academicJournals

Vol. 11(28), pp. 1136-1141, 28 July, 2017
DOI: 10.5897/AJMR2017.8501
Article Number: A487B4165355
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Detection of Chikungunya and West Nile viruses in febrile patients in Ile-Ife Osun State, Nigeria using real time reverse transcription-polymerase chain reaction (RT-PCR)

Adesina O. A.[1*], Japhet M. O.[1] and Omilabu S. A.[2]

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos State, Nigeria.

Received 7 March, 2017; Accepted 26 April, 2017

Most patients presenting with febrile conditions are often treated for malaria, especially in the developing world, whereas some of them may be of arboviral origin as they also present with similar symptoms. Reverse transcription-polymerase chain reaction (RT-PCR) has been found to be the method of choice for the early detection and confirmation of virus in clinical samples, especially where there is an overlap of symptoms. The objective of this study was to detect the involvement of some arboviruses in febrile conditions in humans visiting two health institutions in Ile-Ife, Nigeria. Consenting febrile patients numbering one hundred and sixty five that were to be screened for malaria parasites at the hospitals were recruited for the study. From each patient, 2 ml venous blood was collected and processed for RNA extraction using QiAmp RNA Extraction kit (Qiagen, Hilden, Germany) and amplified using one step RT-PCR and appropriate primers for West Nile, Chikungunya, Dengue and Rift valley fever viruses. The detection was done in 2% agarose gel electrophoresis and viewed using a gel imager. The study reports the detection of West Nile RNA in 6 (3.6%) patients and Chikungunya RNA in 3 (1.8%) out of the 165 serum samples which have been pre-screened for malaria parasite by the hospital where the samples were collected from. Rift valley fever virus RNA and Dengue virus RNA were not detected in any of the samples. Out of the malaria parasite negative patients, 3 tested positive for the West Nile RNA and 1 showed detectable Chikungunya virus RNA, thus suggesting the role of these arboviruses in febrile conditions, first to be reported in Osun state, Nigeria. The involvement of viruses in febrile conditions as shown by this study has buttressed the need to extend laboratory examination of febrile conditions beyond malaria parasite to some of these arboviruses whose vectors are abundant and extending geographical coverage.

Key words: Febrile, malaria, Chikungunya virus, West Nile virus, Dengue virus, reverse transcription-polymerase chain reaction (RT-PCR).

INTRODUCTION

Arboviruses, as far back as 2004, have been considered as a global threat to human and public health (WHO, 2004). This status has not changed as their complex vector-virus-host cycle is leading to unpredictable

epidemiological patterns (Gan and Leo, 2014). They produce conditions ranging from asymptomatic infections to severe undifferentiated fever. In humans, arboviruses can produce three major syndromes which are systemic febrile illness (fever often associated with joint pain or rash), neuroinvasive disease (encephalitis or other infection of the central nervous system) and haemorrhagic fever (fever, generalized bleeding and shock). They can also progress to much more complex secondary conditions, or sequelae, which result in long-term physical and cognitive impairment or in early death. They have been able to progress in their geographical spread through urbanization, migration and climatic change, thereby increasing their impact on both humans and animals. While animal species, other than humans, are for the maintenance of many of these zoonotic viruses (Karabatsos, 2001), humans are incidental or dead end hosts to many of them, yet approximately 134 out of the over 534 viruses known to be transmitted by arthropod vectors have been shown to cause diseases in humans with mosquitoes and ticks being the principal transmitters. Most of these viruses are of public health importance especially members of the *Flavivirus*, *Alphavirus* and *Bunyavirus* genera. Emerging Flaviviruses of particular importance are West Nile (WN), Japanese encephalitis (JE), Chikungunya (CHIK) and Dengue (DEN) viruses which affect many countries of the whole world. Chikungunya virus (CHIKV) is an Alphavirus of importance that emerged in the Indian Ocean regions but is now spreading fast in Africa. Although, the vectors of both arboviruses and malaria are abundant in Africa, reports of coinfections have been scarce in scientific literatures, probably due to the limited number of laboratories capable of diagnosing arboviral infections or because there has not been major epidemic in Africa. Due to the presentation of similar symptoms which is mainly fever, arboviral infections are often taken for malaria thereby having the chance to be propagated the more among humans. Consequently, this may result in the slow identification of an arboviral disease outbreak and potentially high morbidity and mortality (Monlun et al., 1993; WHO, 2010; Baba et al., 2013). Arboviral and malaria parasite co-infections have previously been reported in Papua New Guinea (Senn et al., 2011), Senegal (Robin et al., 1980) and in European travellers in Senegal, Guinea and Sierra Leone (Charrel et al., 2005) but little has been said and known about arboviruses only in patients presenting with fever especially in Africa. Case definition and adequate surveillance, therefore, are major challenges. Treatment for arboviral diseases is mainly supportive (Domingues, 2009; WHO, 2011).

