

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.**

**Annual/Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)**

- 1. Project report No. 1st/2nd/3rd/Final: Final**
- 2. UGC Reference No: 41-518/2012[SR], DT 17/07/2012**
- 3. Period of report: from 2012 to 2015**
- 4. Title of research project: "Identification and epidemiological survey of dengue virus serotypes occurring in South India during 2011-14".**
- 5. (a) Name of the Principal Investigator: Dr Musturi Venkataramana**

(b) Dept. and University/College where work has progressed:
Dept of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad, Prof. C. R Rao Road, Gachibowli-46, Hyderabad, India.
- 6. Effective date of starting of the project: 01-07-2012.**
- 7. Grant approved and expenditure incurred during the period of the report:**
 - a. Total amount approved Rs: 796000-00 (Sanctioned Rs: 723000-00)**
 - b. Total expenditure Rs: 723000-00**
 - c. Report of the work done: (Please attach a separate sheet)**
 - i. Brief objective of the project:**
 - a) Identification of dengue virus serotypes occurring in South India during 2011-14.
 - b) Epidemiological survey of dengue fever in South India during 2011-14.
 - ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication:**
Please find the enclosed Annexure-I at the end.
 - iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons: Yes**
 - iv. Please indicate the difficulties, if any, experienced in implementing the project: No**
 - v. If project has not been completed, please indicate the approximate time by which it is likely to be completed: Completed**
 - vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done**

may also be sent to the Commission
Please find the enclosed Annexure-I at the end.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

(a) Manpower trained:

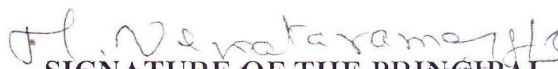
Students registered for Ph. D and M. Sc project students were given the opportunity to procure the consumables and to use the travel money from this project.

(b) Ph. D. Awarded: No

(c) Publication of results:

1. Kishore Vaddadi, Chaitanya Gandikota, Pratul Kumar Jain, V S V Prasad and Musturi Venkataramana. Co-circulation and co-infections of all dengue virus serotypes in Hyderabad, India 2014. *Epidemiology & Infection*. 2017. 145 (12):2563-2574. Doi: 65EBDCCEBCE90A02E56A591F12C127E3.
2. K. Vaddadi, C. Gandikota and M. Venkataramana. Complete genome characterization and evolutionary analysis of serotype-4 associated with severe dengue. *Epidemiol. Infect.* 2017. 145, 1443-1450. doi:10.1017/S0950268817000243.

(d) Other impact, if any:


SIGNATURE OF THE PRINCIPAL
INVESTIGATOR


SIGNATURE OF THE
CO-INVESTIGATOR


REGISTRAR/PRINCIPAL

(P. Sardar Singh)
Registrar
University of Hyderabad
Hyderabad-500 046.

Dr. K. Arun Kumar
Assistant Professor
Department of Animal Biology
School of Life Sciences
University of Hyderabad
P.O. Central University, Gachibowli,
Hyderabad-500 046. T.S. INDIA.

ANNEXURE-I

Work Done

Brief Summary: The data showed the co-circulation and co-infections (in same patient) of all the four dengue virus serotypes in the South Indian states Telangana and Andhra Pradesh. There are more dengue hemorrhagic (DHF) cases than dengue fever (DF) cases and few dengue shock syndrome cases also recorded. We have also initiated the nucleotide sequencing of all the serotypes and for serotype 1 and 2 complete genome sequencing is nearly done. The sequence data is submitted to the NCBI gene bank data base. The data also indicated the significant mutational spectra throughout the genome. Manuscript is under preparation for the publication by compiling all the above obtained data.

Ethics Statement:

This study for using human blood samples was approved by the Institutional Ethics Committee, University of Hyderabad (IEC/UoH/MV/11/06 and UH/IEC/2014/24).

