Metagenomic technologies in neuro-infection

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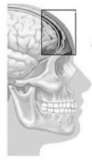
- **Summary**

Challenges in Infectious Meningoencephalitis



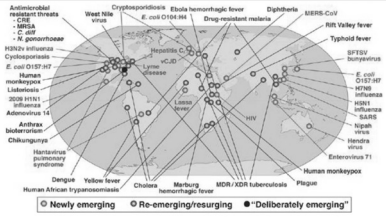
Meningitis / Encephalitis

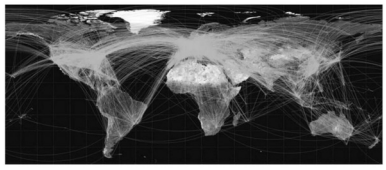
50 – 70%
unknown cause

Viral	*Bacterial*	*Rickettsial*
• HSV-1/2	• *Neisseria meningitis*	• *Rickettsia sp.*
• VZV	• *Streptococcus pneumoniae*	• *Coxiella burnetii*
• EBV	• *Haemophilus influenzae*	• *Ehrlichia chaffeensis*
• CMV	• *Mycoplasma pneumoniae*	
• HHV-6/7	• Group A streptococci	*Parasitic*
• Poliovirus and other enteroviruses incl. Echovirus 22	• *Listeria sp.*	• *Toxoplasma gondii*
• Adenovirus	• Mycobacteria	
• Measles	• *Chlamydia sp.*	
• Mumps	• *Borrelia sp.*	
• Rubella	• *Brucella*	
• LCMV	• *Leptospira*	
• JC virus	• *Treponema pallidum*	
• Influenza A & B	• *Ba*	
• Japanese encephalitis		
• Dengue		
• Tickborne encephalitis		
• Nipah		
• Murray Valley encephalitis		
• St Louis encephalitis		
• West Nile virus		
• Rabies virus		
• European Bat Lyssavirus		
• HIV		
• B-virus		

- Clinical manifestations are not useful for pathogen identification (**Nonspecific symptoms**)
- **Delayed diagnosis** and treatment result in death or severe sequelae

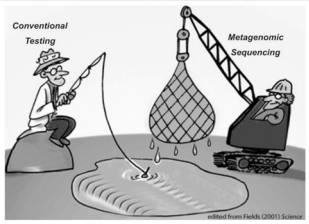
Emerging Infectious Diseases





- **Serological tests?**
- **PCR?**
- **Culture?**

Advantages of Metagenomic Approach



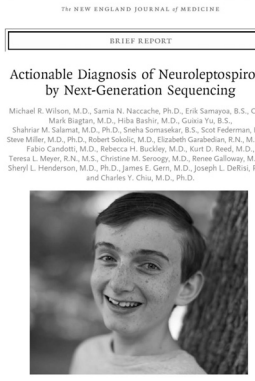
"Meta-" : 더 높은, 초월한
"Metagenomics": 군유전체학

- **Metagenome:** the collective genome of all the microorganisms in an environment (Handelsman et al. 1998)
- **Metagenomics** is the study of genetic material recovered from an environmental sample

- **Advantages**
  - Hypothesis-free, **Universal** pathogen detection
  - Promising to improve diagnostic yield of infections
    - Identification of an **entirely novel microbe**
    - Identification of a **known microbe** that is **not a cause** of particular **disease phenotype**
- **Barriers**
  - High costs
  - Long sequencing times
  - Slow, complicated data analysis tools

Edited from Dr. Charles Chiu's slide

Successful case of Clinical Metagenomics

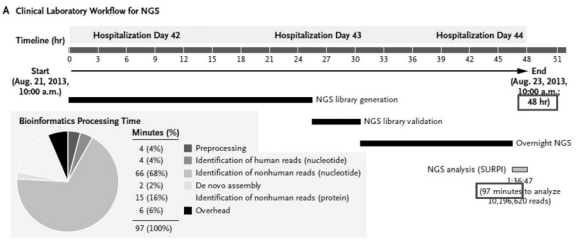


Neuroleptospirosis Diagnosed by Metagenomic NGS

- 3 hospitalizations over 4 months
- 44 days in the ICU
- >100 inconclusive tests
- 3 empiric treatments with no effect
- Brain biopsy and induced coma
- **Cured 2 weeks after NGS diagnosis** with appropriate treatment (PCN G)

(NEJM 2014)