There have been more records of concurrent infection with malaria and dengue (Arya et al., 2005; Deresinski et al., 2006; Carme et al., 2009) after it was first reported in 2005 by Charrel et al. (2005). Although, it was a retrospective study that should be interpreted with caution, Epelboin et al. (2012) opined that, concurrent dengue and malaria infection tends to be more severe than single infections as they were characterized by haematologic abnormalities, such as thrombocytopaenia and anaemia, which are known risk factors of severe dengue fever and/or malaria. In Africa, the impact of most of these arboviruses on the public health has not been properly understood probably because the viruses have not been studied extensively and there has not been any major epidemic known or documented. In view of this, this study was designed to determine the involvement of four arboviruses namely CHIKV, DENV, RVFV and WNV in febrile conditions of patients visiting a private and a public hospital in Ile-Ife, Osun state, Nigeria.

MATERIALS AND METHODS

Sample collection

For the study, 165 venous blood samples from consenting patients presenting with fever in the last two weeks and those going for malaria parasite test at Obafemi Awolowo University Health Centre and Seventh Day Adventist Hospital, Ile-Ife, Osun State, Nigeria were used. Those whose presentation was more than two weeks were excluded from the study. There was neither age limit nor bias for a gender. From each patient, 2 mL of venous blood was collected into plain sample bottles, separated into serum and packed cells and stored at -20°C until analysed. Questionnaires were administered to the patients and the data obtained analysed using SPSS version 20.

RNA extraction

The 165 samples were randomly pooled to 33 with each pool containing five samples. For the extraction, 28 µl of each sample was pipetted into Eppendorf tube to give 140 µl in each pool. The genomic viral RNA extraction procedure was carried out using Qiagen-QIAamp Viral RNA mini spin column extraction kit (Qiagen, Hilden, Germany). The RNA was eluted from the spin columns in a final volume of 60 µl of the elution buffer following the manufacturer's procedure.

Amplification

Being a one-step real time RT-PCR procedure, the master mix was prepared using 4 µl dNTPs, 5 µl of one-step buffer, 0.5 µl probes, 1 µl enzymes and 1.5 µl forward and reverse primers for each of the viruses being screened for as stated in the QIAGEN One-Step RT-PCR Kit procedure used. All these were mixed in 9.5 µl of sterile (RNase free) water in 1.5 ml Eppendorf tube. The template used was 5 µl of the RNA extracted. The initial reverse transcription of RNA to cDNA (at 48 - 50°C for 20 min) and the amplification was done in Applied Biosystem 7300 real time PCR System in 50 cycles

*Corresponding author. E-mail: adesinafat@yahoo.co.uk. Tel: +2348034135898.

