Analyse and Interpret the Data

Descriptive Study

Figure or Table Number: 1

"Official" title for this figure or table (from the caption):

Taxonomic distribution of viral metagenome reads collected from six study sites on the Han River in South Korea.

My (simplified, decoded, in regular language) title for this figure or table:

The classification of different bacterial and viral genomes found in the six study collection sites in the Han River in South Korea

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If we compare panel(s)/column(s) N1, N3, **and** H1 **, we learn about:**

How the viral and bacterial taxonomy is distributed in these samples. More specifically it is seen that all three of these samples have roughly the same distribution in types of bacterial and viruses seen(~80% bacteria and ~12% viral).

If we compare panel(s)/column(s) H4, H6 **and** H7 **, we learn about:**

How the viral and bacterial taxonomy is distributed in these samples. More specifically it is seen that all three of these samples have roughly the same distribution in types of bacterial and viruses seen (~80% bacteria and ~12% viral).

When we make these comparisons, we conclude from this figure:

That only around 12.3−13.8% of the annotated reads were referred to as viral reads, most of which belonged to Myoviridae, Podoviridae, and Siphoviridae of Caudovirales and all of which are commonly found in the environment. Furthermore, the majority of virome reads (82.5 −84.9%) were annotated as bacterial genes that predominantly belonged to the classes Alpha-, Beta-, and Gammaproteobacteria.

Was the hypothesis supported? Why or why not?

Proposed hypothesis: Since bacterial and viral genomes, along with ARGs, have been known to be found in all kinds of diverse marine environments (Calero-Cáceres and Balcázar, 2019), if six surface water samples were collected from the Han River and contain ARGs, then the water's composition should comprise of various types of bacteria and viruses harboring ARGs in their genome.
Yes, the hypothesis was supported because the samples composition did comprise of various types of bacteria and viruses that could harbor ARGs

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Something I am curious about is that the figure has a category of unclassified viruses that is never really mentioned. I wonder if these unclassified viruses have any interesting sequences that could harbor some interesting information we have yet to study.

Descriptive Study

Figure or Table Number: 2

"Official" title for this figure or table (from the caption):

Sequence maps of contigs H4-C441, H1-C74, H4-C244, and H4-C367-bearing β-lactamase genes HRV-1 or HRVM-1

My (simplified, decoded, in regular language) title for this figure or table:

Sequencing maps and structures of 4 of the viral contig samples that harbor β-lactamase ARGs

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If we compare panel(s)/column(s) The top sequence **and** The bottom three sequences **, we learn about:**

How class A β-lactamases and metallo β-lactamases differ in their structure and gene order. For example, class A β-lactamases contain protein modification genes that are not present in metallo β-lactamases. Furthermore, the location of β-lactamases in each type of viral contig is different, as it is in the middle of the metallo β-lactamases genomes and located at the end of class A β-lactamases.

When we make these comparisons, we conclude from this figure:

That of the 4 viral contigs they analyzed in detail, three have a clearly similar sequences and homology that aligns with metallo β-lactamases and the other one has a structure of a class A β-lactamases.

Was the hypothesis supported? Why or why not?

Proposed Hypothesis: Since ARGs have been known to be transferred via phage-related mobile elements (Brown et al., 2015) and phages are known to be found in aquatic environments (Calero-Cáceres and Balcázar, 2019), if viral contig samples from water samples of the Han River contain ARGs, then a genome analysis should reveal sequence regions harboring ARGs.

Yes, the hypothesis was supported, because when they analyzed the viral contigs, they did find regions haboring β-lactamases that are ARGS.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The sequence map has large sections of hypothetical proteins with no function. I am curious if the authors have any ideas as to what these genes/proteins are and if they have any important function within the virus. For example, could they be knocked out and would the virus still be fine?