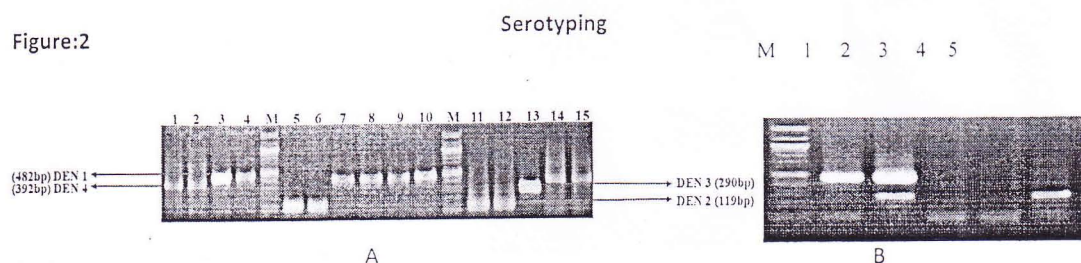
Sample collection:

We are in touch with different hospitals in the states of Telangana and Andhra Pradesh, South India, for dengue virus infected/suspected blood samples since 2009-10. In connection with the present study we have collected nearly 100 samples during the years 2013-14 and 2014-15. Infected blood samples are collected from the Mahatma Gandhi Memorial (MGM) hospital, Warangal and Lotus Children's hospital, Hyderabad, Telangana. Clinical information of the patients was procured and saved. Nearly 20 healthy samples were also procured during the same period from the same hospitals. Warangal and its surroundings is the place from where frequent virus outbreaks were reported and caused significant number of deaths. As per the recorded patient information, we have noticed that the patients recorded in Lotus hospital, Hyderabad are from different places of the states Telangana and Andhra Pradesh. The samples were stored at -20°C until use.

Serotyping and whole genome sequencing:

Serotyping was performed using 50 samples, and we have identified serotypes in the tested samples. Few samples showed individual serotypes where as some showed more than one serotype in a single patient's sample. 80% of DENV1 and 70% of DENV2 gene segments were amplified and cloned successfully. Sequencing of the above gene segments was also carried.

Figure:2



Agarose gel analysis of the product from RT-PCR followed by second-round nested PCR of RNA samples. From left to right (100 bp molecular marker) Fig A: lanes 3,4,7,8,9 and 10 of sample positive for DEN-1 (482 bp), lanes 5,6,11 and 12 of sample positive for DEN-2 (119 bp), lane 13, sample positive for DEN-3 (290 bp), lanes 1,2,14 and 15 of sample positive for DEN-4 (392 bp). Fig B: lane 1 (DV 1 and 2), lane 2 (DV1 and DV3), lane 3 and 4 (DV2&DV4) lane 5: DV 3.

Dr. Musturi Venkataramana
Associate Professor
Department of Microbiology
University of Hyderabad
Campus
H. Hyderabad
G. 500 046, India.