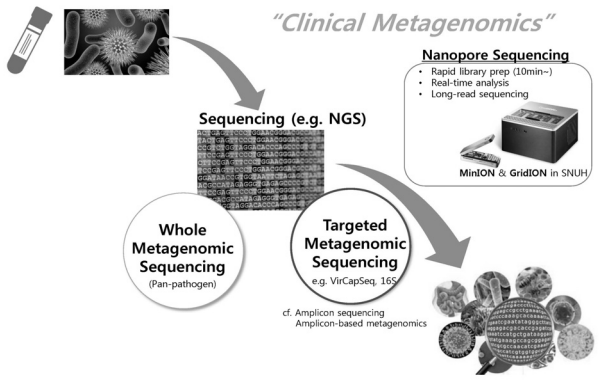
Rapid identification of Leptospira sequence in CSF



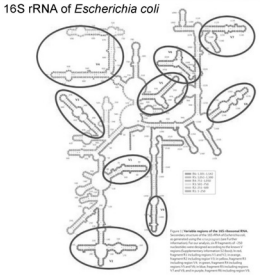
- Sample-to-answer turnaround time of **48 hours**
  - SURPI analysis for **97 min**
- Majority of bacterial reads (475 of 589 reads; 80.6%) corresponded to the **Leptospiraceae family**
- **High dose of penicillin G was started immediately** before confirmatory testing
- **Confirmatory test and phylogenetic analysis** revealed **L. santarosai** from CSF

(NEJM 2014)

Metagenomic Pathogen Detection



16S rRNA gene Sequencing for Bacterial identification



- A small subunit of the ribosome present in **prokaryotes (bacteria and archaea)**
- Combination of **conserved, variable** and **hypervariable** regions (≒1500 bp)
- 16S rRNA gene has become the **most sequenced taxonomic marker** over the past decades
  - Living Tree Project (LTP)
  - SILVA rRNA database
- **Full length sequencing** is required for accurate classification of taxonomy

DNA extraction from sample → PCR with universal primers → Library preparation → Sequencing → Data analysis (Bacterial identification)

(Nat Rev Microbiol 2014)

Advantages of 16S rRNA gene sequencing

- Diagnosis of **unculturable** bacteria
- **Rapid** diagnosis of bacterial infection (with metagenomic sequencing)
  - Can reduce the time required for culture
- Diagnosis of **polymicrobial** infection at once (with deep sequencing)

- Detection of bacteria from **unculturable specimens?**
  - **Small amount** of specimens
  - Specimens obtained **after antibiotics** use

16S Sequencing in Bacterial Meningitis

- **Can we skip the bacterial culture step?**
  - Direct sequencing from **blood or CSF** (after 16S rRNA PCR)
  - Can significantly reduce the turnaround time

- Culture positive specimens stored at SNUH
  - From **SNUH infectious encephalitis registry**
    - Several patients with bacterial culture (+) results

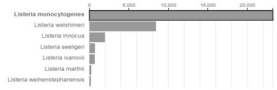
Sex/Age	Clinical diagnosis	Blood Cx.	CSF Cx.
M/67	Listeria meningitis	L. monocytogenes	L. monocytogenes
M/77	Pseudomonas meningitis	(-)	P. aeruginosa
M/79	Streptococcus meningitis	S. agalactiae	(-)
F/78	Streptococcus meningitis	S. oralis	(-)
F/57	Klebsiella meningitis with ventriculitis	(-)	(-)

Outside blood Cx: K. pneumoniae
Transferred to SNUH after 3-day antibiotics

Case> Bacterial meningitis

- **M/67**
  - Library preparation: using **DNA extracted from CSF**
  - 16S Sequencing run time: **1h 25min**
  - Total reads: 53,154, **Analyzed reads: 53,076**

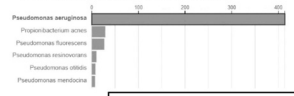
SNUH CSF Cx (+), Blood Cx (+)
: L. monocytogenes



Species: Listeria monocytogenes

- **M/77**
  - Library preparation: using **DNA extracted from CSF**
  - 16S Sequencing run time: **3h**
  - Total reads: 14,896, **Analyzed reads: 14,886**

SNUH CSF Cx (+), Blood Cx (-)
: P. aeruginosa



Species: Pseudomonas aeruginosa

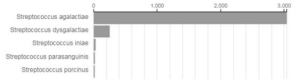
MinION 16S sequencing may not require culture step!

(Moon et al, Int J Med Microbiol 2019)

Case> Bacterial meningitis

- **M/79**
  - Library preparation: using **DNA extracted from CSF**
  - 16S Sequencing run time: **3h**
  - Total reads: 11,157, **Analyzed reads: 11,154**

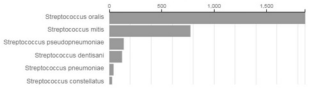
SNUH Blood Cx (+)
: Streptococcus agalactiae
SNUH CSF Cx (-)



Species: Streptococcus agalactiae

- **F/78**
  - Library preparation: using **DNA extracted from CSF**
  - 16S Sequencing run time: **3h**
  - Total reads: 14,274, **Analyzed reads: 14,272**

SNUH Blood Cx (+)
: Streptococcus oralis
SNUH CSF Cx (-)



Species: Streptococcus oralis

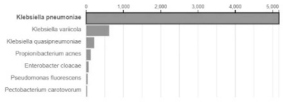
MinION 16S sequencing is more sensitive than culture studies!