Descriptive Study

Figure or Table Number: 3

"Official" title for this figure or table (from the caption):

Maximum-likelihood phylogenetic tree of HRV-1 with representative enzymes of classes A, C, and D β-lactamases

My (simplified, decoded, in regular language) title for this figure or table:

Phylogenetic tree of the enzyme classes A, C, and D β-lactamases and where HRV-1 groups in these classes

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If we compare panel(s)/column(s) Class A, Class C **and** Class D **, we learn about:**

How each of these classes group into distinct clades with a high probability. We also learn that the ARG HRV-1 very clearly groups into class A enzymes and forms a clade within class A.

When we make these comparisons, we conclude from this figure:

The ARG HRV-1 very clearly groups into class A enzymes and forms a clade within class A, which supports evidence found in sequencing showing conserved active sites and motifs specific to class A β-lactamases

Was the hypothesis supported? Why or why not?

Proposed Hypothesis: Since sequence analysis of the ORF H4-C441- ORF28 carried the conserved active sites and motifs specific to class A β-lactamases standards(Ambler et al., 1991), if ORF H4-C441- ORF28 does belong to class A β-lactamases, then the ORF should group into a clade of class A β-lactamases in a phylogenetic tree analysis.

Yes, because after a phylogenetic analysis ORF H4-C441- ORF28 very clearly grouped into class A β-lactamases. It should be noted that while it did group into class A, it still grouped into its own distinct clade.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

I am curious where the other closely related genomes on the tree originated from. Are they from phages/viruses found in similar areas to where these viral samples where isolated, such as the water and in Asia.

Descriptive Study

Figure or Table Number: 4

"Official" title for this figure or table (from the caption):

Maximum-likelihood phylogenetic tree of HRVM-1 with representative enzymes of subclasses B1, B2, and B3

My (simplified, decoded, in regular language) title for this figure or table:

Phylogenetic tree of the enzyme subclasses of B1, B2, and B3 β-lactamases and where HRVM-1 groups in these classes

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If we compare panel(s)/column(s) B1, B2 **and** B3 **, we learn about:**

How each of these subclasses group into distinct clades with a high probability. We also learn that the ARG HRVM-1 very clearly groups into subclass B3 enzymes and forms a clade within class B3.

When we make these comparisons, we conclude from this figure:

The ARG HRVM-1 very clearly groups into subclass B3 enzymes and forms a clade within subclass B3, which supports evidence found in sequencing showing conserved active sites and motifs specific to class B3 metallo-β-lactamases.

Was the hypothesis supported? Why or why not?

Proposed Hypothesis: Since sequence analysis of the ORFs, H1- C74-ORF21, H4-C244-ORF21, and H4-C367-ORF18 revealed conserved active sites and motifs specific to metallo β-lactamases (Ambler et al., 1991), if the ORFs, H1- C74-ORF21, H4-C244-ORF21, and H4-C367-ORF18 do belong to a particular subclass of metallo β-lactamases, then the ORFs should group into a distinct subclass of metallo β-lactamases in a phylogenetic analysis.
Yes, because ORFs, H1- C74-ORF21, H4-C244-ORF21, and H4-C367-ORF18 all grouped with metallo-β-lactamases in a phylogenetic analysis. Furthermore, all grouped together in a distinct B3 clade.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Similar to the previous figure, I am curious where the other closely related genomes on the tree originated from, especially PNGM-1. Are they from phages/viruses found in similar areas to where these viral samples where isolated, such as the water and in Asia.

Experimental Test

Figure or Table Number: 5

"Official" title for this figure or table (from the caption):

Minimum inhibitory concentrations (MICs) of β-lactams for Escherichia coli BL21 (DE3) transformants producing HRV-1 [E. coli BL21 (DE3)- pET-30a(+)-HRV-1] or HRVM-1 (E. coli BL21 (DE3)-pET-28a(+)-HRVM-1) or harboring the expression vectors pET-28a(+) or pET-30a(+) [E. coli BL21 (DE3)-pET-28a(+)/E. coli BL21 (DE3)-pET-30a(+)]

My (simplified, decoded, in regular language) title for this figure or table:

Minimum inhibitory concentration (MIC) assay results of β-lactams for Escherichia coli expressing the two ARGs of interest, HRV-1 and HRVM-1

The controls in this experiment are:

E. coli not containing plasmids expressing either HRV-1 and HRVM-1

They are represented (in which part of the chart or graph, or what figure panels?)

