Novel Approaches to the Diagnosis of Central Nervous System Infections

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Ho Chi Minh City
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Case study

September 16th 2016

A previously healthy 16-year-old boy in Southern Vietnam became ill: fever, back pain, diarrhea and limb weakness

September 18th 2016

Admitted to our hospital in Ho Chi Minh City: high fever, neck stiffness and flaccid paralysis of 4 limbs.

Acute cerebrospinal fluid (CSF) lab results:

- Pleocytocis
- Normal glucose
- Normal lactase
- Elevated protein

Open Forum Infectious Diseases, 2017

Initial laboratory investigations

Routine diagnosis

All negative

- Gram stain
- Bacterial culture
- Cryptococcal antigen test (LFA)
- Acid-fast bacilli examination
- HSV PCR
- Dengue NS1
- Japanese encephalitis virus IgM

CSF
Plasma
Urine
Rectal swab

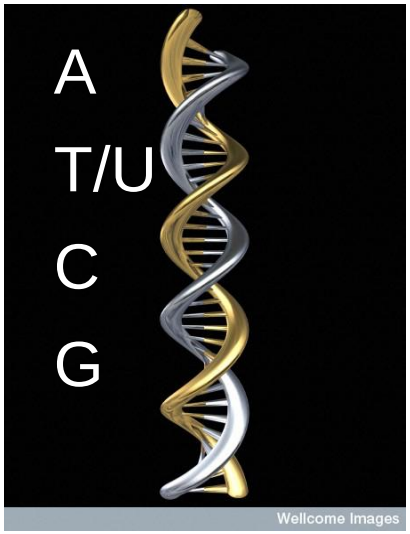
OUCRU lab diagnosis

All negative

- Enterovirus RT-PCR
- Dengue RT-PCR
- Zika RT-PCR

Pathogen detection by next-generation sequencing of clinical samples: the principle

Patient samples



Millions of DNA sequences are generated

Pathogen sequence identification by ‘simple’ bioinformatics tools

Viral metagenomics results

	CSF	Rectal swab	Plasma
No of reads	550,644	597,032	754
Pathogen	Not found	Not found	Not found
No of reads mapped	Not done	Not done	Not done

Was the detection of JEV in urine just a contamination?

Clinical Infectious Diseases IDSA Infectious Diseases Society of America hivma hiv medicine association

Japanese Encephalitis Virus RNA Not Detected in Urine

FREE

Hui Zhao ✉ ✉, Yu-Guang Wang, Yong-Qiang Deng, Ke-Yu Song, Xiao-Feng Li, Hong-Jiang Wang, Chao-Min Zhu, E-De Qin, Cheng-Feng Qin

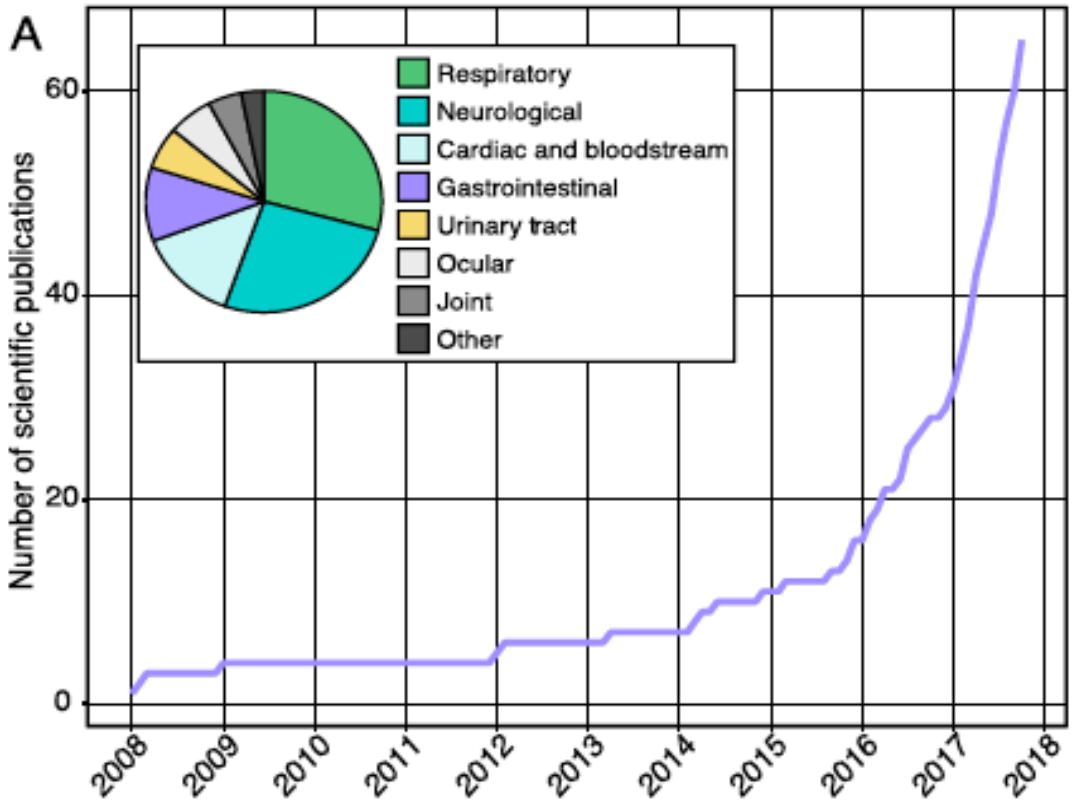
Clinical Infectious Diseases, Volume 57, Issue 1, 1 July 2013, Pages 157–158,
https://doi.org/10.1093/cid/cit169
Published: 26 March 2013

