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Evaluation of Diagnostic Performance of a Multiplex RT-qPCR Method for Detecting DENV Serotypes and CHIKV in Clinical Samples, Ouagadougou, Burkina Faso

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Abstract

Introduction: Arbovirus diseases such as dengue and chikungunya threaten public health worldwide. Early and rapid diagnosis and surveillance of dengue virus (DENV) and chikungunya virus (CHIKV) infections are essential to the control of these diseases. In this study, we evaluate the diagnostic performance of our new in-house multiplex RT-qPCR method for detecting DENV serotypes and CHIKV in an external laboratory. **Methodology:** The evaluation study was conducted on 200 clinical samples of suspected patients for arbovirus disease infection, collected in Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso. Our new multiplex RT-qPCR was compared to the commercial kit, the Zika, Dengue, and Chikungunya (ZDC) Real-Time PCR Assays kit (Bio-Rad, California, USA). **Results and Conclusions:** Among 200 samples, 21.5% (43/200) were DENV-positive by multiplex RT-qPCR, and 21.5% (43/200) were also DENV-positive by reference real-time RT-PCR. 157 (78.5%) samples tested negative for DENV by

both tests (new mRT-qPCR and reference test). The sensitivity and specificity of mRT-qPCR were 100%. The DENV serotypes detected were DENV-1 60.5% (26/43) and DENV-3 39.5% (17/43). CHIKV was not detected in this study. Our new mRT-qPCR is sensitive, cost-effective, simple, and can be used in developing country laboratories.

Keywords

Evaluation, Multiplex RT-qPCR, Dengue Virus, Chikungunya, Burkina Faso

1. Introduction

Chikungunya virus (CHIKV) and dengue virus (DENV) are arboviruses, transmitted to humans by the *Aedes* mosquito, particularly by *Aedes aegypti* and *Aedes albopictus* [1]. Arbovirus diseases impact millions of people every year and are highly prevalent in tropical and sub-tropical countries. DENV comprises four different serotypes including DENV1 to 4 and is a member of the *Flavivirus* genus, CHIKV is a member of the *Togaviridae* family and the *Alphavirus* genus [2]. According to the World Health Organization, 3.9 billion people in more than 128 countries, are at risk of DENV infection. CHIKV is now present in more than 110 countries on four continents including Asia, America, Africa, and Europe [3]. DENV is the main arbovirus in Burkina Faso, With the highest prevalence and a substantial morbidity and fatality rate. 1061 DENV cases have been reported in 2016 in Burkina [4]. In Ouagadougou, Burkina Faso, 28.54% of pregnant women had dengue fever in 2017 [5]. 29.1% seroprevalence of CHIKV was found in seroepidemiological research conducted in Ouagadougou using donor blood sampling in 2015 [6]. Two outbreaks occurred in Burkina Faso between August and November 2023. The CHIKV outbreak was concentrated in the city of Pouytenga, and the DENV outbreak mainly affected the cities of Ouagadougou and Bobo-Dioulasso. [7].

DENV infection can lead to severe dengue, including dengue hemorrhagic fever and dengue shock syndrome [2]. Generally, many patients infected with CHIKV recover within a few weeks, but reports of neurologic illness and death have been made [1]. The probability of severe dengue disease is increased when numerous DENV serotypes co-circulate with CHIKV [8]. Therefore, the identification of DENV serotypes and CHIKV detection is necessary for disease management and public health surveillance. In the absence of specific and effective antivirals and vaccines, strong surveillance is essential to control CHIKV and DENV infections [9].

For rapid and simultaneous detection and serotyping of DENV and CHIKV, we have previously developed one-step real-time multiplex RT-PCR (mRT-qPCR) assays. The process of diagnostic method development, adoption, or recommendation by health authorities requires independent evaluation by other clinical laboratories. This study aims to evaluate independently the diagnostic performance

of our new multiplex RT-qPCR method for detecting DENV serotypes and CHIKV in clinical samples in an external laboratory.