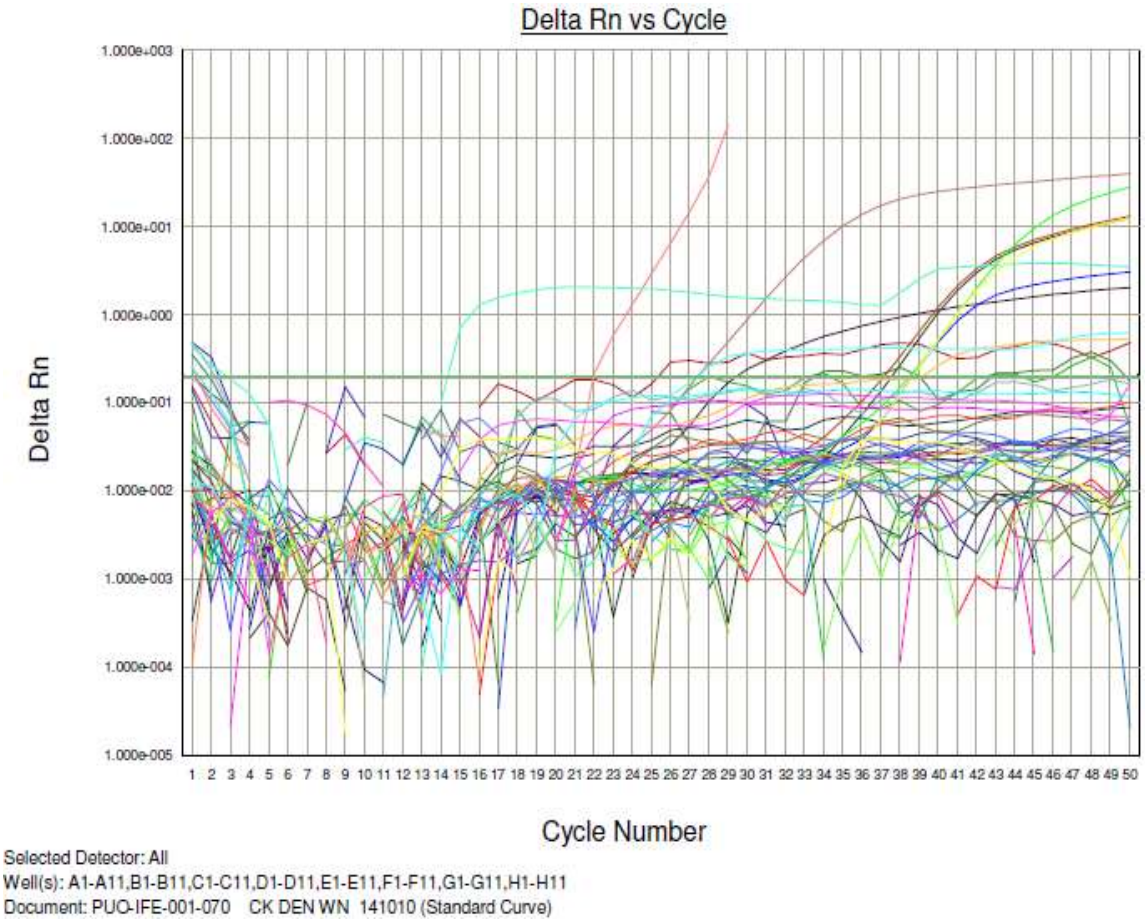

Figure 1. Real time result of some of the pools.

of denaturation (at 95°C for 15 min), annealing (at 60°C for 30 s), Elongation (at 56°C for 30 - 60 s). All the samples in the pools that showed positive results in the real time were separately extracted and the amplicons subjected to a one-step real time RT-PCR as was done for the pools (Figure 1).

Detection in agarose gel electrophoresis

This was carried out using a 2% agarose gel and TAE (Tris acetate EDTA) buffer to further confirm the result of the real time RT-PCR and to show the distribution of the selected arboviruses in the samples. Loading dye (5 µl) was added to the amplicon before loading on the cast and set gel. Appropriate ladders were used as the controls to indicate the band size expected from each virus (Dengue - 75 bp, West Nile - 200 bp, Chikungunya - 120 bp) where present. The gel was run at 100 V for 25 min after which it was stained in ethidium bromide for 10 min before viewing on the UV Invitrogen safe Imager. The expected band size was then compared to the ladder to check for positive cases and for which of the viruses.

RESULTS

The result of the malaria parasite pre-screening was collected from the hospitals where the samples were collected and it showed 125 (76%) having detectable malaria parasite (MP) in them and 40 (24%) were MP negative. Out of the 125 that had MP in their blood, 9 (7.2%) showed the presence of the nucleic acid of one of the arboviruses being studied in them but none had more than one. No patient showed the presence of Rift Valley Fever and Dengue viruses infections as the nucleic acids of these two viruses were not detected in any of the patients screened. Among the 40 MP negative patients, 4 (10%), making 2.4% of the patients studied, had either CHIKV or WNV detectable in them. In all, there was no case of the involvement of two arboviruses in any one of the febrile patients studied. It was also observed that there were patients who had both MP and at least one of either CHIKV or WNV. There were 2 cases of Chikungunya-malaria and 3 West Nile-malaria co-infections. There was only one male (making 20%) out of these five, who showed detectable viral RNA which in this case is Chikungunya virus. The remaining four were females. Table 1 shows the details of the results, while Table 2 shows the distribution of the arboviruses in relation to their socio-demographic data. The analysis of the data showed no statistically significant correlation (at $P < 0.01$ and $P < 0.05$) between the arboviruses detected

Table 1. Summary of the distribution of CHIKV, WNV and malaria in febrile patients.