(Moon et al, Int J Med Microbiol 2019)

Case> Bacterial meningitis

- **F/57, Transferred patient**
  - Library preparation: using **DNA extracted from CSF**
    (After antibiotics use ~ 3days, SNUH CSF Culture result: negative)
  - 16S Sequencing run time: **3h**
  - Total reads: 31,572, **Analyzed reads: 31,554**

Blood Cx from previous hospital
: K. pneumoniae
SNUH CSF Cx (-), Blood Cx (-)

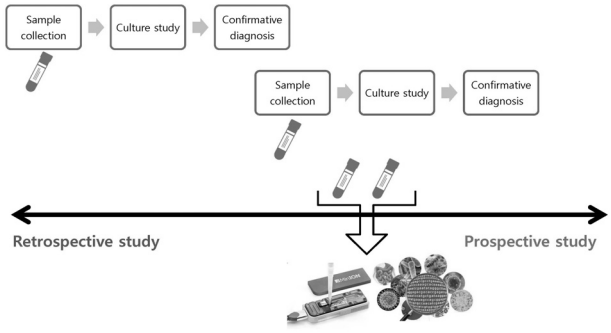


Species: Klebsiella pneumoniae

MinION 16S sequencing is more sensitive than culture studies!
(even useful in antibiotics-treated samples)

(Moon et al, Int J Med Microbiol 2019)

Potential for Prospective application



Case> Bacterial meningitis (prospective)

- **F/36**
  - BT: 38.6℃, WBC: 10,090, CRP: 11.9
  - CSF study: WBC 1,548 (P90%, L8%, O2%), Prot 131, **Glu 2**, CSF/Serum Glu = 0.02
  - **CSF 16S Seq (3h): Streptococcus oralis**
  - **Blood Cx: Viridans Streptococci (same day), CSF Cx: Viridans Streptococci (1 day later)**

Species: Streptococcus oralis

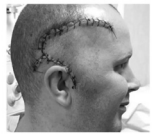
- **F/83**
  - BT: 39.0℃, WBC: 32,700, CRP: 25.5
  - CSF study: WBC 16,704 (P91%, O9%), Prot 172, **Glu 1**, CSF/Serum Glu = 0.01
  - **CSF 16S Seq (3h): Streptococcus pneumoniae**
  - **Blood Cx: no growth, CSF Cx: no growth**

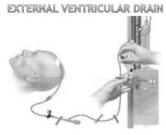
Species: Streptococcus pneumoniae

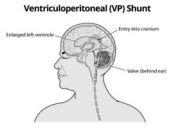
MinION 16S sequencing can be applied prospectively

(Moon et al, Int J Med Microbiol 2019)

Diagnosis of CNS infection in NS patients



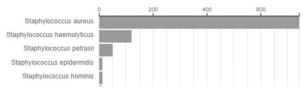




- **Catheter-related infection** (e.g. EVD, Shunt etc.) or **Post-Op infection** suspected patients
- 16S Sequencing from CSF samples (& Abscess)

Case> Catheter-related CNS infection

- **M/64: NPH with VP shunt**
  - 16S Sequencing run time: 1h
  - Total reads: 12,262, **Analyzed reads: 12,260**
  - Result: **Staphylococcus aureus**
  - Confirmed by CSF culture study **2 days later!**



- **M/28: Traumatic ICH with Lumbar drain**
  - 16S Sequencing run time: 3h
  - Total reads: 6,631, **Analyzed reads: 5,431**
  - Result: **Staphylococcus hominis**
  - Confirmed by CSF culture study **1 day later!**

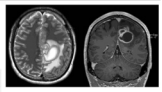
Taxon	Cumulative Reads
Staphylococcus hominis	3,790
Staphylococcus petrasii	638
Comamonas denitrificans	52
Staphylococcus epidermidis	47
Staphylococcus devriesei	45

Nanopore 16S sequencing is faster than culture studies!