2. Materials and Methods

2.1. Patients and Samples

Between August 2023 and January 2024, we enrolled 200 patients suspected of having arbovirus disease who presented at the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso. Patients with one or more of the following symptoms: fever (>39˚C), headache, nausea, vomiting, diarrhea, rash, myalgia, arthralgia, and retroorbital pain, and who gave consent to participate were included in this study. Our exclusion criteria were patients who withdrew their consent for further participation in this study. Whole blood was collected from all patients and centrifuged to separate the serum. The serum samples were aliquoted into 1.5 mL vials and stored at −80˚C before all analysis. This study was approved by the institutional ethical committees of the Health Science Research, Burkina Faso No. A026-2023/CEIRES/IRSS. All participants gave informed consent before inclusion and sample collection.

2.2. Study Design

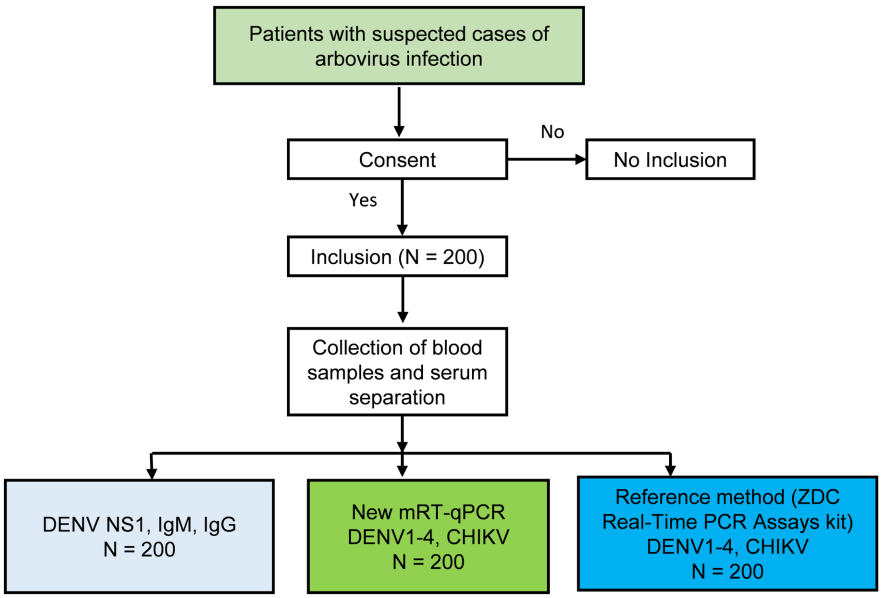

Figure 1. Study flow diagram.

First, all samples were screened for detection of DENV non-structural 1(NS1) antigen and DENV antibodies (IgM and IgG) using the diagnostic kit (Wondfo, Guangzhou, China) according to the manufacturer's instructions. Viral ribonucleic acid (RNA) was then extracted from serum using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and RNA extracts were used for molecular screening of DENV1-4 and CHIKV by our new multiplex RT-qPCR assay using

the QuantiTect SYBR Green kit (Qiagen, Hilden, Germany) according to protocol developed by Belem *et al.* [10]. All samples were also screened using the reference method, the Zika, Dengue, and Chikungunya (ZDC) Real-Time PCR assay kit (Bio-Rad, California, USA) (Figure 1). Multiplex RT-qPCR was performed using QuantStudio 5 real-time PCR system with software version 1.5.1. (Applied Biosystems™, Thermo Fisher Scientific, Massachusetts, USA)

2.3. Data Analysis

Descriptive demographic variables were done by calculating median age, percentages, and frequencies. The screening of DENV1-4 and CHIKV (molecular and serological test) was carried out by 3 methods, the new mRT-q PCR, the reference real-time RT-PCR and the serological test. The sensitivity and specificity of the new test were determined by comparing positive and negative cases detected by new mRT-q PCR and reference real-time RT-PCR. R software version 4.3.1 was used for the analysis of all data.

