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Metagenomic Next-Generation Sequencing of Nasopharyngeal Swabs in Acute Febrile Illness in Cambodia

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Background

- Next-generation sequencing (NGS) is increasingly available in low-resource settings.[1]
- Potential applications of NGS include pathogen detection for disease mapping, which can inform diagnostic algorithms and identify emerging pathogens.[2]

Objectives

Use metagenomic NGS to characterize the respiratory disease landscape in Cambodia.

Methods

Study participants: From December 2020, individuals aged 2 months to 65 years presenting to 4 tertiary hospitals in Cambodia with fever and respiratory symptoms were prospectively enrolled.

Sample processing: Nasopharyngeal (NP) swabs were obtained within 24 hours of documented fever. Paired serum samples were obtained for the first 6 months of the study. Total nucleic acids were extracted from biospecimens and metagenomic RNA libraries prepared and sequenced on a NextSeq2000 instrument.

Analysis: Raw sequence reads were stripped for host reads and aligned to NCBI nucleotide and protein databases using a cloud-based bioinformatics platform.

Ethics and protocol registration and Ethics: The study protocol was approved by NIH and Cambodian IRB and the trial prospectively registered on clinicaltrials.gov (NCT04034264).

Results

Study Cohort

Table 1. Cohort characteristics.

Characteristic	Total N= 436
Male gender, N (%)	248 (57%)
Age in years, Median (IQR)	2 (1-7)
Urban location	261 (60%)
Symptom, N (%)	
Cough	357 (82%)
Rhinorrhea	303 (69%)
Dyspnea	132 (30%)
Symptom duration, N (%)	
<= 1 day	175 (40%)
2-4 days	196 (45%)
>= 5 days	65 (15%)
Pathogen hit, N (%)	238 (55%)

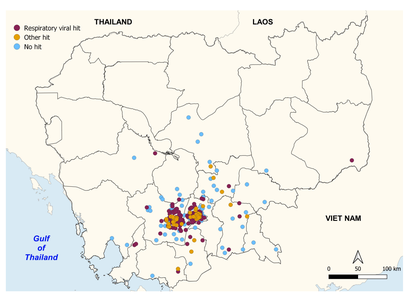


Fig 1. Map of Cambodia with geolocation of sampled cases.
Purple= respiratory virus, orange= other pathogen, blue= no pathogen.

Results

Temporal variability of respiratory virus detection in Cambodia, December 2020 – July 2022

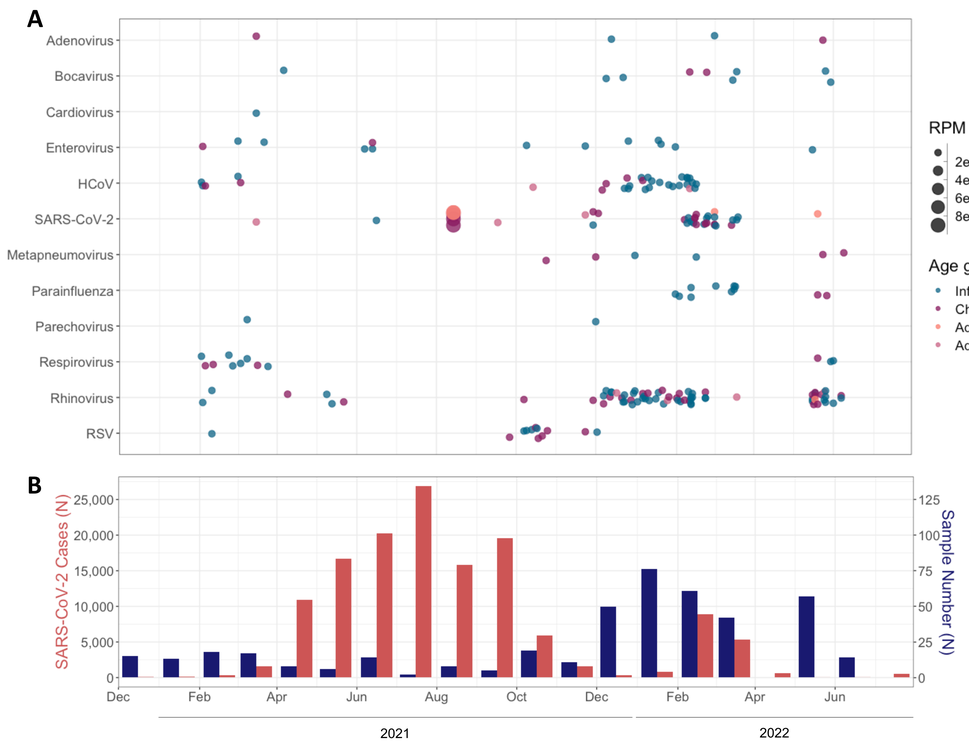
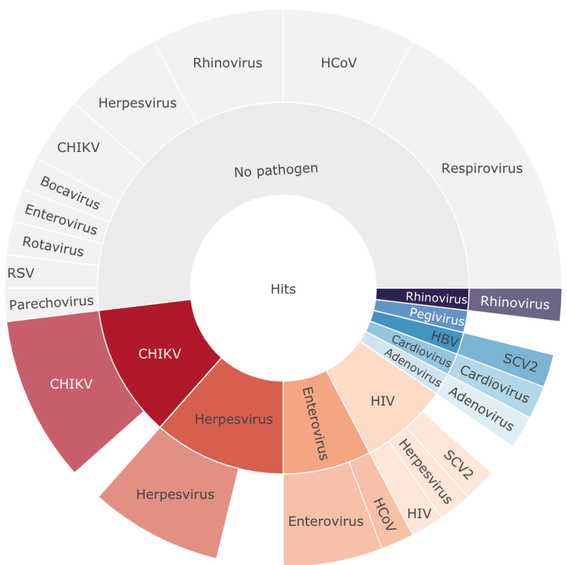


