Development of computationally-guided workflow for designing therapeutic phage cocktail: targeting multidrug-resistant (MDR) bacteria

Rabia Nawaz (dr.rabia.nawaz8@gmail.com)  
Superior University  
Ali Husnain  
Superior University  
Muhammad Ali  
Superior University  
Moin Sajjad  
Superior University  
Ammara Ahad  
Superior University  
Muhammad Shahid  
University of the Punjab  
Uqba Mehmood  
Superior University  
Attea Razzaq  
Superior University  
Idrees Khan  
University of Peshawar  

Research Article

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Abstract

Background:
Antibiotic misuse and overuse have contributed to the emergence of multi-drug resistant bacteria (MDR), a serious public health problem across the globe. Phage cocktails, which combine several phages to destroy various bacterial strains, offer a more thorough and efficient method of battling MDR illnesses. This might revolutionize the looming threat of reemergence of untreatable bacterial diseases. To provide a focused strategy to tackle the rising incidence of MDR bacterial infections, a phage cocktail against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was intended to be made computationally. Predicting a group of prophages which can successfully lyse and disrupt these three MDR bacterial strains and might help to lessen the severity and occurrence of illnesses caused by these notorious pathogens.

Methods:
The genomes of selected MDR bacteria were accessed through NCBI GenBank, and prophages targeting them were selected. The prophages were further annotated for ORFs, putative promoters, virulence factors, transcriptional terminators, and tRNAs. Dot plot was created to investigate the similar phages and phylogenetic analysis was performed.

Results:
A total of 11 prophages were predicted from three MDR bacterial genomes, the investigation identified 472 ORFs and CDS, rRNA, and tRNA regions in 11 prophage genomes were predicted. The presence of 3 tRNAs encoded by the predicted prophages suggests a possible reliance on the host translation machinery for protein synthesis. The presence of transcription terminators and promoters were detected to understand the transcriptional and translational regulation of prophage genes. The comparative genomic and phylogenetic analyses of predicted prophages provided important insights into diversity and relatedness of the phages. The final selected five prophages included *Acinetobacter baumannii* prophage (2759376-2809756), *Acinetobacter baumannii* prophage (3311844-3364667), *Klebsiella pneumoniae* (1288317-1338719), *Klebsiella pneumoniae* prophage (1778306-1808606), and *Klebsiella pneumoniae* prophage (2280703-2325555).

Conclusion:
In conclusion, our work provides an example of developing a phage cocktail to combat multidrug resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Sequence similarity analyses revealed that the cocktail is capable of targeting *Enterobacter hormaechei* and other carbapenemase-producing *K. pneumoniae* strains also. The phage cocktail indicates the possibility of being employed as a therapeutic agent for reducing harmful bacterial infections, where conventional antibiotic therapeutics fail.

Background
Antibiotic resistance is a major public health concern worldwide. MDR microorganisms make treating illnesses they cause challenging since they have developed resistance to a variety of treatments [1]. Overuse and incorrect use of antibiotics in both humans and livestock is a major factor of the creation and spread of antibiotic-resistant bacteria. MDR infections in humans can cause a wide range of diseases, including skin infections, pneumonia, sepsis, and urinary tract infections. These infections can be difficult to treat, resulting in longer hospital stays, higher healthcare costs, and a higher mortality risk. MDR infections are growing increasingly widespread over the world, and in certain cases, there are no viable treatments available. [2]

Bacteriophage therapy which utilizes phage cocktails is a promising strategy for treating illnesses caused by MDR bacteria. Bacteriophages are viruses that attack and destroy specific bacterial strains, whereas phage cocktails are phage mixtures that target many bacterial strains. People in Eastern Europe and the former Soviet Union have used phage therapy to treat bacterial infections for over a century. In recent years, phage therapy has received significant interest as a potential solution to the problem of antibiotic resistance. [3]

Carbapenem-resistant *Acinetobacter baumannii* (CRAB), Carbapenem-resistant *Klebsiella pneumoniae*, and multidrug resistance Common multidrug resistant microorganisms (MDR) that are causing problems include *Pseudomonas aeruginosa*. As a result, these microorganisms are chosen for the phage cocktail-based bacteriophage treatment. [4, 5]

The genomic sequences of multi-drug resistant bacteria *Acinetobacter baumannii*, *K. pneumoniae*, and *P. aeruginosa* were investigated for prophages in this work. The prophages were further characterized in order to better understand their possible effects on the biology and pathogenicity of their bacterial hosts. The study revealed 11 prophages, three from *Acinetobacter baumannii*, seven from *K. pneumoniae*, and one from *P. aeruginosa*, with likely activities and biological functions for encoded proteins.

The findings of the study indicated that these prophages had rRNA genes and tRNA regions, suggesting that they may interact with the host cell's protein production and translation machinery [3]. The scientists uncovered probable promoter sequences and areas in the prophage genomes, which provide information on prospective regulatory mechanisms for these prophages and their interactions with the host bacteria. The presence of virulent genes were discovered in various prophages, suggesting that these genes may play a role in the pathogenicity of their bacterial hosts.

This study on phage cocktail creation is a crucial addition to current attempts to tackle multidrug-resistant (MDR) bacteria [6]. Because of the growth in antibiotic resistance, the use of bacteriophages as a viable alternative to antibiotics has attracted significant interest in recent years. The discovery of phage cocktails, which contain a mix of phages that may attack different regions of the bacterial cell, presents a viable method for targeting numerous strains of MDR bacteria. By taking this method, we may be able to overcome the constraints of employing individual phages, such as the formation of resistance to a
single phage. Our work on phage cocktail creation has the potential to make a big difference in the battle against MDR bacteria, as well as give a crucial addition to the arsenal of tools for treating bacterial illnesses.

