Supplementary Materials

Characterization and preclinical *in silico* safety assessment of three virulent bacteriophages targeting carbapenem-resistant uropathogenic *Escherichia coli*

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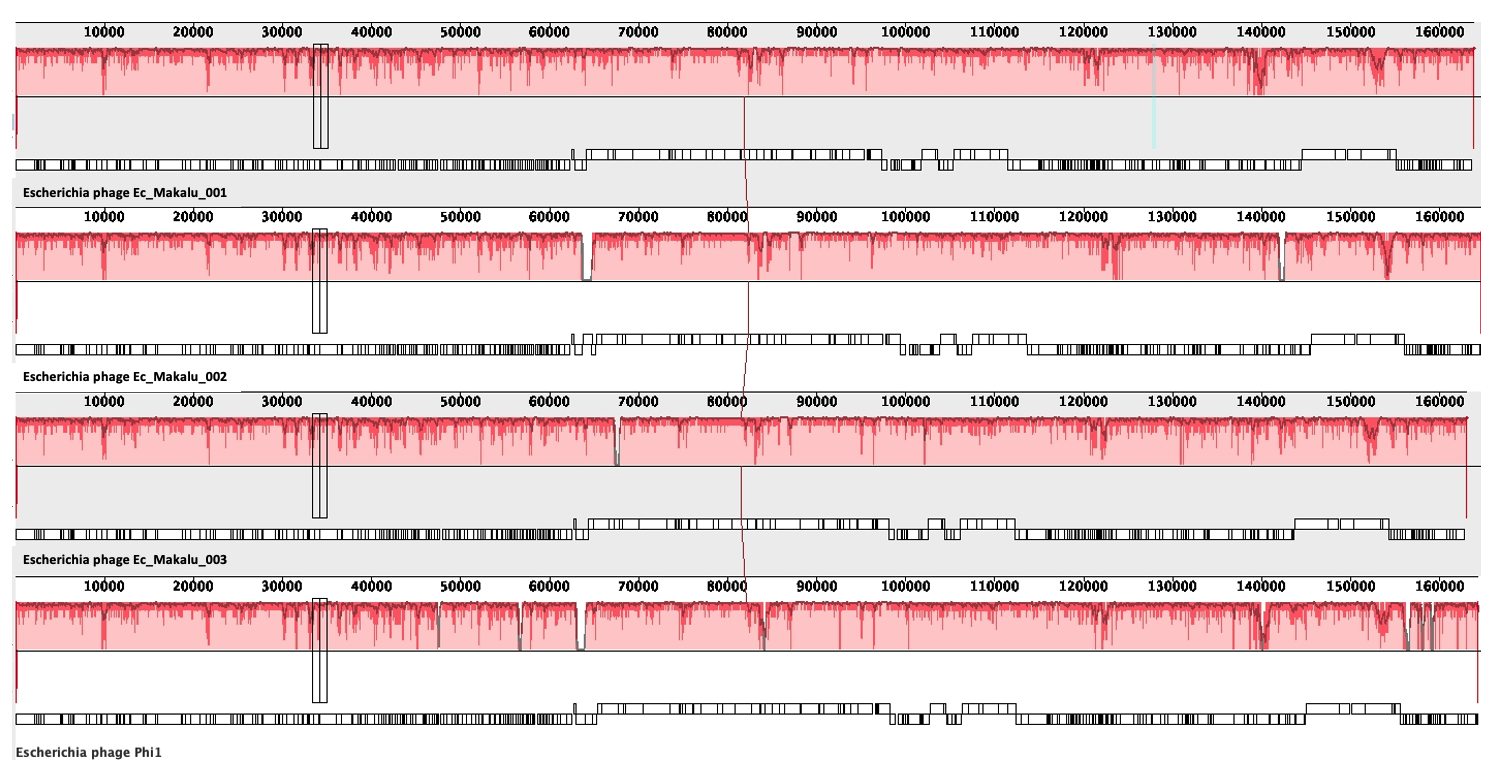
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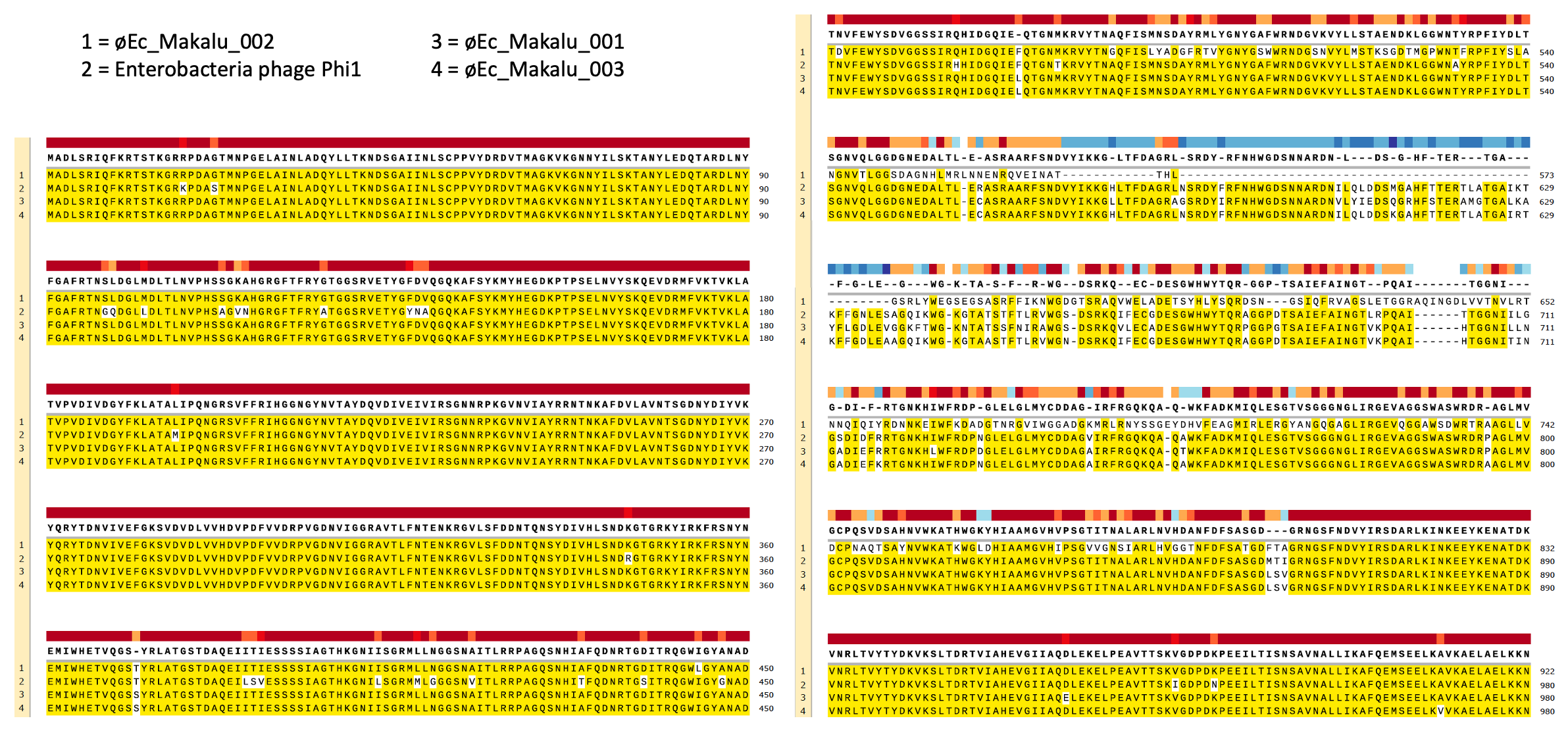
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#### Supplementary Figures