Sample code	Sex	Age range (years)	Occupation	CHIKV +VE	WNV +VE	Malaria Parasite	
						+ve	-ve
HC041	Male	21-30	Student	✓		✓	
HC077	Male	11-20	Student	✓			✓
HC125	Female	51-60	Private Business	✓		✓	
HC123	Female	11-20	Student		✓		✓
HC071	Female	31-40	Teaching		✓		✓
HC080	Female	21-30	Student		✓	✓	
HC094	Male	21-30	Student		✓		✓
SD002	Female	61-70	None		✓	✓	
SD008	Female	41-50	Private Business		✓	✓	

CHIKV – Chikungunya virus; WNV – West Nile virus; MP– Malaria parasite.

Table 2. Socio-demography and the distribution of the detected arboviruses.

Parameter		WNV result			CHIK V Result		
		+ ve (%)	-ve (%)	Total	+ve (%)	-ve (%)	Total
Month of sample collection	August 2010	0 (0)	69 (41.8)	69	1 (0.6)	68 (41.2)	69
	September 2010	5 (3.0)	72 (43.6)	77	2 (1.2)	75 (45.5)	77
	October 2010	1 (0.6)	18 (10.9)	19	0 (0.0)	19 (11.5)	19
Age category (years)	Under 1	0 (0)	1 (0.6)	01	0 (0.0)	1 (0.6)	01
	1 - 10	0 (0)	4 (2.4)	04	0 (0)	4 (2.4)	04
	11 - 20	1 (0.6)	24 (14.5)	25	1 (0.6)	24 (14.5)	25
	21 - 30	2 (1.2)	71 (43.0)	73	1 (0.6)	72 (43.6)	73
	31 - 40	1 (0.6)	26 (15.8)	27	1 (0.6)	26 (15.8)	27
	41 - 50	1 (0.6)	18 (10.9)	19	0 (0)	19 (11.5)	19
	51 - 60	0 (0)	10 (6.1)	10	0 (0)	10 (6.1)	10
	61 - 70	1 (0.6)	5 (3.0)	06	0 (0)	6 (3.6)	06
Sex	Female	5 (3.0)	85 (51.5)	90	1 (0.6)	89 (54.0)	90
	Male	1 (0.6)	74 (44.8)	75	2 (1.2)	73 (44.2)	75
Occupation	Students	3 (1.8)	97 (58.8)	100	2 (1.2)	98 (59.4)	100
	Health worker	0 (0)	2 (1.2)	2	0 (0)	2 (1.2)	02
	Teacher	1 (0.6)	9 (5.4)	10	0 (0)	10 (6.1)	10
	Other civil servants	0 (0)	29 (17.6)	29	1 (0.6)	28 (17.0)	29
	Retiree	0 (0)	3 (1.8)	3	0 (0)	3 (1.8)	03
	Private business	1 (0.6)	14 (8.5)	15	0 (0)	15 (9.1)	15
	Artisan	0 (0)	2 (1.2)	2	0 (0)	2 (1.2)	2
	None	1 (0.6)	3 (1.8)	4	0 (0)	4 (2.4)	4
Highest academic qualification	None	2 (1.2)	8 (4.8)	10	0 (0)	10 (6.1)	10
	Primary	0 (0)	18 (10.9)	18	0 (0)	18 (10.9)	18
	Secondary	3 (1.8)	85 (51.5)	88	1 (0.6)	87 (52.7)	88
	Tertiary	1 (0.6)	48 (29.1)	49	2 (1.2)	47 (28.5)	49
Location	Within Osun	6 (3.6)	153 (92.7)	159	3 (1.8)	156 (94.5)	159
	Outside Osun	0 (0)	6 (3.6)	6	0 (0)	6 (3.6)	6

and the sociodemographic factors considered.