Methods

Sequence retrieval from NCBI

A total of three whole genome sequences of bacteria were selected on basis of their pathogenicity in humans which are causing multi drug resistant diseases. NCBI GenBank was used to obtain the nucleotide sequences of *Acinetobacter baumannii* AB0057 (accession number: NC_011586.1), *Klebsiella pneumoniae* subsp. pneumoniae HS11286 (accession number: NC_016845.1), and *Pseudomonas aeruginosa* UCBPP-PA14 (accession number: NC_008463.1) (Table. 1).

Prophage selection

The presence of prophages in the genomes of *Acinetobacter baumannii* AB0057, *Klebsiella pneumoniae* subsp. pneumoniae HS11286, and *Pseudomonas aeruginosa* UCBPP-PA14 were investigated by utilizing the results of prophage prediction from a previous study conducted by Mageeney et al., 2020 [7]. In their study, prophage prediction was performed using two bioinformatics tools: Islander [8] and TIGER [9]. The predicted prophages were extracted from the output files generated by these tools and were used as input for further analysis (Table 2).

Open Reading Frames (ORFs) prediction

Open reading frames encoded by the selected 11 prophage sequences were predicted by Prodigal [10], Phanotate [11], and GeneMark.hmm [2].

Functional annotation using Prokka

To find the transfer RNAs, CDs, and ribosomal RNAs from the predicted prophages Prokka [21] annotation tool was utilized. To locate the locations of genomic features inside contigs, Prokka uses external feature prediction algorithms.

Annotation of ORFs

Predicted ORFs were annotated using the BLASTP algorithm against the non-redundant (nr) protein database of the NCBI (National Center for Biotechnology Information) [3].

Putative promoter sequence detection

The putative promoter sequences and regions on the phage genomes were identified using BPROM [4] which is a promoter online analysis tool of Softberry [12].

Virulent gene prediction

Virulent genes on the predicted prophage sequences were detected by using the VFDB: Virulence Factor Database [5].

Prediction of Rho-independent transcription terminators

Rho-independent transcription terminators on predicted prophage sequences were detected using ARNold program [6] [13].

tRNA prediction on prophage sequences

tRNAs carried by prophage sequences were detected using the online program tRNAscan-SE [7] [14] and ARAGORN [8].

Blastn analysis

To find out the highest similar phages we performed Blastn [9] analysis against standard databases of nucleotides using NCBI.

Comparative genomic analysis

Comparative genome analysis of the predicted prophages was conducted using EasyFig 2.2 [15]. Genome maps of selected prophage sequences were constructed with their highest similar phages using the tBlastx analysis and minimum selected e value was 0.001.

Phylogenetic analysis

Phylogenetic analysis of the selected phages was performed based on the nucleotide sequences of their large terminase subunits. A neighbor-joining phylogenetic tree was constructed for nucleotide sequences of finally selected prophages using MEGA 11 software with 1000 bootstrap replications [16].

Results

Retrieval of sequences
The whole genome sequences of *Acinetobacter baumannii* AB0057, *Pseudomonas aeruginosa* UCBPP-PA14, and *Klebsiella pneumoniae* subsp. pneumoniae HS11286 were retrieved from NCBI with accession numbers "NC_011586.1", "NC_008463.1" and "NC_016845.1" respectively (Table 1).

### Table 1. Bacterial strains with their accession numbers.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>NC_011586.1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> UCBPP-PA14</td>
<td>NC_008463.1</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae HS11286</td>
<td>NC_016845.1</td>
</tr>
</tbody>
</table>

### Prophage selection

The selected prophage genomes integrated into the genomes of bacteria were detected by two Integrated Genetic Element (IGE) discovery algorithms, islander and TIGER by Mageeney et al., 2020 [7]. A total of 11 prophages were detected from the selected bacterial genomes including 7 prophages from *Klebsiella pneumoniae* subsp. pneumoniae HS11286, 3 prophages from *Acinetobacter baumannii* AB0057, and 1 prophage from *Pseudomonas aeruginosa* UCBPP-PA14. Island length and coordinates of predicted prophages were also predicted (Table 2).

### Table 2. Results of TIGER and islander showing the predicted prophages with their reference genome Accession numbers, source, genome coordinates, island or phage length, and island type.

<table>
<thead>
<tr>
<th>DNA accessions</th>
<th>Source</th>
<th>Left Coordinate</th>
<th>Right Coordinate</th>
<th>Island Length</th>
<th>Island Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC_016845.1</td>
<td>TIGER, Islander</td>
<td>581777</td>
<td>594029</td>
<td>12253</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER</td>
<td>1062574</td>
<td>1073328</td>
<td>10755</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER, Islander</td>
<td>1288317</td>
<td>1338719</td>
<td>50403</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER</td>
<td>1778306</td>
<td>1808606</td>
<td>30301</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER</td>
<td>2280703</td>
<td>2325555</td>
<td>44853</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER</td>
<td>4049881</td>
<td>4085225</td>
<td>35345</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_016845.1</td>
<td>TIGER</td>
<td>4819189</td>
<td>4835185</td>
<td>15997</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_011586.1</td>
<td>TIGER</td>
<td>1464206</td>
<td>1483241</td>
<td>19036</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_011586.1</td>
<td>TIGER, Islander</td>
<td>2759376</td>
<td>2809756</td>
<td>50381</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_011586.1</td>
<td>TIGER</td>
<td>3311844</td>
<td>3364667</td>
<td>52824</td>
<td>Phage</td>
</tr>
<tr>
<td>NC_008463.1</td>
<td>TIGER</td>
<td>4345129</td>
<td>4355794</td>
<td>10666</td>
<td>Phage</td>
</tr>
</tbody>
</table>