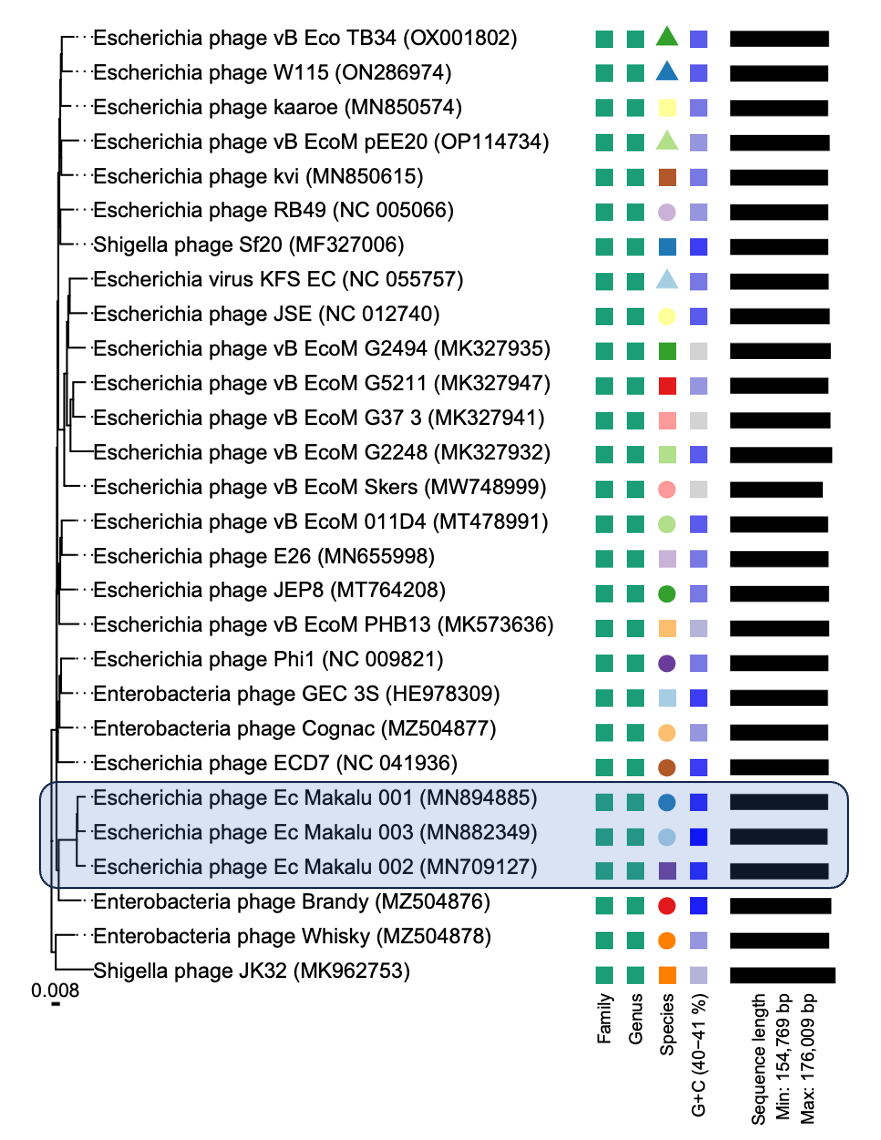
#### Figure S1 | MAUVE alignment of three Escherichia phages (øEc\_Makalu\_001, øEc\_Makalu\_002 and øEc\_Makalu\_003) compared with the most closely related Escherichia phage Phi1.



**Figure S2 | Consensus alignment of long tail fiber protein.** Amino acids that match the reference are marked with yellow highlighting. Consensus threshold = 50%*.*



**Figure S3 | The Genome-BLAST Distance Phylogeny (GBDP) tree of phages** ø**Ec\_Makalu\_001,** ø**Ec\_Makalu\_002, and** ø**Ec\_Makalu\_003 inferred using formula D4 and yielding average support of 6 %.** The analysis reveals that all three phages belong to the same family and genus level and share maximum sequence similarity with other phages in the NCBI database, including Shigella phages.