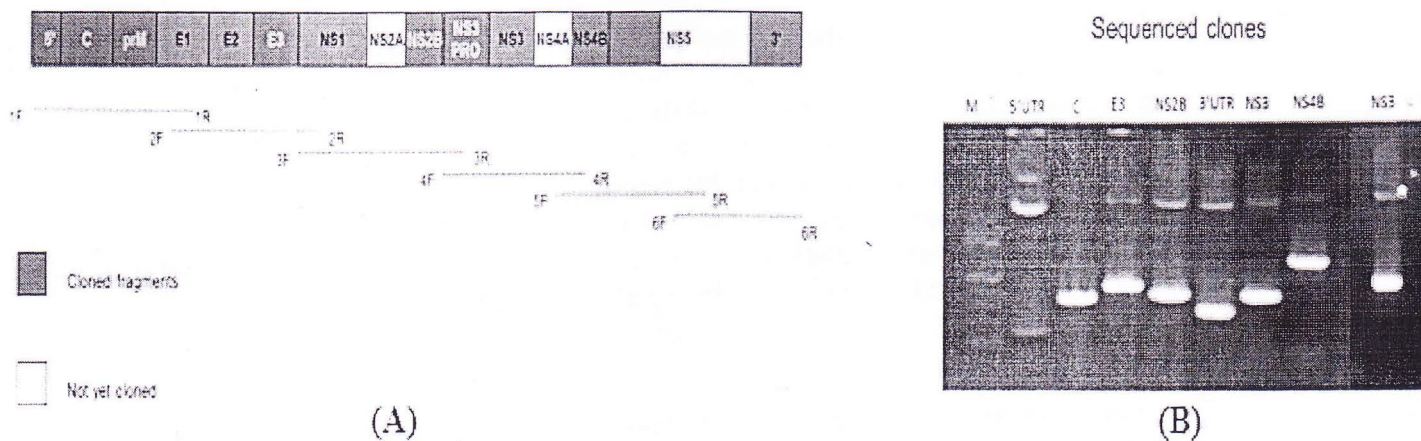


Figure 2: Complete genome sequencing (A) DENV 1 Complete genome sequencing. (B) Agarose gel analysis of the PCR amplified sequenced products.

Table 1: Complete genome sequencing of Dengue virus serotypes

Genes	DEN1	DEN2	DEN3	DEN4
Capsid	Yes	Yes	-	-
PrM	Yes	Yes	Yes	Yes
Enveloped	Yes	Yes	-	-
NS1	Yes	-	-	-
NS1-2	Yes	-	-	-
NS2A	-	-	-	-
NS2B	Yes	Yes	-	Yes
NS3	Yes	Yes	-	Yes
NS3 Hel	Yes	Yes	-	-
NS3-4	Yes	-	-	-
NS4A	Yes	-	-	-
NS4B	Yes	-	-	-
NS5	--	-	-	-
3'UTR	Yes	Yes	-	-

Dr. Musturi Venkataraman
 Associate Professor
 School of Biotechnology
 School of Life Sciences
 University of Hyderabad
 Gachibowli, Hyd-500 046, India.

Table: List of dengue virus nucleotide sequences carried out in our laboratory and submitted to the Gene Bank

1	Dengue virus 1/ IN/ UOH_3041/ 2014, NS2B partial cds	1841527 (ID)	Chaitanya Gandikota, Kishore Vaddadi, Venkataramana Musturi
2	Dengue virus 1/ IN/ UOH_3041/ 2014, NS3 partial cds	1841520 (ID)	“
3	Dengue virus 4 / IN/ UOH_3095/ 2014, NS2B Partial cds	KT282376	“
4	Dengue virus 2 / IN/ UOH_2986/ 2014, NS2B Partial cds	KT282377	“
5	Dengue virus 2 / IN/ UOH_2986/ 2014, NS3 Partial cds	Not yet submitted	-
6	Dengue virus 4 / IN/ UOH_3095/ 2014, NS3 Partial cds	Not yet submitted	-
7	Dengue virus 1/ IN/ UOH_3041/ 2014, 5'UTR	KP974820	Kishore Vaddadi, Chaitanya Gandikota, Venkataramana Musturi
8	Dengue virus 2 / IN/ UOH_4276/ 2014, 5'UTR	KT282378	“
9	Dengue virus 2 / IN/ UOH_7446/ 2014, 5'UTR	KT282379	“
10	Dengue virus 2 / IN/ UOH_7446/ 2014, 3'UTR	KT282380	“
11	Dengue virus 1/ IN/ UOH_3041/ 2014, 3'UTR	KP974821	“
12	Dengue virus 1/ IN/ UOH_2690/ 2014, CprM partial cds	KR780674	“
13	Dengue virus 1/ IN/ UOH_2899/ 2014, Env DIII partial cds	KR780675	“
14	Dengue virus 2 / IN/ UOH_7446/ 2014, CprM Partial cds	KT282374	“
15	Dengue virus 3 / IN/ UOH_2355/ 2014, CprM Partial cds	KT282375	“
16	Dengue virus 1/ IN/ UOH_3041/ 2014, NS4B partial cds	KR349064	Barnwal. A, “
17	Dengue virus 1/ IN/ UOH_3041/ 2014, Capsid partial cds	KR349065	Jain. P, “

Dr. Venkataramana Musturi
 Dr. Musturi Venkataramana :
 Associate Professor
 Department of Biology
 Life Sciences
 University of Hyderabad
 Gachibowli, Hyderabad-500 046, India