Additional JEV testing

Test	Admission samples		
	CSF	**plasma**	**Urine**
JEV IgM ELISA	Neg	Neg	ND
Deep Sequencing	Neg	Neg	Pos
JEV RT-PCR	Neg	Neg	Pos

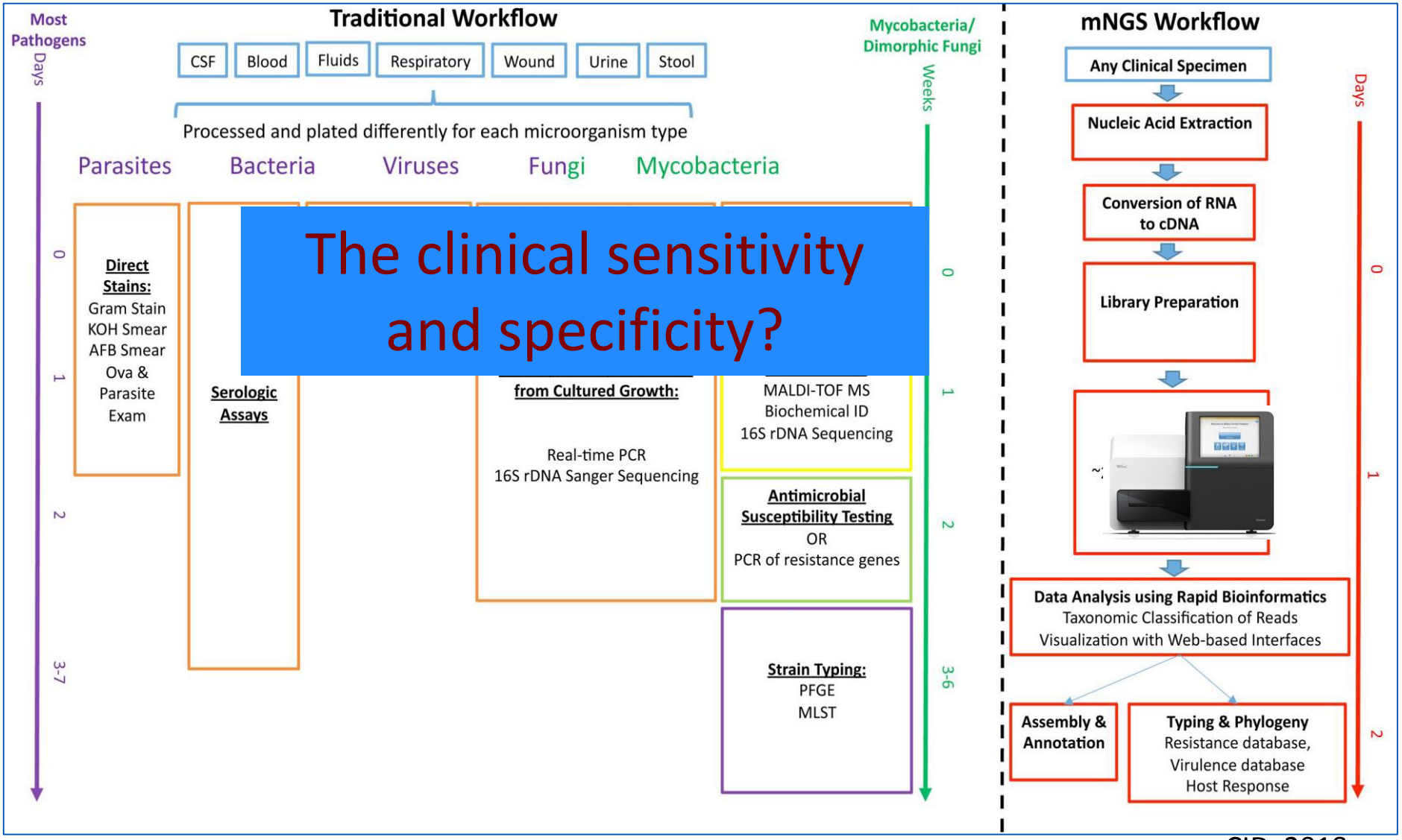
- JEV was responsible for the cause of paralysis.
- 9th November 2016: transferred to rehab hospital, marked with weakness of all limbs.

The use of next-generation sequencing for diagnostic purposes: current gaps in knowledge



Computational and Structural Biotechnology Journal 2018

The use of next-generation sequencing for diagnostic purposes: current gaps in knowledge



CID, 2018

A prospective study to evaluate the utility of new technologies in patients with brain infections

Research questions:

- Can metagenomics detect a broad range of known/unknown pathogens in the CSF, thereby improving upon current standard laboratory assays?
- Can NGS can provide rapid whole-genome sequencing and prediction of antimicrobial susceptibility for *M. tuberculosis* and *S. pneumoniae*, the two commonest causes of bacterial meningitis worldwide?
- Do CSF samples contain discriminating protein/peptide signatures for the common bacterial, fungal and viral causes of CNS infection?

Sample size: 750 patients

Duration: ~36 months (2017 – 2020)

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In summary

- Urine can be useful for early diagnosis of Japanese encephalitis virus

- Next-generation sequencing can be a pan-pathogen assay for clinical diagnosis, especially cases of unknown etiology.

- Prospective study is needed to evaluate the clinical utility of next-generation sequencing, and is ongoing.

Acknowledgements

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The CNS infections diagnosis study team