#### Supplementary Table

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table S1 | Sequences producing significant alignments with reference genome of Escherichia phage Ec\_Makalu\_001 | | | | | | | | |
| SN | **Scientific Name** | **Accession** | **Max Score** | **Total Score** | **Query Coverage** | **Per. identity** | **Total Per. identity** | **Acc. Length** |
| 1 | Escherichia phage Ec\_Makalu\_001 | [MN894885.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN894885.1?report=genbank&log$=nucltop&blast_rank=1&RID=TXADWNTA013) | 3.02E+05 | 3.05E+05 | 100% | 100 | 100.00 | 163752 |
| 2 | Escherichia phage Ec\_Makalu\_002 | [MN709127.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN709127.1?report=genbank&log$=nucltop&blast_rank=2&RID=TXADWNTA013) | 1.12E+05 | 2.87E+05 | 98% | 98.49 | 96.52 | 164674 |
| 3 | Escherichia phage Ec\_Makalu\_003 | [MN882349.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN882349.1?report=genbank&log$=nucltop&blast_rank=3&RID=TXADWNTA013) | 77484 | 2.89E+05 | 98% | 98.79 | 96.81 | 162966 |
| 4 | Escherichia phage Phi1 | [EF437941.1](https://www.ncbi.nlm.nih.gov/nucleotide/EF437941.1?report=genbank&log$=nucltop&blast_rank=4&RID=TXADWNTA013) | 62462 | 2.59E+05 | 96% | 97.07 | 93.19 | 164270 |
| 5 | Escherichia phage kvi | [MN850615.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN850615.1?report=genbank&log$=nucltop&blast_rank=5&RID=TXADWNTA013) | 58362 | 2.55E+05 | 94% | 96.74 | 90.94 | 163673 |
| 6 | Escherichia phage JEP8 | [MT764208.1](https://www.ncbi.nlm.nih.gov/nucleotide/MT764208.1?report=genbank&log$=nucltop&blast_rank=6&RID=TXADWNTA013) | 57899 | 2.59E+05 | 96% | 96.51 | 92.65 | 165295 |
| 7 | Escherichia phage vB\_EcoM\_PHB13 | [MK573636.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK573636.1?report=genbank&log$=nucltop&blast_rank=7&RID=TXADWNTA013) | 55118 | 2.56E+05 | 96% | 97.31 | 93.42 | 165641 |
| 8 | Enterobacteria phage GEC-3S | [HE978309.1](https://www.ncbi.nlm.nih.gov/nucleotide/HE978309.1?report=genbank&log$=nucltop&blast_rank=8&RID=TXADWNTA013) | 51849 | 2.61E+05 | 97% | 95.04 | 92.19 | 163424 |
| 9 | Escherichia phage ECD7 | [NC\_041936.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_041936.1?report=genbank&log$=nucltop&blast_rank=9&RID=TXADWNTA013) | 49807 | 2.59E+05 | 97% | 97.29 | 94.37 | 164706 |
| 10 | Escherichia virus KFS-EC | [NC\_055757.1](https://www.ncbi.nlm.nih.gov/nucleotide/NC_055757.1?report=genbank&log$=nucltop&blast_rank=10&RID=TXADWNTA013) | 49748 | 2.47E+05 | 94% | 93.29 | 87.69 | 164715 |
| 11 | Escherichia phage vB\_EcoM\_011D4 | [MT478991.1](https://www.ncbi.nlm.nih.gov/nucleotide/MT478991.1?report=genbank&log$=nucltop&blast_rank=11&RID=TXADWNTA013) | 49543 | 2.59E+05 | 96% | 95.1 | 91.30 | 163764 |
| 12 | Escherichia phage RB49 | [AY343333.1](https://www.ncbi.nlm.nih.gov/nucleotide/AY343333.1?report=genbank&log$=nucltop&blast_rank=12&RID=TXADWNTA013) | 43674 | 2.60E+05 | 96% | 97.33 | 93.44 | 164018 |
| 13 | Escherichia phage kaaroe | [MN850574.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN850574.1?report=genbank&log$=nucltop&blast_rank=13&RID=TXADWNTA013) | 43120 | 2.60E+05 | 96% | 96.94 | 93.06 | 163719 |
| 14 | Escherichia phage E26 | [MN655998.1](https://www.ncbi.nlm.nih.gov/nucleotide/MN655998.1?report=genbank&log$=nucltop&blast_rank=14&RID=TXADWNTA013) | 42911 | 2.60E+05 | 96% | 96.43 | 92.57 | 164572 |
| 15 | Escherichia phage vB\_EcoM\_G2248 | [MK327932.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK327932.1?report=genbank&log$=nucltop&blast_rank=15&RID=TXADWNTA013) | 42475 | 2.38E+05 | 93% | 95.48 | 88.80 | 170678 |