3. Results

3.1. Characteristics of Patient

During the study period, a total of 200 patients with suspected cases of arbovirus disease were included in CERBA. Females are the majority of patients including 56.5% (113/200) against 43.5% (87/200) of males. The median age of 27 years has been found among the study population.

3.2. Serological Diagnostic of DENV

All sera were only screened for DENV NS1 antigen and IgM and IgG antibodies using the combined diagnostic kit NS1, IgM and IgG (Wondfo, Guangzhou, China). Among the 200 samples, 20% (40/200) were positive for DENV NS1, while 1.0 % (2/200) and 2.5 % (5/200) were positive for DENV IgM and IgG antibodies respectively. Additionally, 0.5% (1/200) were positive for both DENV NS1 and IgM, 0.5% (1/200) positive for (NS1 + IgM + IgG) and 0.5% (1/200) for both IgM and IgG (Table 1).

The overall seroprevalences for DENV (considering NS1, IgM, and IgG antibodies) was 25% (50/200). DENV NS1 positive in the sample was associated with symptoms onset within 0 - 7 days, while IgM and IgG positivity were observed from 7 days onwards.

Table 1. Serological diagnostic of DENV.

Serology	NS1 DENV, n/N (%)	NS1 + IgM DENV, n/N (%)	NS1 + IgM + IgG DENV, n/N (%)	IgM DENV, n/N (%)	IgG DENV n/N (%)	IgM + IgG DENV, n/N (%)
Number of patients	40/200 (20)	1/200 (0.5)	1/200 (0.5)	2/200 (1.0)	5/200 (2.5)	1/200 (0.5)

3.3. Performance Diagnostic of the New mRT-qPCR in CERBA

A total of 200 samples were analyzed at CERBA, of which 21.5% (43/200) were DENV-positive by the new multiplex RT-qPCR and the same 21.5% (43/200) were also DENV-positive by reference real-time RT-PCR. 157 (78.5%) samples tested negative for DENV by both tests (New mRT-qPCR and reference test). The sensitivity and specificity of mRT-qPCR were 100% (Table 2). CHIKV was not detected in this study.

Table 2. Comparison of new mRT-qPCR and reference RT-qPCR.

Performance	mRT-qPCR, n/N (%)		Reference RT-qPCR, n/N (%)	
	DENV	CHIKV	DENV	CHIKV
	43/200 (21.5)	0/200 (0.0)	43/200 (21.5)	0/200 (0.0)
Sensibility mRT-qPCR	100%		NA*	
Specificity mRT-qPCR	100%		NA	

NA = Not Applicable.

The serotypes detected were DENV-1 60.5% (26/43) and DENV-3 39.5% (17/43) (Table 3). All samples positive for DENV-1 and DENV-3 by reference test were also positive by new mRT-qPCR. DENV-2 and DENV-4 were not detected by both tests. The sensitivity and specificity were also 100% in determination of the DENV serotype.

Table 3. DENV serotypes detected by mRT-qPCR.

	Serotypes			
	DENV-1, n/N (%)	DENV-2, n/N (%)	DENV-3, n/N (%)	DENV-4, n/N (%)
Number of patients	26/43 (60.5)	0/43 (0.0)	17/43 (39.5)	0/43 (0.0)

4. Discussion

Due to their similar modes of transmission and shared mosquito vectors, DENV and CHIKV are increasing in endemic and epidemic transmission worldwide. Patients with similar clinical signs are difficult to diagnose because of the large overlap between the two viruses. In addition, both viruses have the potential to cause severe, long-lasting diseases with a significant impact on global public health. Thus, it is crucial to transfer rapid and accurate molecular methods for detecting these for appropriate patient management, and public health surveillance. This study evaluated a new multiplex RT-qPCR method previously developed for one-step detection and serotyping of DENV and CHIKV [10]. This study was carried out in the laboratory of CERBA, Ouagadougou, Burkina Faso, a region where dengue fever is endemic.