DISCUSSION

The concerted efforts to combat malaria has resulted in its mortality falling by 42% globally since 2000, and by 49% in the WHO African Region as well as mortality rates among children in Africa have been reduced by an estimated 54% since 2000 (WHO, 2014). However, the issue of the involvement of and co-infection with one or more arboviruses has called for taking febrile conditions more serious than before. Sow et al. (2016) carried out a study to investigate co-infection with malaria among arbovirus-infected patients in Senegal. Out of these patients, they reported that 48.7 % (20/41) were co-infected with malaria parasites with CHIKV having 18.7% (3/16) among other arboviruses with fever being the only sign or symptom associated with the dual malaria parasite/arbovirus infection. This is higher than 9 (7.2%) that is being reported in this study. In a study to ascertain the etiologic agent causing an outbreak of febrile illness with symptoms similar to chikungunya fever in Chiapas State, Mexico, Kautz et al. (2015) found that 79% of febrile illness cases with polyarthralgia in Chiapas State during late 2014 were caused by CHIKV. This is a further confirmation of the need to extend diagnosis of fever/febrile conditions beyond malaria as the pathogen as well as the vector seem to be thriving in virtually all the continents of the world.

The global spread of mosquito vectors of these pathogens via global demographic and societal changes, and modern transportation have provided the mechanisms for the vectors as well as the pathogens they transmit to break out of their natural ecology and become established in new geographic locations where susceptible arthropod vectors and hosts provide permissive conditions for them to cause major epidemics (Bonizzoni et al., 2013).

Presently in Africa, the epidemiology and public health impact of CHIKV and WNV is still unclear owing to the scanty information available but the current geographical distribution of their primary vectors and the likely further spread, increasing human population growth, unplanned urbanisation especially in developing world and increased international travel have all made transmission likely and successful (Amarasinghe et al., 2011 and Caglioti et al., 2013). Also, they present with similar symptoms thereby making it possible for them to be misdiagnosed. Furthermore, where malaria is endemic and the majority of febrile illnesses are diagnosed as such, often without laboratory confirmation, both viral infections may go undetected and so continue to perpetuate themselves (Amexo et al., 2004). It is very important that more extensive studies be carried out on the diagnosis and pathogenesis of arboviruses as some of them namely DFV, Zika virus and CHIKV infections have shown ocular manifestations which can be present at the time of fever or may manifest after many weeks. Anterior uveitis, optic neuritis and retinitis are the most common manifestations during the acute infection of these infections (de Andrade et al., 2017).

It is interesting to know that all the age groups are represented in those who were malaria parasite negative but positive for one arbovirus or the other. Arbovirus-*Plasmodium* infections can be said therefore to have nothing to do with age as all groups are susceptible. This is another reason why arboviruses should be suspected in febrile conditions. It was observed in this study that all the samples that had one arbovirus or the other but void of malaria parasite were collected in September. Although, more studies need to be conducted to establish the reason for this, but it is in agreement with what Forshey et al. (2010) reported where the arboviral infections showed a rise in the number of people infected in September. It is important to improve on the research capacity of many of the affected countries so as to correctly diagnose and manage these arboviral infections. The prevalence reported in this study could be higher if a larger population is studied and if patients with febrile conditions report early enough and their sample collected in the earlier stage of the infection than two weeks used in this study. This is calling for a more extensive study of the involvement of arboviruses in febrile conditions as a way to control their further spread since it appears that the spread of the vectors of these arboviruses, mosquitoes, has not been properly checked. A more involvement of the laboratory in the diagnosis of febrile conditions as a way to minimize the effect and spread of the pathogens is therefore necessary. It is also important that these arboviruses should be considered in conditions with symptoms similar to their infections.

CONFLICT OF INTERESTS

The authors hereby declare that there is no conflict of interest.

REFERENCES

Amarasinghe A, Kuritsk JN, Letson GW, Margolis HS (2011). Dengue virus infection in Africa. Emerg Infect. Dis. 17(8):1349-1354.

Amexo M, Tolhurst R, Barnish G, Bates I (2004). Malaria misdiagnosis: Effects on the poor and vulnerable. Lancet 364 (9448):1896-1898.

Arya CS, Mehta KL, Agarwal N, Agarwal BK, Mathai G, Moondhara A (2005). Episodes of concurrent dengue and malaria. Dengue Bull. 29:208-209.

