Viral Diversity of Wastewater in Kampala, Uganda
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Introduction

Waterborne viruses are a significant cause of human disease, especially in developing countries. The development of wastewater treatment facilities in Uganda is one step in combating exposure to waterborne viruses. Still, more robust detection and characterization of the waterborne viruses found in wastewater is necessary to better protect human health. Both conventional and metagenomic analyses can assist in this task. Quantitative PCR (qPCR) can evaluate the concentrations of viral pathogens known to exist in wastewater samples, whereas metagenomic analyses can help to identify other viruses that may be impactful to human health. These methods can also be utilized for wastewater-based epidemiology in order to identify and predict disease outbreaks.

Objectives:
1) Determine the concentrations of Adenovirus, Enterovirus, Hepatitis A virus, and Rotavirus in wastewater using qPCR
2) Assess the viral diversity of wastewater and identify other human viruses of importance using metagenomic analyses
3) Develop analytical methods for broader application to other environmental samples from the Kampala area

Methods

Wastewater Sample
- Filtration (Argonide electropositive cartridge filters)
- Elution and Concentration

DNA & RNA Extraction
- Reverse Transcription
- Quantitative PCR
- Next-Generation Whole Genome Sequencing (Illumina HiSeq)

Sequence Reads Quality Control: Trimming (Trimmomatic)
- DeNovo Assembly (IDBA-UD)
- Sequence Alignment & Coverage (BWA-MEM)
- Annotation and Taxonomic Classification (BLAST, MEGAN)

Sampling was performed on-site following EPA protocol for viral isolation. Samples were shipped on ice to MSU and processed within 72 hrs using developed protocol. Nucleic acids were extracted and qPCR was performed using prior assays. To allow for sequencing of both RNA and DNA viruses, reverse transcription was performed using random primers to convert RNA to double-stranded DNA. Samples were sequenced on an Illumina HiSeq platform, and sequencing results were used for further metagenomic analyses. BLAST results were computed using an max E-value of 10^-3.

Results

Three samples were collected from the influent of the Bugolobi wastewater utility in summer of 2016. All samples were positive for all four viruses quantified with qPCR as shown in Figure 1.
- Enterovirus and Adenovirus were present in higher concentrations than Hepatitis A virus and Rotavirus
- One sample was analyzed with Illumina HiSeq.

Among annotated viral sequences, 72.5% infect bacteria, while 7.0% infect vertebrates
Numerous human viruses detected with BLAST:
- Enterovirus (3 hits)
- Adenovirus (1)
- Rotavirus (1)
- Polyomavirus (1)
- Astroivirus (1)
- Papillomavirus (3)
- Herpesvirus (4 hits)
- Norovirus (1)
- Lyssavirus (1)
- Rubella virus (1)
- Sapporo virus (1)

Of all sequences, only 1.6% were annotated as viral using BLAST - nearly all others unaffiliated

Table 1: Summary of human viruses with significant coverage from BWA-MEM alignment.

<table>
<thead>
<tr>
<th>Human Virus</th>
<th>Number of Species</th>
<th>Maximum Coverage</th>
<th>Average Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus</td>
<td>9</td>
<td>43.39%</td>
<td>7.21%</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>22</td>
<td>3.61%</td>
<td>0.95%</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>14</td>
<td>2.05%</td>
<td>1.28%</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>5</td>
<td>4.13%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>7</td>
<td>8.03%</td>
<td>3.75%</td>
</tr>
<tr>
<td>Influenza</td>
<td>46</td>
<td>6.49%</td>
<td>1.93%</td>
</tr>
<tr>
<td>Astroivirus</td>
<td>9</td>
<td>4.74%</td>
<td>2.45%</td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>52</td>
<td>4.56%</td>
<td>2.04%</td>
</tr>
<tr>
<td>Norovirus</td>
<td>5</td>
<td>4.45%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Summary of human viruses with significant coverage from BWA-MEM alignment.

References & Acknowledgements


- Funding for his work was provided by MSU International Institute of Health, College of Engineering, and College of Veterinary Medicine. Special thanks to the Research Technology Support Facility for the sequencing resources and assistance provided.

Sampling Location

Above: Map of sampling location in Kampala, Uganda. Below: Bugolobi wastewater influent & sampling.

Conclusions & Future Work

- Concentrations of Adenovirus, Enterovirus, Hepatitis A virus, and Rotavirus are present in significant levels in wastewater influent. These viruses were also detected using metagenomic analyses, lending confidence to the results. Numerous other human viruses were detected using metagenomic methods, expanding knowledge of potential viral exposure from wastewater.
- The presented methods have proven to be valuable and effective in quantifying and identifying human pathogens. The methods will be applied to samples taken at other locations near the wastewater utility in order to assess the overall impact of the wastewater utility on the surrounding environment and to assess potential disease using wastewater-based epidemiology methods.