The proportion of females in our study population was higher than males. Although the risk of contracting an arbovirus infection is not gender-specific but rather depends on exposure to mosquito bites [11] [12]. The young, active population was represented by a median age of 27 years. As demonstrated by Sondo *et al.*, this may result in a decline in the workforce due to illness and socioeconomic repercussions [13].

RDTs are the primary diagnostic tool for DENV in Burkina Faso. A significant portion (25%) tested positive for DENV (NS1, IgM, and IgG), were found. The sample positive for IgG indicates a past infection. NS1 is detected in patients during the acute phase where the patient is viremic. These results highlight the importance of using a combination of diagnostic methods depending on the stage of infection for effective epidemiological and seroepidemiological surveillance

In this study, only DENV was detected by molecular assays, with the circulation of DENV-1 and DENV-3. Finding aligns with recent outbreaks in 2023, Burkina Faso [7]. Dengue outbreaks due to these serotypes have been found in Nepal in 2022 and in Nigeria in 2019.

In our study, CHIKV was not detected, maybe because the sample collection period predated the CHIKV outbreak reported in September 2023 by the Reference National Laboratory of Viral Hemorrhage Fever (LNR FHV) of Burkina Faso.

Molecular detection of DENV or CHIKV depends on the acute phase where patients are viremic, due to immune response allows for substantial antibody formation after 7 days from the onset of symptoms, and may result in virus neutralization and lack of detection by molecular methods.

The evaluation of the new mRT-qPCR at CERBA confirmed the high sensitivity (100%) and specificity (100%) of the assay. The study showed that our new method can be reproduced in other laboratories with different equipment. mRT-qPCR was carried out using several models of thermal cycler, including the iCycler Real-Time PCR (BioRad, Hercules, California, USA) at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, the CFX96 Real-Time System (BioRad, Hercules, California, USA) at the Centre Muraz molecular biology laboratory and QuantStudio 5 real-time PCR system at CERBA molecular biology laboratory [7] [10]. The various thermal cyclers achieved good, sensitive, and specific amplification.

Appropriate management of the disease and care of the patient depends on differential diagnosis. Dengue serotyping helps predict the intensity of future outbreaks and provides evidence of the shift in the main circulating serotypes over time. By detecting connected outbreaks across borders, precise serotyping would enable a better understanding of traveling waves in dengue fever transmission from an international standpoint. Research on the multi-annual cross-country periodicity of dengue, which is believed to be connected to the cycling of host immunity to various serotypes, can also be informed by serotyping data.

Cost is another crucial factor to take into account while implementing assays in

developing countries. Molecular detection of arboviruses is generally carried out using commercial real-time RT-PCR kits based on TaqMan technology. This method is costly, especially if it is used for monitoring large numbers of samples. The in-house mRT-qPCR based on the SYBR green method has the benefits of sensitivity, affordability, and ease of use.

The mRT-qPCR is cost-effective and can be used in clinical applications for DENV and CHIKV differential diagnostics for the administration of supportive care and public health surveillance to control outbreaks.

This study's limits are, that the assay's results with clinical diagnoses and patient outcomes have not correlated and the evaluation has not been carried out in multicenter. The next study will be a multicenter evaluation of this assay for the detection of DENV1-4 and CHIKV in human and *Aedes* mosquitos and expanding its capability to include Zika and yellow fever virus detection.

5. Conclusion

This independent evaluation study confirms the good performance, sensitivity and specificity of our new mRT-qPCR compared to a reference method. It can be reproduced in other laboratories with different real-time PCR equipment for the diagnosis and epidemiologic surveillance of CHIKV and DENV. This tool is cost-effective, simple, and can be used in developing country laboratories.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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