Fig 2. (A) Dot plot of respiratory viruses in NP swabs of febrile individuals. **(B)** Monthly new SARS-CoV-2 cases in Cambodia (red) and study samples (blue).
HCoV = Human (non-SARS-associated) coronaviruses; RSV = Respiratory syncytial virus.

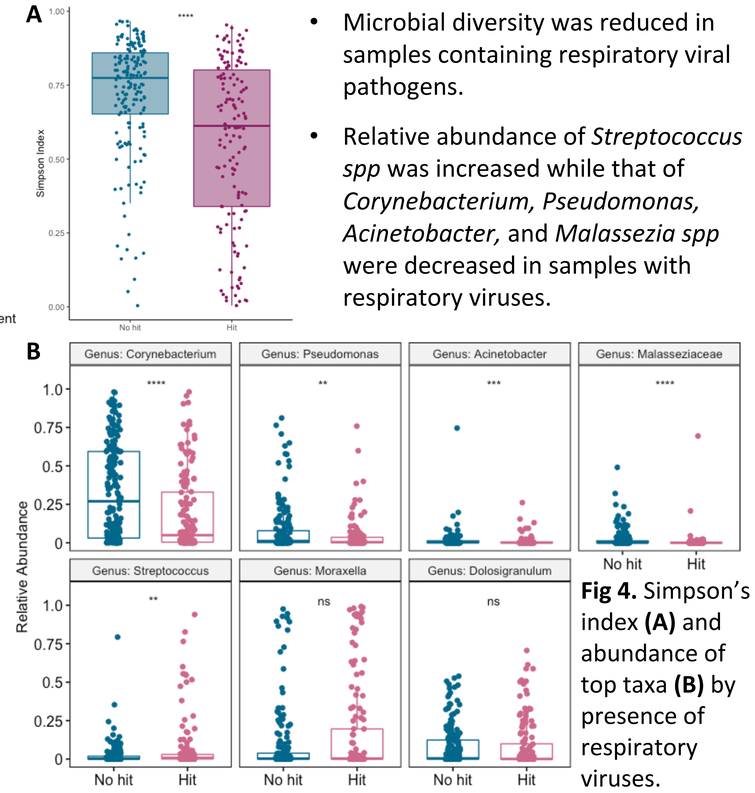
Detection of systemic viruses in nasopharyngeal swabs

Fig 3. Sunburst plot of pathogen hits in paired serum and NP samples for individuals with any pathogen identified. Inner and outer circles represent sera and NP hits, respectively.
CHIKV = Chikungunya virus; HBV = Hepatitis B virus; SCV2 = SARS-CoV-2; HIV = Human immunodeficiency virus.

- Pathogens were detected in NP swabs of 27 cases without hits in sera.
- CHIKV was detected in NP swabs in 5 of 6 individuals and HIV in 1 of 4 individuals with corresponding pathogen detection in sera.



Composition of nasopharyngeal microbiome by presence of respiratory viruses



- Microbial diversity was reduced in samples containing respiratory viral pathogens.
- Relative abundance of *Streptococcus spp* was increased while that of *Corynebacterium, Pseudomonas, Acinetobacter,* and *Malassezia spp* were decreased in samples with respiratory viruses.

Fig 4. Simpson's index **(A)** and abundance of top taxa **(B)** by presence of respiratory viruses.

Conclusions

- Metagenomic NGS can be successfully implemented in low-resource settings to identify common and emerging pathogens.
- Nasopharyngeal sampling may reveal potential pathogens not present in sera.
- Certain viral pathogens causing systemic disease, even if not classically associated with respiratory disease, may be detected in nasopharyngeal samples.
- The composition of the nasopharyngeal microbiome varies with presence/ absence of respiratory viruses.

References

[1]Yek C, Pacheco AR, Vanaerschot M, *et al.* Metagenomic pathogen sequencing in resource-scarce settings: Lessons learned and the road ahead. Front Epidemiol. 2022 Aug 15.
[2]Bohl JA, Lay S, Chea S, *et al.* Discovering disease-causing pathogens in resource-scarce Southeast Asia using a global metagenomic pathogen monitoring system. Proc Natl Acad Sci U S A. 2022 Mar 15;119(11):e2115285119.