### Open reading frame (ORFs) prediction

Open reading frames of selected prophages genomes were predicted using two command line tools: Prodigal and Phanotate and online program GeneMark.hmm. There were 29 ORFs predicted from *Acinetobacter baumannii* AB0057 prophages (1464206-1483241), 79 ORFs from *Acinetobacter baumannii* AB0057 prophages (2759376-2809756) and 85 ORFs from *Acinetobacter baumannii* AB0057 prophages (2759376-2809756). While only 14 ORFs were predicted from *Pseudomonas aeruginosa* UCBPP-PA14 Prophage (4345129-4355794), there were 14, 13, 73, 37, 63, 43, and 22 ORFs predicted from 7 *Klebsiella pneumoniae* subsp. pneumoniae HS11286 prophages (Table 3). Their positions, strands type and length of gene were also annotated (Additional file 1: Tables S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11).
Table 3. Bacterial Strains with their prophage regions and their genomic analysis including ORFs, predicted tRNAs and rRNAs, predicted promoters and terminators regions are given.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Prophage position</th>
<th>Open Reading Frame</th>
<th>Promotors</th>
<th>Terminators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>1464206-1483241</td>
<td>29</td>
<td>47</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2759376-2809756</td>
<td>79</td>
<td>126</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>3311844-3364667</td>
<td>85</td>
<td>133</td>
<td>61</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae HS11286</td>
<td>581777-594029</td>
<td>14</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1062574-1073328</td>
<td>13</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1288317-1338719</td>
<td>73</td>
<td>108</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1778306-1808606</td>
<td>37</td>
<td>67</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2280703-2325555</td>
<td>63</td>
<td>95</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>4049881-4085225</td>
<td>43</td>
<td>78</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>4819189-4835185</td>
<td>22</td>
<td>37</td>
<td>9</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> UCBPP-PA14</td>
<td>4345129-4355794</td>
<td>14</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

**Annotations of ORFs**

BLASTP algorithm was used to annotate ORFs in prophage sequences of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. By comparing them with known protein sequences in the NCBI non-redundant protein database, putative functions and biological roles were identified. A total of 29, 79, and 85 ORFs were identified in 3 prophages of *Acinetobacter baumannii* with maximum identity percentages of 100% and minimum identity percentages ranging from 30.84% to 42.82%. Similarly, 14, 13, 73, 37, 63, 43, and 22 ORFs were identified in 7 prophages of *Klebsiella pneumoniae* with maximum identity percentages of 100% and minimum identity percentages ranging from 27.51% to 93.83%. Finally, 14 ORFs were identified in 1 prophage of *Pseudomonas aeruginosa*, with maximum identity percentages of 100% and minimum identity percentages of 38.22%. ORFs with their maximum and minimum identities were also predicted (Table 4).

Table 4. Cluster BLASTP percentage similarity of predicted ORFs of prophages using the BLASTP algorithm against the non-redundant (nr) protein database of the NCBI.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Prophage position</th>
<th>Open Reading Frame</th>
<th>Cluster BLASTP percentage similarity with nr database</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nt)</td>
<td></td>
<td>Maximum identity</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>1464206-1483241</td>
<td>29</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2759376-2809756</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3311844-3364667</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae HS11286</td>
<td>581777-594029</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1062574-1073328</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1288317-1338719</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1778306-1808606</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2280703-2325555</td>
<td>63</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4049881-4085225</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4819189-4835185</td>
<td>22</td>
<td>90.91</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> UCBPP-PA14</td>
<td>4345129-4355794</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

**Annotation of prophages using prokka**
Annotations of prophages genomes gives the CDs, tRNAs and rRNAs carried by prophage genomes. 1 tRNA was found from *Acinetobacter baumannii* AB0057 prophage (3311844-3364667) and 2 tRNAs from *Klebsiella pneumoniae* subsp. pneumoniae HS11286 prophage (1288317-1338719). The host bacteria normally supply the tRNA molecules required for translation, this result raises the possibility that these prophages rely on the host translation machinery for protein synthesis. However, no ribosomal RNAs were found in the prophage sequences (Table 5).

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Prophage position</th>
<th>CDS</th>
<th>tRNA</th>
<th>rRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>1464206-1483241</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2759376-2809756</td>
<td>78</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3311844-3364667</td>
<td>80</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae HS11286</td>
<td>581777-594029</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1062574-1073328</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1288317-1338719</td>
<td>72</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1778306-1808606</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2280703-2325555</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4049881-4085225</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4819189-4835185</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> UCBPP-PA14</td>
<td>4345129-4355794</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Putative promoter sequences detection

Softberry promoter online analysis tool was employed to identify putative promoter sequences and regions in 11 prophage genomes. The results revealed that three prophages of *Acinetobacter baumannii* had 47, 126, and 133 putative promoters, respectively. Among the seven prophages of *Klebsiella pneumoniae*, the number of putative promoters were 25, 19, 108, 67, 95, 78 and 37. For the single prophage of *Pseudomonas aeruginosa*, we identified 19 putative promoters (Table 3). The list of promoter sequences of 11 prophages with their transcription factors (TF), binding site, position and binding scores were recorded (Additional file 1: Table S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, and S22).