They are represented in gray bars on the barchart.

The experimental conditions are:

They are represented as:

Minimum inhibitory concentrations (MICs) of β-lactams for Escherichia coli in the presence and absence of novel ARGs	The axes on the bar graph and the bars in the graph

We need to compare the controls in Figure 5 (red and blue bars) **with the experimentals in** Figure 5 (gray bars) **to find out:**

Whether or not expressions of these particular ARGs(HRV-1 and HRVM-1) confer any type of antibiotic resistance to the E. coli.

When we make these comparisons, we conclude from this figure:

The two strains expressing either HRV-1 or HRVM-1 showed reduced susceptibility, ranging from 2- to 16-fold reductions, to extended-spectrum cephalosporins and carbapenems, as well as to penicillin and narrow-spectrum cephalosporins, displaying typical characteristics of extended-spectrum β-lactamases (ESBL) or carbapenemase.

Was the hypothesis supported? Why or why not?

Proposed Hypothesis: Since ARGs belonging to the β-lactamases classes have been known to confer resistance to the antibiotic lactamase (Blanco et al., 2020), if HRV-1 and HRVM-1 genes found in the viral contig samples do provide antibiotic resistance, then expressing these genes within E. coli should increase the level of resistance to antibiotics when compared to controls.

Yes, the hypothesis is supported because the ARGs being studied did confer a noticeable amount of resistance for the antibiotics that the E. coli was exposed to, as seen by the much higher level of MIC needed to kill the bacteria.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The authors mention finding other ARGs, however, there is no figure or supplemental figure showing MIC tests for those ARGs. I know they said that these other ARGS weren't as promising as the ones they did study in depth, but I think it may have been interesting to further study them to see if maybe they were novel ARGs that confer resistance to bacteria.

Descriptive Study

Figure or Table Number: 6

"Official" title for this figure or table (from the caption):

Genomic maps of the Han River bacterial metagenomic contigs that harbor homologous ORFs to HRV-1 or HRVM-1

My (simplified, decoded, in regular language) title for this figure or table:

Genomic structure maps of bacterial metagenome contigs found in the Han River that have homologous ORFs to the ARGs of interest, HRV-1 or HRVM-1

If we compare panel(s)/column(s) Figure 6 H4-C441 **and** Figure 6 5-N3-2000013, 5-H1-2-0007454, 5-H6-2-0011724 **, we learn about:**

How these identified bacterial contigs share significant sequence similarity and structure to the ARG HRV-1 found in the viral sample

If we compare panel(s)/column(s) The red viral contigs in the big box in the **and** The black bacterial contigs in **, we learn about:**

corner	the big box in the corner

How these identified bacterial contigs share significant sequence similarity and structure to the ARG HRVM-1 found in the viral sample

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When we make these comparisons, we conclude from this figure:

That the metagenome contigs with these ORFs showed high synteny to the viral contigs containing HRV-1 and HRVM-1, suggesting the presence of infectious phages or prophages carrying HRV-1 and HRVM-1 in the Han River bacterial communities

Was the hypothesis supported? Why or why not?

Proposed Hypothesis: Since ARGs have been known to spread via horizontal gene transfer in microbial ecosystems (von Wintersdorff, 2016), if ARGs are being transferred from phages to bacteria in the Han River of South Korea via horizontal gene transfer, then analysis of the bacterial contigs from Han River Samples should contain ARG sequences with high similarity to ARG sequences found in viral contigs isolated from the same Han River.
Yes, the hypothesis was supported because the bacterial contigs they studied did contain genome structure and ARG sequences with high similarity to ARG sequences found in viral contigs isolated from the same Han River.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The simplified sequence similarity map is nice, but does not give as much detail as to how much the sequences are similar, like how a phamerator analysis does with different coloring for different levels of similarity.