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Development of an RT-LAMP assay for the detection of Lassa viruses in southeast and south-central Nigeria

Abstract

Background

Lassa fever is a viral hemorrhagic fever responsible for an acute and often fatal illness. Lassa Fever is endemic in Western African countries. Approximately 100,000 to 300,000 clinical infections and 5000 deaths per year are estimated in those regions. Nigeria is the most affected country. LASV strains are genetically diverse and clustered into six main lineages according to their geographic location. The clinical recognition of Lassa Fever represents a challenge due to the symptom similarities with other febrile illnesses prevalent in the LASV affected areas such as Malaria and Typhoid fever. To confirm a diagnosis of Lassa Fever, a laboratory test is required. In this study, a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed for the detection of LASV lineage II in southeast and south-central Nigeria.

Method

LAMP primers for the detection of LASV lineage II were designed based on the coding sequences from GPC gene. The nucleotide sequences of LASV lineage II isolates, which are available in the GenBank database, were aligned to identify conserved regions. Three primer sets specific for the lineage II strains were designed: primers specific for the isolates from Ebonyi, Anambra and Edo states. The amplification of LASV RNAs was performed in a constant temperature (63 °C) using the mixture of the three primer sets. The assay was carried out using a portable battery-powered fluorescence device for 30 minutes.

Results

The limit of detection of the developed assay was evaluated using *in vitro* RNAs of six representative isolates of LASV lineage II. The assay was able to detect 20 to 2000 copies of RNA per reaction depending on the isolate. The assay was further evaluated for detection in 73 plasma samples collected from patients with suspected Lassa fever from southern Nigeria. Clinical samples with a viral titer of ≥2,303 geq/mL (Ct value ≤ 32) could be detected by both RT-LAMP assay and RT-PCR method. Using the conventional RT-PCR as the reference test, the assay revealed a sensitivity of 50% in general with 100% for samples with a viral titer of 9500 geq/mL and higher. The assay showed 98% specificity with no cross-reactivity to other viruses and pathogens which cause symptoms similar to Lassa fever.

Significance

The RT-LAMP assay is a useful diagnostic test for Lassa fever during the acute phase of the disease, contributing to improve the patient management and to speed up outbreak response. This assay offers the possibility of use in the field or in ill-equipped laboratory settings and an accessible diagnostic test of Lassa Fever to the most affected population living in rural and remote areas.