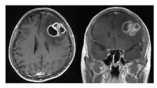
(Moon et al, Unpublished)

Case> Brain abscess

- **M/26: Right side numbness, r/o brain abscess**
  - Library preparation: DNA extracted from **navigation guided abscess drainage**
  - 16S Sequencing run time: 3h
  - Result: **Aggregatibacter aphrophilus**
  - Confirmed by culture study **6 days later!**



Aggregatibacter aphrophilus	3,179
Actinobacillus ignieresii	5
Mannheimia granulomatis	4
Mannheimia haemolytica	3
Aggregatibacter segnis	2



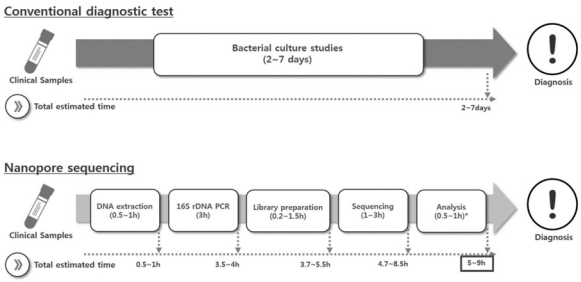
- **M/57: Motor aphasia**
  - Myelofibrosis/Chronic neutrophilic Leukemia from Polycytemia vera
  - Library preparation: DNA extracted from **navigation guided abscess drainage**
  - 16S Sequencing run time: 2h
  - Result: **Nocardia cyriacigerorgica**
  - **Requested for longer duration of culture studies**
  - Confirmed by culture study **13 days later!**

Nocardia cyriacigeorgica	37
Acinetobacter baumannii	11
Enterococcus faecium	11
Corynebacterium striatum	9
Nocardia testacea	6

Nanopore 16S sequencing enables the rapid detection of rare bacteria that are difficult to culture!

(Moon et al, Unpublished)

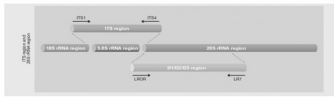
Estimated turnaround time of Nanopore 16S Seq

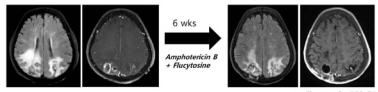


(Moon et al, Int J Med Microbiol 2019)

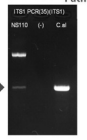
Fungal identification

- **Fungal PCR**





- **F/64: Decreased visual acuity**
  - HTN, Arrhythmia on meds
  - r/o PRES with ICH (10MA), no evidence of embolic infarction, malignancy, vascular malformation
  - Aggravation of decreased visual acuity, recurrence of intracranial lesions
  - **Brain biopsy** performed for accurate diagnosis
  - **Pathology (PAS stain)**: hyphae vs. pseudohyphae, **r/o fungal infection**



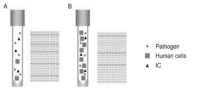
Homo sapiens	78,205
Candida albicans	3,409
Cryptococcus neoformans	403
Schizosaccharomyces octosporus	101
Phycomyces blakesleeanus	86
Paracoccidioides brasiliensis	56
Candida dubliniensis	54
Candida tropicalis	35

ITS1 Seq: Candida albicans
▶ **Prev. hospital C-line tip culture (7MA): C. albicans**

(Moon et al, Unpublished)

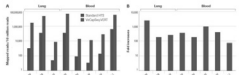
Viral identification

- **Virus sequence enrichment is essential**
  - Still troubleshooting..
  - VirCapSeq, DASH



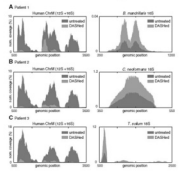
- **VirCapSeq**
  - Cover the genomes of 207 viral taxa known to infect vertebrates
  - **Licensing Agreement, Roche, 2017 Jan**

(mBio 2015)

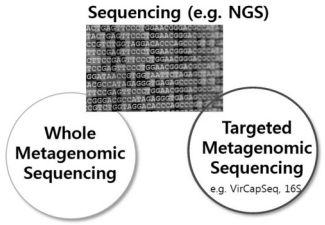


- **Depletion of Abundant Sequences by Hybridization (DASH)**
  - Using Cas9 to remove unwanted high-abundance species in sequencing libraries
  - DASH **depletes human rRNA transcripts** that comprise the bulk of NGS library

(Genome Biol 2016)



Metagenomics for Pan-pathogen detection



Summary

- **Metagenomic analysis can be useful** in the pathogen detection in Neuro-infectious diseases
  - Hypothesis-free
  - Unbiased universal pathogen detection
  - Potential improvement in diagnostic yield of infections
  - **Rapid turnaround time/Actionable diagnosis!**
- Pathogen detection **technologies are rapidly improving**
  - VirCapSeq, Nanopore sequencing, etc
- Promising role of **16S rRNA gene sequencing**
  - Diagnosis directly from clinical samples (**can skip culture**)
  - Unculturable bacteria / Unculturable samples
  - **Rapid and actionable diagnosis**

Thank you for listening

Any questions?