemnge, M.M. (2011) Prospective study on severe malaria among in-patients at Bombo regional hospital, Tanga, north-eastern Tanzania. *BMC Infectious Diseases* 11: 256.

Mwanziva, C., Manjurano, A., Mbugi, E., Mweya, C., Mkali, H., Kivuyo, M. P., Sanga, A., Ndaro, A., Chambo, W., Mkwizu, A., Kitau, J., Kavishe, R., Dolmans, W., Chilongola, J. and Mosha, F. W. (2011) Defining malaria burden from morbidity and mortality records, self treatment practices and serological data in Magugu, Babati district, northern Tanzania. *Tanzania Journal of Health Research*, 13: 1–12.

Nielsen, M.V., Sarpong, N., Krumkamp, R., Dekker, D., Loag, W., Amemasor, S., Agyekum, A., Marks, F., Huenger, F., Krefis, A.C., Hagen, R.M., Adu-Sarkodie, Y., May, J. & Schwarz, N.G. (2012) Incidence and characteristics of bacteremia among children in rural Ghana. *PLOS ONE* 7: e44063.

Njama-Meya, D., Clark, T.D., Nzarubara, B., Staedke, S., Kamya, M.R. & Dorsey, G. (2007) Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. *Malaria Journal* 6: 7.

Njozi, M., Amuri, M., Selemani, M., Masanja, I., Kigahe, B., Khatib, R., Kajungu, D., Abdula, S. & Dodoo, A. N. (2013) Predictors of antibiotics co-prescription with antimalarials for patients presenting with fever in rural Tanzania. *BMC Public Health* 13: 1097.

Obaro, S., Lawson, L., Essen, U., Ibrahim, K., Brooks, K., Otuneye, A., Shetima, D., Ahmed, P., Ajose, T. & Olugbile, M. (2011) Community acquired bacteremia in young children from central Nigeria-a pilot study. *BMC infectious diseases* 11: 1.

Okwara, F.N., Obimbo, E.M., Wafula, E.M. & Murila, F.V (2004) Bacteraemia, urinary tract infection and malaria in hospitalised febrile children in Nairobi: is there an association? *East African Medical Journal* 81: 47–51.

Perlmutter, B.L., Glaser, J.B. & Oyugi, S.O. (1999) How to recognize and treat acute HIV syndrome. *American Family Physician* 60:535-542.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A. & Olomi, R. (2004) Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* 329: 1212.

Schacker, T., Collier, A.C., Hughes, J., Shea, T. & Corey, L. (1996) Clinical and epidemiologic features of primary hiv infection. *Annals of Internal Medicine* 125: 257–264.

Shaw, K.N., Gorelick, M., McGowan, K.L., Yakscoe, N.M. & Schwartz, J.S. (1998) Prevalence of urinary tract infection in febrile young children in the emergency department. *Pediatrics* 102: e16.

Vairo, F., Mboera, L.E.G., De Nardo, P., Oriyo, N.M., Meschi, S., Rumisha, S.F., Colavita, F., Mhina, A., Carletti, F., Mwakapeje, E., Capobianchi, M.R., Castilletti, C., Di Caro, A., Nicastri, E., Malecela, M.N. & Ippolito, G. (2016) Clinical, virologic, and epidemiologic characteristics of Dengue Outbreak, Dar es Salaam, Tanzania, 2014. *Emerging Infectious Diseases* 22: 895-899.

WHO (2015) *World Malaria Report*. World Health Organization, Geneva, Switzerland

Wilson, M.L., Mitchell, M., Morris, A.J., Murray, P.R., Reimer, L.G., Reller, L.B., Towns, M., Weinstein, M.P., Wellstood, S.A. & Dunne, J.W.M. (2007) Principles and procedures for blood cultures; approved guideline. *CLSI document M47-A. Clinical and Laboratory Standards Institute, Wayne, PA*.