Virulent genes Prediction

The study employed the Virulence Search program using the VFDB to detect virulent genes in the prophage genomes. The analysis identified Peptidoglycan DD-metalloendopeptidase family protein and undecaprenyl-phosphate glucose phosphotransferase in one prophage of *Klebsiella pneumoniae*, both of which are known virulence factors. Additionally, the prophage of *Pseudomonas aeruginosa* was found to contain Phenazine biosynthesis protein PhzF, which is involved in phenazine biosynthesis and has been linked to virulence in *P. aeruginosa*.

Prediction of Rho-independent transcription terminators

To identify Rho-independent transcription terminators in the prophage genomes, we employed the ARNold program. The results showed the presence of terminators in various prophages, with 3 prophages of *Acinetobacter baumannii* containing 18, 53, and 61 terminators, 7 prophages of *Klebsiella pneumoniae* containing 10, 8, 35, 25, 31, 29, and 9 terminators, and 1 prophage of *Pseudomonas aeruginosa* containing 2 terminators (Table 3). The prediction of Rho-independent terminators is essential for understanding the transcriptional regulation of prophage genes and their integration into the host genome. The predicted transcription terminators, the position of terminators in prophage genome, strand type and sequence of predicted terminators are given (Additional file 1: Table S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, and S33).

tRNAs prediction

tRNAs prediction in prophages genomes are detected by online software tRNA-SCAN-SE and ARAGORN. The presence of 3 tRNAs encoded by 11 prophages were discovered through examination of the tRNA regions in the prophage genomes (Table 5). Given that the host bacteria normally supply the tRNA molecules required for translation, this result raises the possibility that these prophages rely on the host translation machinery for protein synthesis. The structure of predicted tRNAs was also predicted (Figure 1,2).

Dot Plot generation

The dot plot of predicted prophage genomes was created using the Gepard software. Dot plot enables us to check the similarity between sequences. The dot plot for selected *Acinetobacter baumannii* AB0057 prophages, and selected *Klebsiella pneumoniae* subsp. pneumoniae HS11286 prophages (Figure 3). *Klebsiella pneumoniae* subsp. pneumoniae HS11286 prophage (1778306-1808606) and *Klebsiella pneumoniae* subsp. pneumoniae HS11286 prophage (1778306-1808606) were rejected as being similar to each other.
Selection of viable prophages

The viable prophages were selected and non-viable prophages were excluded from the further study, based on the annotations and optimum threshold values (Table 6). The prophage positions, sizes and cutoff values, tRNAs, rRNAs, virulence factors, terminator, and promoter regions detected in prophages from *Acinetobacter baumannii* AB0057, *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286, and *Pseudomonas aeruginosa* UCBPP-PA14 were taken into consideration.

Comparative genomic analysis

Comparative genome analysis of the prophages was conducted using EasyFig 2.2. The selected 5 prophages with their highest similar phage sequences were analysed using the EasyFig 2.2. This allowed us to visualize the similarities and differences between the prophages and their closest homologs. Genome maps of *Acinetobacter baumannii* AB0057 phages (Figure 4,5). Genomes maps of *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 prophages with their highest similar phage (Figure 6,7,8).

### Table 6. Selection of viable and exclusion of non-viable prophages on the basis of all probable parameters:

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Detected prophage position</th>
<th>Size</th>
<th>tRNAs detected using ARAGORN and tRNAscan-SE</th>
<th>rRNA detected using Prokka and Bammap</th>
<th>Virulence factors detected using VFDB</th>
<th>Terminator regions detected using Softberry BPROM</th>
<th>Promoter regions detected using ARNold</th>
<th>Dotplot alignment similarities detected from Gep</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em> AB0057</td>
<td>1464206-1483241</td>
<td>19.04*</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>18</td>
<td>47</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2759376-2809756</td>
<td>50.38</td>
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<td>581777-594029</td>
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<td>Peptidoglycan DD-metalloendopeptidase family protein, undecaprenylphosphate glucose phosphotransferase*</td>
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<td><em>Pseudomonas aeruginosa</em> UCBPP-PA14</td>
<td>4345129-4355794</td>
<td>10.67</td>
<td>0</td>
<td>0</td>
<td>Phenazine biosynthesis protein PhzF, isomerase*</td>
<td>2</td>
<td>19</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values that did not met the inclusion criteria

BLASTn analysis

BLASTn was performed for selected prophages. The most identical phages were selected for further analysis to check the viability of prophages used in this study. Identical phages were found to be involved in phage therapy. The sequences of identical phages were with their accession numbers were also retrieved (Table 7).
Table 7. BLASTn analysis of selected 5 prophages: Phages having sequence similarities with our final selected prophages with their accession numbers and similarities percentages. The phages sharing 100% and 99.9% similarities are highlighted.