| 16 | Escherichia phage vB\_EcoM\_Skers | [MW748999.1](https://www.ncbi.nlm.nih.gov/nucleotide/MW748999.1?report=genbank&log$=nucltop&blast_rank=16&RID=TXADWNTA013) | 41539 | 2.33E+05 | 88% | 95.08 | 83.67 | 154769 |
| 17 | Enterobacteria phage Brandy | [MZ504876.1](https://www.ncbi.nlm.nih.gov/nucleotide/MZ504876.1?report=genbank&log$=nucltop&blast_rank=17&RID=TXADWNTA013) | 40470 | 2.57E+05 | 96% | 95.83 | 92.00 | 169232 |
| 18 | Escherichia phage W115 | [ON286974.1](https://www.ncbi.nlm.nih.gov/nucleotide/ON286974.1?report=genbank&log$=nucltop&blast_rank=18&RID=TXADWNTA013) | 39548 | 2.61E+05 | 96% | 95.18 | 91.37 | 163997 |
| 19 | Shigella phage JK32 | [MK962753.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK962753.1?report=genbank&log$=nucltop&blast_rank=19&RID=TXADWNTA013) | 38428 | 2.29E+05 | 93% | 93.65 | 87.09 | 176009 |
| 20 | Escherichia phage vB\_EcoM\_G37-3 | [MK327941.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK327941.1?report=genbank&log$=nucltop&blast_rank=20&RID=TXADWNTA013) | 37096 | 2.44E+05 | 94% | 96 | 90.24 | 167832 |
| 21 | Enterobacteria phage Whisky | [MZ504878.1](https://www.ncbi.nlm.nih.gov/nucleotide/MZ504878.1?report=genbank&log$=nucltop&blast_rank=21&RID=TXADWNTA013) | 34915 | 2.46E+05 | 95% | 95.62 | 90.84 | 165615 |
| 22 | Shigella phage Sf20 | [MF327006.1](https://www.ncbi.nlm.nih.gov/nucleotide/MF327006.1?report=genbank&log$=nucltop&blast_rank=22&RID=TXADWNTA013) | 33787 | 2.60E+05 | 96% | 97.75 | 93.84 | 163982 |
| 23 | Escherichia phage vB\_Eco\_TB34 | [OX001802.1](https://www.ncbi.nlm.nih.gov/nucleotide/OX001802.1?report=genbank&log$=nucltop&blast_rank=23&RID=TXADWNTA013) | 33606 | 2.62E+05 | 96% | 96.56 | 92.70 | 165220 |
| 24 | Enterobacteria phage Cognac | [MZ504877.1](https://www.ncbi.nlm.nih.gov/nucleotide/MZ504877.1?report=genbank&log$=nucltop&blast_rank=24&RID=TXADWNTA013) | 31490 | 2.53E+05 | 95% | 96.94 | 92.09 | 164031 |
| 25 | Escherichia phage vB\_EcoM\_G5211 | [MK327947.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK327947.1?report=genbank&log$=nucltop&blast_rank=25&RID=TXADWNTA013) | 31242 | 2.40E+05 | 93% | 96.21 | 89.48 | 164278 |
| 26 | Escherichia phage vB\_EcoM\_G2494 | [MK327935.1](https://www.ncbi.nlm.nih.gov/nucleotide/MK327935.1?report=genbank&log$=nucltop&blast_rank=26&RID=TXADWNTA013) | 28801 | 2.45E+05 | 94% | 97.15 | 91.32 | 168327 |
| 27 | Escherichia phage JSE | [EU863408.1](https://www.ncbi.nlm.nih.gov/nucleotide/EU863408.1?report=genbank&log$=nucltop&blast_rank=27&RID=TXADWNTA013) | 27253 | 2.45E+05 | 95% | 96.97 | 92.12 | 166418 |
| 28 | Myoviridae sp. | [BK017805.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK017805.1?report=genbank&log$=nucltop&blast_rank=28&RID=TXADWNTA013) | 24912 | 59131 | 22% | 97.7 | 21.49 | 40781 |
| 29 | Myoviridae sp. | [BK016552.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK016552.1?report=genbank&log$=nucltop&blast_rank=29&RID=TXADWNTA013) | 16460 | 47240 | 24% | 89.23 | 21.42 | 43086 |
| 30 | Myoviridae sp. | [BK016985.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK016985.1?report=genbank&log$=nucltop&blast_rank=30&RID=TXADWNTA013) | 11300 | 11300 | 5% | 90.13 | 4.51 | 12469 |
| 31 | Myoviridae sp. | [BK024305.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK024305.1?report=genbank&log$=nucltop&blast_rank=31&RID=TXADWNTA013) | 7638 | 46483 | 21% | 92.82 | 19.49 | 38377 |
| 32 | Escherichia phage RB49 | [AF203974.1](https://www.ncbi.nlm.nih.gov/nucleotide/AF203974.1?report=genbank&log$=nucltop&blast_rank=32&RID=TXADWNTA013) | 3251 | 3251 | 1% | 98.79 | 0.99 | 1824 |