Baba M, Logue CH, Oderinde B, Abdulmaleek H, Williams J, Lewis J, Laws TR, Hewson R, Marcello A, D' Agaro P (2013). Evidence of arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. J. Infect. Dev. Ctries. 7:51-59.

Bonizzoni M, Gasperi G, Chen X, James AA (2013). The invasive mosquito species Aedes albopictus: Current knowledge and future perspectives. Trends Parasitol. 29(9):460-468.

Caglioti C, Lalle E, Castilletti C, Carletti F, Capobianchi MR, Bordi L (2013). Chikungunya virus infection: an overview. New Microbiol. 36(3):211-27.

Carme B, Matheus S, Donutil G, Raulin O, Nacher M, Morvan J (2009). Concurrent dengue and malaria in Cayenne Hospital, French Guiana. Emerg Infect Dis. 15:668-671.
Charrel RN, Brouqui P, Foucault C, De Lamballerie X (2005). Concurrent dengue and malaria. Emerg. Infect Dis. 11:1153-1154.
de Andrade GC, Ventura CV, Mello Filho PA, Maia M, Vianello S, Rodrigues EB (2017). Arboviruses and the eye. Int. J. Retina Vitreous 3:4.
Deresinski S (2006). Concurrent Plasmodium vivax malaria and dengue. Emerg. Infect. Dis. 12(11):1802.
Domingues RB (2009). Treatment of viral encephalitis. Central Nervous Syst Agents Med. Chem. 9:56-62.
Epelboin L, Hanf M, Dussart P, Ouar-Epelboin S, Djossou F, Nacher M, Carme B (2012). Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. Malar. J. 11:142
Forshey BM, Guevara C, Laguna-Torres VA, Cespedes M, Vargas J, et al (2010). Arboviral Etiologies of Acute Febrile Illnesses in Western South America, 2000-2007. PLoS Negl. Trop. Dis. 4(8): e787.
Gan VCH, Leo YS (2014). Current epidemiology and clinical practice in arboviral infections - implications on blood supply in South-East Asia. ISBT Science Series 9:262-267
Karabatsos N (2001). International catalogue of arboviruses, including certain other viruses of vertebrates. Am. Soc Trop. Med. Hyg. (San Antonio, TX, USA); 1985. 2001 update.
Kautz TF, Díaz-González EE, Erasmus JH, Malo-García IR, Langsjoen RM, Patterson EI, Auguste DI, Forrester NL, Sanchez-Casas RM, Hernández-Ávila M, Alpuche-Aranda CM (2015). Chikungunya Virus as Cause of Febrile Illness Outbreak, Chiapas, Mexico, 2014. Emerg. Infect. Dis. Vol. 21, No. 11.
Monlun E, Zeller H, Le Guenno B, Traoré-Lamizana M, Hervy JP, Adam F, Ferrara L, Fontenille D, Sylla R, Mondo M, Digoutte JP (1993). Surveillance of the circulation of arbovirus of medical interest in the region of eastern Senegal (1988-1991)] (In French). Bull. Soc. Pathol. Exot. 86:21-28.
Robin Y, Cornet M, Heme G, Le Gonidec G (1980). Isolement du virus de la dengue au Sénégal. Ann. Virol. (Inst. Pasteur). 131E:149-54.
Senn N, Suarkia DL, Manong D, Siba PM, McBride WJH (2011). Contribution of dengue fever to the burden of acute febrile illnesses in Papua New Guinea: an age-specific prospective study. Am. J. Trop. Med. Hyg. 85:132-137.
Sow A, Loucoubar C, Diallo D, Faye O, Ndiaye Y, Senghor CS, Dia AT, Faye O, Weaver SC, Diallo M, Malvy D, Sall AA (2016). Concurrent malaria and arbovirus infections in Kedougou, southeastern Senegal. Malar. J. 15:47.
WHO (2011). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever - revised and expanded edition. New Delhi**:** WHO Regional Office for South East Asia 2011; p. xiv+196.
WHO (2014). Malaria Fact sheet N°94 Updated March 2014 http://www.who.int/mediacentre/factsheets/fs094/en.
WHO global burden disease 2004 update. Available from: http://www.who.int/healthinfo/global burden disease/2004 report update/en/index.html
WHO (2010). World malaria report 2010. Geneva: Word Health Organization.