<table>
<thead>
<tr>
<th>Prophages</th>
<th>Similar phages</th>
<th>Accession numbers</th>
<th>Similarity (%)</th>
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<tbody>
<tr>
<td>Acinetobacter baumannii (2759376-2809756)</td>
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<td>NC_019541.1</td>
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<td>Acinetobacter phage Acba_1</td>
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<td>OQ101257.1</td>
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<td>Acinetobacter baumannii (3311844-3364667)</td>
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<td>Acinetobacter phage Ab105-2phideltaC1404ad</td>
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<td>Enterobacter phage LAU1</td>
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<td>Klebsiella phage ST258-KPC3phi16.2</td>
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<td>Klebsiella phage ST512-KPC3phi13.2</td>
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Phylogenetic Analysis

Phylogenetic analysis of predicted prophages was conducted on the basis of their large terminase subunits. Neighbour-joining phylogenetic tree was generated with 1000 bootstrap replications using MEGA 11 (Figure 9). Phylogenetic analysis of our selected prophages along with the similar phages provided important insights into their diversity and relatedness. The Klebsiella pneumoniae prophages were found to be present with other known Klebsiella pneumoniae phages showing close relatedness, whereas the prophages from Acinetobacter baumannii AB0057 sequences were found to be closely related in a monophyletic order.

Discussion

Antibiotic-resistant bacteria, such as Carbapenem-resistant Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa, are a growing problem due to factors such as overuse and misuse of antibiotics, gene transfer, and inadequate diagnostics [17]. Gram-negative bacteria are particularly difficult to treat, and infections caused by multidrug-resistant bacteria can lead to increased healthcare costs, morbidity, and mortality. Phage therapy, which uses viruses that attack and replicate inside bacterial cells, is a potential substitute for antibiotics due to its high specificity and ability to target harmful microorganisms without harming human cells [18]. In a previous study by Gallet et al., 2022 and Adams et al., 2008 they conducted a whole genome analysis of Carbapenem-Resistant Klebsiella pneumoniae genomes and multidrug resistant Acinetobacter baumannii, respectively. They found that these strains are causing serious problems in human heath worldwide [19, 20].

In this study, bacteriophage therapy is considerably constructed on phage cocktail. These cocktails can be formulated by combination of various isolates or phages to increase the efficiency to lyse and they also can be re tailored if resistance develops [21]. To lyse the multiple targeted strains of bacteria, instead of one phage, phage cocktails are formulated. There are very specific phages, only infect a single and specific bacterium. A mixture of phages with perfect host ranges called phage cocktail, to increase targeted strains and useful for broader range application can be predicted [22]. In a previous study by Breijyeh et al., 2020 they conducted a study on how antimicrobial resistant occurs and also provide treatment process. They also suggested that instead of using antibiotics, we can provide phage therapy against multi drug resistant bacteria [23].

In current study, multidrug resistant strains of bacteria were selected to start the designing of phage cocktail. The selected strains were Acinetobacter baumannii AB0057, Klebsiella pneumoniae HS11286, Pseudomonas aeruginosa UCBPP-PA1 which are causing serious health problems. Carbapenem resistance was found to be dominant in these bacterial strains. In a previous study by Chen et al., 2019 and Maria et al., 2020 the therapeutic cocktail to combat staphylococcal strain and Vibrio sp. Va-F3 were designed respectively [24, 25].
A total of 11 prophages were detected from three bacterial genomes by Mageeney et al., 2020 including 3 prophages from Acinetobacter baumannii, 7 prophages from Klebsiella pneumoniae and 1 prophage from Pseudomonas aeruginosa. In another study by Mageeney et al., 2020 they predicted the IGEs from 2168 genomes of bacterial strains but they did not perform the designing of phage cocktail for the bacterial strains, which were included in our current study [26]. In a previous study by Bose et al., 2021 they performed the annotation of prokaryotic genomes using the prophage finder program for the prediction of prophages [27].

We used TIGER and Islander algorithms, which were used by Magneeney et al., 2020 for IGE detection including prophage genomes, however, we used these predicted prophages for designing of therapeutic cocktail against the multi drug resistant strains of bacteria. Prophages were identified and characterized to learn more about their possible effects on the biology and pathogenicity of their bacterial hosts. It may be possible to create novel methods for the treatment and prevention of bacterial illnesses by knowing the genetic components which contribute to the pathogenicity of bacteria.

In a previous study by Martínez-Vaz et al., 2020 they used other methods for hunting of phages from bacterial genome. They used PHASTER, a program for detection of phages from bacterial genomes [28]. In another study by Song et al., 2019, Prophage Hunter, an algorithm for hunting of active prophages was used for the detection of prophages [29]. In our study, integrated genomic element-based algorithms were applied, which detected the different islands from bacterial genomes including prophages.

The small prophages having sizes below 11 kb are considered to be non-functional and unable to assemble a mature and viable bacteriophage because an intact phage with complete functional units can disrupt a target bacterium completely [30]. The prophages having size below 30 kb are also considered small and it is very difficult to differentiate between them and other mobile genetic elements [31]. This can pose a challenge when attempting to accurately identify and classify small prophages within prokaryotic genomes, therefore, we have included only those prophages in our study which had a size above 30 kb.

In a previous study by Happel et al., 2022 they found that prophages that were beyond the average size range for bacteriophages in the NCBI Caudovirales database (less than 11.6 kb), were found using five algorithms for identifying phages. These prophages were excluded from further analysis since it was considered improbable that they would perform their function properly. Also, they discarded those phages which have size larger than 800kb, much larger than size of known phage genome size. None of our selected prophages were larger than 60 kb, which suggests that these prophages can code for complete and viable bacteriophages.

In a previous study by Bobay et al., 2014 they performed the probability distributions for genome size of the 68 double stranded DNA temperate caudophages infecting enterobacteria, and discarded those phages which had a size lower than 30 kb, considering them to be non-functional [32].