| 33 | Escherichia phage RB49 | [AJ489481.1](https://www.ncbi.nlm.nih.gov/nucleotide/AJ489481.1?report=genbank&log$=nucltop&blast_rank=33&RID=TXADWNTA013) | 3020 | 3020 | 1% | 97.25 | 0.97 | 1783 |
| 34 | Escherichia phage RB49 | [AB084150.1](https://www.ncbi.nlm.nih.gov/nucleotide/AB084150.1?report=genbank&log$=nucltop&blast_rank=34&RID=TXADWNTA013) | 2900 | 2900 | 1% | 95.73 | 0.96 | 1803 |
| 35 | Escherichia coli | [CP071263.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP071263.1?report=genbank&log$=nucltop&blast_rank=35&RID=TXADWNTA013) | 2765 | 3398 | 1% | 85.4 | 0.85 | 5241795 |
| 36 | Escherichia phage RB49 | [AJ550428.1](https://www.ncbi.nlm.nih.gov/nucleotide/AJ550428.1?report=genbank&log$=nucltop&blast_rank=36&RID=TXADWNTA013) | 2488 | 2488 | 0% | 96.78 | 0.00 | 1491 |
| 37 | Escherichia coli | [CP057368.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP057368.1?report=genbank&log$=nucltop&blast_rank=37&RID=TXADWNTA013) | 2183 | 3209 | 2% | 81.65 | 1.63 | 4743602 |
| 38 | Escherichia coli | [CP028733.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP028733.1?report=genbank&log$=nucltop&blast_rank=38&RID=TXADWNTA013) | 1978 | 2617 | 1% | 80.31 | 0.80 | 4816573 |
| 39 | Escherichia coli | [CP063720.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP063720.1?report=genbank&log$=nucltop&blast_rank=39&RID=TXADWNTA013) | 1930 | 2541 | 1% | 79.99 | 0.80 | 4771390 |
| 40 | Escherichia coli | [CP057861.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP057861.1?report=genbank&log$=nucltop&blast_rank=40&RID=TXADWNTA013) | 1925 | 2585 | 1% | 79.95 | 0.80 | 4899428 |
| 41 | Escherichia coli | [CP057166.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP057166.1?report=genbank&log$=nucltop&blast_rank=41&RID=TXADWNTA013) | 1842 | 2901 | 2% | 79.41 | 1.59 | 4935063 |
| 42 | Escherichia coli | [CP091038.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP091038.1?report=genbank&log$=nucltop&blast_rank=42&RID=TXADWNTA013) | 1646 | 3133 | 2% | 84.75 | 1.70 | 4911746 |
| 43 | Enterobacteria phage MV BS | [DQ485346.1](https://www.ncbi.nlm.nih.gov/nucleotide/DQ485346.1?report=genbank&log$=nucltop&blast_rank=43&RID=TXADWNTA013) | 1290 | 1290 | 0% | 95.76 | 0.00 | 799 |
| 44 | Escherichia coli | [CP024131.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP024131.1?report=genbank&log$=nucltop&blast_rank=44&RID=TXADWNTA013) | 1229 | 2819 | 1% | 83.54 | 0.84 | 5084741 |
| 45 | Escherichia fergusonii | [CP057093.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP057093.1?report=genbank&log$=nucltop&blast_rank=45&RID=TXADWNTA013) | 1201 | 2777 | 1% | 82.99 | 0.83 | 4730551 |
| 46 | Myoviridae sp. | [BK030387.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK030387.1?report=genbank&log$=nucltop&blast_rank=46&RID=TXADWNTA013) | 1184 | 2199 | 1% | 82.03 | 0.82 | 36350 |
| 47 | Escherichia fergusonii | [CP079891.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP079891.1?report=genbank&log$=nucltop&blast_rank=47&RID=TXADWNTA013) | 1109 | 2645 | 2% | 83.67 | 1.67 | 4934492 |
| 48 | Shigella dysenteriae | [CP055055.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP055055.1?report=genbank&log$=nucltop&blast_rank=48&RID=TXADWNTA013) | 1086 | 1086 | 0% | 83.35 | 0.00 | 5075418 |
| 49 | Shigella dysenteriae | [CP055052.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP055052.1?report=genbank&log$=nucltop&blast_rank=49&RID=TXADWNTA013) | 1086 | 1086 | 0% | 83.35 | 0.00 | 5075418 |
| 50 | Myoviridae sp. | [BK043428.1](https://www.ncbi.nlm.nih.gov/nucleotide/BK043428.1?report=genbank&log$=nucltop&blast_rank=50&RID=TXADWNTA013) | 1081 | 1081 | 0% | 83.25 | 0.00 | 36352 |