A total of 472 ORFs were predicted form the 11 prophages. In a previous study by Lavigne et al., 2018 PHIRE algorithm was presented to predict the regulatory elements in bacteriophage genomes [33]. We run GeneMark.hmm program for prediction of open reading frames.

Prokka annotation of these prophage enabled us to acknowledge the features of these prophages including CDS regions, rRNAs (ribosomal RNAs), and tRNAs (transfer RNAs). Mageeney et al., (2020) performed prokka annotation only for P aeruginosa strains Pae5 and Pae1505, but in current study different strains of bacteria i.e., Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa were used for cocktail designing. In another study about phage cocktail designing by Maria et al., 2020 the auto annotation of phages were performed using Rapid Annotation Using Subsystem Technology. Their work contributed in determination of Podoviridae and Myoviridae bacteriophages against staphylococcal strains of bacteria (34).

This annotation enabled us to learn more about the diversity and development of these mobile genetic elements, as well as their possible interactions with bacterial hosts and other mobile genetic elements, by examining the CDS, rRNA, and tRNA sections of prophages. This knowledge can aid in the creation of fresh tactics for the management and avoidance of bacterial illnesses.

The presence of 3 tRNAs encoded by 11 prophages were discovered through examination of the tRNA regions in the prophage genomes. Given that the host bacteria normally supply the tRNA molecules required for translation [35], this result raises the possibility that these prophages rely on the host translation machinery for protein synthesis. In a past study by Chen et al., tRNAs from vibrio phages were predicted using ARAGORN software. Also, tRNA-Scans-SE was employed for the prediction of tRNAs with their colorful structure [36].

We also detected rRNA genes, which can interact with the host cell’s translation and protein production machinery. We did not find any tRNA in predicted prophages, but it does not have any effect on phage functioning because a bacteriophage hijacks host translation machinery and replicates inside the host cell [37].

We used the BLASTP algorithm to annotate the predicted ORFs in the prophage genomes. This allowed us to identify putative functions and potential biological roles of the encoded proteins. The use of the NCBI non-redundant protein database for the BLASTP search ensured that we had access to a comprehensive collection of known protein sequences for comparison. We identified a total of 29, 79, and 85 Open Reading Frames in 3 prophages of Acinetobacter baumannii with maximum identity percentages of 100% and minimum identity percentages of 30.84%, 40.68%, and 42.82% respectively. The Acinetobacter phage 5W was most identical to our query prophages, which is very effective against multidrug-resistant Acinetobacter baumannii.

Chen et al., 2019 performed annotation of the protein sequences of predicted ORFs using BLASTP algorithm. Out of 530 ORFs analyzed, approximately 66.2% (n = 351) demonstrated amino acid sequence identities ranging from 26–100% with sequences present in the NCBI GenBank database [38].

We identified a total of 14, 13, 73, 37, 63, 43, and 22 Open Reading Frames in 7 prophages of Klebsiella pneumoniae with maximum percentage identities of 100% and minimum identity percentages of 93.76%, 27.51%, 87.79%, 93.83%, 59.29%, 70.18%, and 86.84% respectively. BLAST analysis of Klebsiella prophages showed identical results with Klebsiella phage Mulock. We found that Klebsiella phage KPP5665-2 and Klebsiella phage Mulock were used for
bacteriophage therapy [39]. In a study conducted by Herridge et al., 2020 many phages including Klebsiella phage Mulock was used against the resistant strains of Klebsiella [36].

A total of 14 ORFs were identified in the prophage of Pseudomonas aeruginosa which showed maximum identity percentage of 100% and minimum identity percentage of 38.22%. We found that Pseudomonas phage Pf1 was most identical, which is effective against other strain of Pseudomonas [40]. We employed the Softberty phage promoter online analysis tool to identify putative promoter sequences and regions in 11 prophage genomes. Our results revealed that three prophages of Acinetobacter baumannii had 47, 126, and 133 putative promoters, respectively. Among the seven prophages of Klebsiella pneumoniae, the number of putative promoters ranged from 19 to 108. For the single prophage of Pseudomonas aeruginosa, we identified 19 putative promoters. These findings provide insights into the potential regulatory mechanisms of these prophages and their interactions with the host bacteria. Due to their minimum sized genome and presence of virulence factor they were rejected as discussed by Happel et al., 2022.

We also identified Rho-independent transcription terminators in the prophage genomes. The results showed the presence of terminators in various prophages, with 3 prophages of Acinetobacter baumannii containing 18, 53, and 61 terminators, 7 prophages of Klebsiella pneumoniae containing 10, 8, 35, 25, 31, 29, and 9 terminators, and 1 prophage of Pseudomonas aeruginosa containing 2 terminators. The prediction of Rho-independent terminators is essential for understanding the transcriptional regulation of prophage genes and their integration into the host genome [41]. In a previous study by Chen et al., 2020 they predicted terminators for vibrio phages but not perform any analysis for other strains of bacteria which are used in this study.

The Virulence Search program using the Virulence Factor Database (VFDB) was employed to detect virulent genes in the prophage genomes. The analysis revealed that one prophage of Klebsiella pneumoniae harbored Peptidoglycan DD-metalloendopeptidase family protein and undecaprenyl-phosphate glucose phosphotransferase, which are known virulence factors [42]. Additionally, the prophage of Pseudomonas aeruginosa was found to contain Phenazine biosynthesis protein PhzF, which is an isomerase involved in phenazine biosynthesis and has been linked to virulence in P. aeruginosa [43]. Therefore, those prophages which are detected to producing virulent genes are rejected.

In this study, we selected 5 prophages for phage therapy. These prophages met all the inclusion criteria. The size of selected prophages was neither less than 30kb nor higher than 800kb. Apart of this the phages which contain virulence factors were also excluded. Dot plot of selected phages was generated, which enabled us to reject K. pneumoniae Prophage (4049881–4085225 as being similar to K. pneumoniae Prophage (1778306–1808606).

Comparative genome analysis of the prophages was conducted using EasyFig and genome maps were created showing the similarities between two identical genomes of prophages. The selected prophages with their highest similar prophage sequences were analyzed using the EasyFig 2.2. This allowed us to visualize the similarities and differences between the prophages and their closest homologs. Their highest identical prophage 95.67% (NC0391949.1) Acinetobacter phage YC#06 which is discussed by Luo et al., 2022 they isolated and characterized and used this virulent phage against multidrug-resistant Acinetobacter baumannii strains, moreover the also made antibiotic mixtures with that phage to enhance the effects of phage therapy. [44]

After performing BLASTn analysis we found that our selected prophage Acinetobacter baumannii 2759376–2809756 was similar with Podoviral Bacteriophage YMC/09/02/B1251 ABA BP (NC_019541.1) by 96.06% identity. This similar bacteriophage belongs to the family Podoviridae and has a double-stranded circular DNA genome with a length of 45,364 bp and a 39.05% GC content. In a study by Jeon et al., 2012 they perform a complete analysis of Bacteriophage YMC/09/02/B1251 ABA-BP and reported that this phage causes the lysis of an isolate of Carbapenem-Resistant Acinetobacter baumannii strain from a septic patient [45].

In a study by Baginska et al., 2023 they purposed a alternative methods of phage therapy instead of using supplement and other antibiotics. They conducted a study on determination of morphology and biological properties of 12 phages including Acinetobacter phage Acba_1, Acinetobacter phage Acba_3, Acinetobacter phage Acba_11, Acinetobacter phage Acba_15 specific against MDR Acinetobacter baumannii. Moreover, after analysis of morphology and biological properties of these phages they were determined to use these phages against MDR Acinetobacter baumannii [46]. These phages are very similar to those phages of Acinetobacter baumannii used in current study.

In a study by Bleriot et al., 2020 genonomic analysis of 40 Klebsiella phage which were integrated in 16 strains of Klebsiella pneumoniae which are resistant to carbapenem. They also included those phages which are highest similar to Klebsiella pneumoniae prophages used in current study. They revealed that these phages contain different proteins which were mainly involved in structure of virion, replications, transcription of virion and regulation of lysogenic and lytic cycle [47].

We reviewed available studies on prophages having 100% and 99.99% similarity with our prophages, targeting any additional bacterial species or strains in addition to the mentioned strains (Table. 7). The phage (MN688132.1) targeting Klebsiella pneumoniae (1283817–1338719) is reported by Chen et al., 2022, to be capable of targeting Enterobacter hormaechei as well [51]. E. hormaechei is responsible for nosocomial infections, urinary tract diseases and bacteremia [53]. Bleriot et al., 2020 also reported phages targeting a wide range of Klebsiella strains, among which some of the phages were also included in our study. The phage (MK416019.1) was found to target K. pneumoniae ST11-VM1 and phage (MK433582.1) was found to target K. pneumoniae ST258-KPC3. In the same way, phages (MN166823.1 and NC_049452.1) were reported for targeting K. pneumoniae ST512-KPC3 and phage (NC_049449.1) was found targeting K. pneumoniae ST437-OXA245 [47][52]. These Klebsiella strains are found to be carbapenemase producing and are majorly responsible for nosocomial infections, pneumonia, and urinary tract infections leading to high morbidity and mortality rates [47]. This investigation suggests that the phage cocktail we have designed, has the potential to target the above-mentioned bacterial strains also, in addition to our selected MDR bacterial strains.

Phylogenetic analysis was conducted on the prophages isolated from Acinetobacter baumannii and Klebsiella pneumoniae, as well as one prophage from Pseudomonas aeruginosa. The sequences chosen for the analysis were selected based on BLASTn, and the neighbour-joining method with 1000 bootstrap replications was used for the analysis using MEGA 11 software. Highest similar sequences prophages were selected and phylogenetic tree was constructed
based on large terminase subunits of selected phages. Two critical tasks are carried out by the terminase large subunit during the viral replication cycle. First of all, it acts as an ATP-powered molecular motor to help viral DNA move into empty capsids. It also serves as an endonuclease, cleaving the viral DNA to start and stop the packing activity [48].

The comparative genome analysis and phylogenetic analysis of the isolated phages provide important insights into the diversity and relatedness of the phages. The *Klebsiella* prophages were found to be present with other known *Klebsiella* prophages showing close relatedness, whereas the prophages from *Acinetobacter baumannii* genomes were closely found to be monophyletic. In this study, the highest similar sequences for *Acinetobacter baumannii* ranged from 99.91–75.38%, indicating a relatively high degree of diversity among the phages. For *Klebsiella pneumoniae*, a total of 25 highest similar phages were selected with similarities ranging from 99.99–78.95%. This indicates a relatively high diversity within this species, which is consistent with previous studies that have reported a high level of diversity in *Vibrio* phages and prophages form *Pseudomonas* strains.

Phage cocktails have the potential to become an important tool in the fight against multidrug-resistant bacteria in the future [49]. One of the main benefits is their specificity, which allows for targeted and personalized treatment. This reduces the risk of disrupting the beneficial bacteria in the body, unlike traditional antibiotics which can lead to collateral damage to the microbiome [50]. Additionally, phages can be isolated and propagated relatively quickly, and can be tailored to target specific strains or combinations of strains. This makes them a promising alternative to antibiotics, especially in cases where conventional antibiotics are ineffective or unavailable. Overall, the use of phage cocktails could revolutionize the treatment of multidrug-resistant bacterial infections and provide new hope for patients with limited treatment options.

**Conclusion**

This work provides a rigorously developed protocol for developing a phage cocktail against Multidrug resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* and might be very useful to combat these bacterial strains that are resistant to a variety of drugs. Through extensive sequence similarity analyses, it was discovered that this phage cocktail exhibits an ability of targeting *Enterobacter hormaechei* and various carbapenemase-producing *Klebsiella pneumoniae* strains as well, in addition to the initially selected multidrug-resistant bacteria. The phage cocktail indicates the possibility of also being employed as a therapeutic agent for reducing harmful bacterial infections because the use of different antibiotics in aquaculture is frequently prohibited.

**Abbreviations**

- MDR Multi drug resistant bacteria
- BLAST Basic Local Alignment Search Tool
- CDS Coding sequences
- CRAB Carbapenem-resistant *Acinetobacter baumannii*
- TIGER Target Integrated Genetic Element Retriever
- IGE Integrated Genetic Elements

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets supporting the conclusions of this article are included within the article (and its additional files).

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

A.H. and M.A.A. performed the computational analyses. A.H., M.A.A., M.S., M.S., and A.A. assisted in data retrieval process. A.H., M.A.A., and S.A. contributed to results interpretation. A.A. and M.A.A. wrote the manuscript text. R.N. and I.K. supervised this study. All authors read and approved the final manuscript.
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References

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42. Chen LX, Jaffe AL, Borges AL, Penev PI, Nelson TC, Warren LA, Banfield JF. Phage-encoded ribosomal protein S21 expression is linked to late-stage phage replication. ISME Communications. 2022 Mar 30;2(1):31. https://doi.org/10.1038/s43705-022-00111-w


45. Jeon J, Kim JW, Yong D, Lee K, Chong Y. Complete genome sequence of the podoviral bacteriophage YMC/09/02/B1251 ABA BP, which causes the lysis of an OXA-23-producing carbapenem-resistant Acinetobacter baumannii isolate from a septic patient. https://doi.org/10.1128/jvi.02132-12


Footnotes
2. http://exon.gatech.edu/genemark/gmhmmp.cgi
5. http://www.mgc.ac.cn/VFs/

Figures

Structure of predicted tRNA carried by Acinetobacter baumannii prophage 3311844-3364667
Figure 2

Structure of predicted tRNAs carried by Klebsiella pneumoniae prophage 1288317-1338719
Figure 3

Figure 4
Genome map of *Acinetobacter baumannii* (2759376-2809756) with their highest identical prophage 95.67% (ON391949.1) Acinetobacter phage YC#06. The gray region indicates the high similarity among genomic sequences. CDs region is shown in orange color. Green color shows the maximum blast similarity while red color shows the minimum blast similarity.
Figure 5
Genome map of *Acinetobacter baumannii* prophage (3311844-3364667) with their highest identical prophage. The gray region indicates the high similarity 99.86% (OQ101248) *Acinetobacter phage Acba_*3. CDs region is shown in orange color. Green color shows the maximum blast similarity while red color shows the minimum blast similarity.

Figure 6
Genome map of *Klebsiella pneumoniae* prophage (1288317-1338719) with their highest similar phage MN688132.1 (100%). Phage LAU1, The gray region indicates the high similarity among genomic sequences. CDS region is shown in orange color and blue color shows the other mics_features. Green color shows the maximum blast similarity while red color shows the minimum blast similarity.
Figure 7

Genome map of *Klebsiella pneumoniae* prophage (1778306-1808606) with its highest similar phage NC_049449.1 (100%), *Klebsiella* phage ST437-OXA245phi4.2. The gray region indicates the high similarity among genomic sequences. CDS region is shown in orange color and blue color shows the other mics_features. Green color shows the maximum blast similarity while red color shows the minimum blast similarity.

Figure 8

Genome map of *Klebsiella pneumoniae* prophage (2280703-2325555) with their highest similar phage MK416019.1 (99.99%) *Klebsiella* phage ST11-VIM1phi8.3. The gray region indicates the high similarity among genomic sequences. CDS region is shown in orange color and blue color shows the other mics_features. Green color shows the maximum blast similarity while red color shows the minimum blast similarity.
Figure 9

A neighbor-joining phylogenetic tree of selected 5 prophages; *A. baumannii* Prophage (2759367-2809756), *A. baumannii* Prophage (2759367-2809756), *K. pneumoniae* Prophage (1288317-1338719), *K. pneumoniae* Prophage (1778306-1808606), *K. pneumoniae* Prophage (2280703-2326555), *K. pneumoniae* Prophage (4049881-4085225) was constructed with 1000 bootstrap replications on MEGA 11. Number of clades from A to M were found. Circled sequences are 5 selected prophages which are showing similarity with other phages. The sequences were selected after performing the BLASTn of 5 prophages and highest similar phages with maximum identity was selected. The similarity of selected phages for two prophages of *Acinetobacter baumannii* was ranging from 93% to 99%. While the similarity of phages for 3 prophages of *Klebsiella pneumoniae* was 80% to 100%.

Supplementary Files

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- **AdditionalFile1Tables.docx**