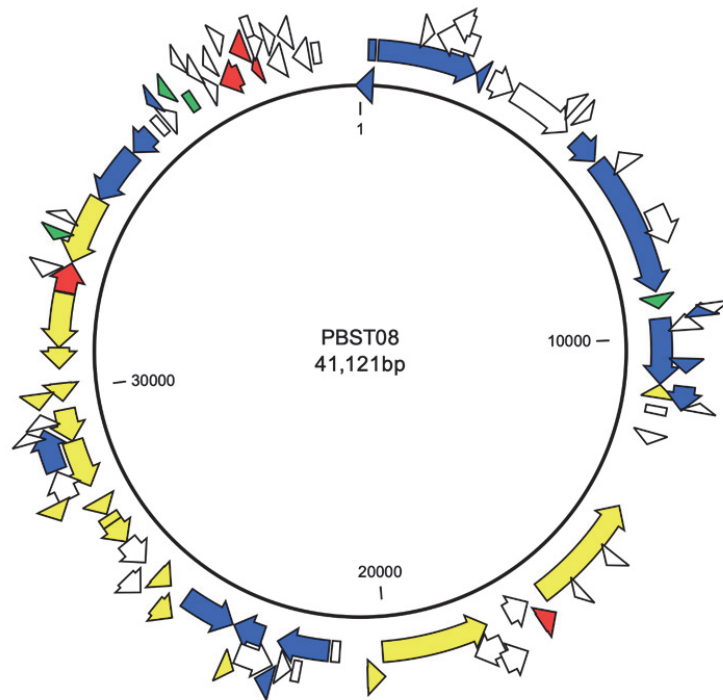


**Table S1. Comparison of genome sequences of phage PBST08 by BLAST analysis.**

| Description   | Query cover | Per. Ident | Accession No. |
|---|-------------|------------|---------------|
| <i>Salmonella</i> phage TS3, complete genome          | 99%         | 98.66%     | MK249126.1    |
| <i>Salmonella</i> phage MA12, complete genome         | 99%         | 98.96%     | KX245013.1    |
| <i>Salmonella</i> phage ST1, partial genome           | 99%         | 98.57%     | MF001366.1    |
| <i>Salmonella</i> phage ST3, partial genome           | 99%         | 98.84%     | MF001364.1    |
| <i>Salmonella</i> phage PIZ SAE-01E2, complete genome | 96%         | 91.61%     | MN336266.1    |
| <i>Salmonella</i> phage vB_SenS_ER22, complete genome | 95%         | 92.28%     | MW355464.1    |
| <i>Salmonella</i> phage vB_SenS_ER23, complete genome | 95%         | 92.49%     | MW355465.1    |
| <i>Salmonella</i> phage vB_SenS_ER21, complete genome | 95%         | 91.39%     | MW355463.1    |
| <i>Salmonella</i> phage f18SE, complete genome        | 94%         | 91.01%     | KR270151.1    |

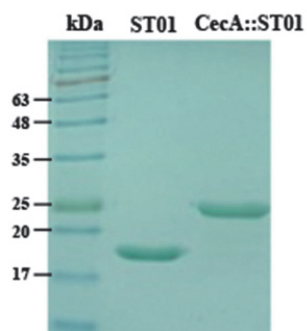


**Figure S1. Open reading frame (ORF) map of PBST08 phage.** Open reading frame (ORF) was analyzed using NCBI BLAST and map was drawn using CLC workbench 20. Each arrow represents a functionally annotated ORF and is colored according to its function. Red, lysis; yellow, structural protein; green, DNA packaging; blue, replication and regulation; white, uncharacterized protein.

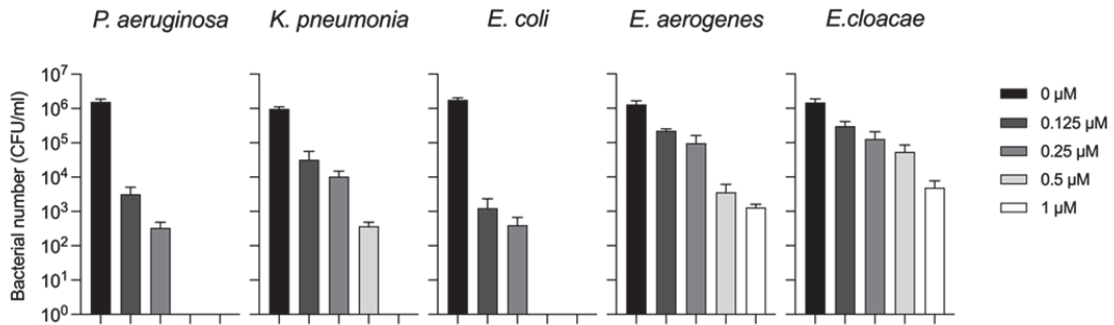
**Table S3. Functional annotation of open reading frames (ORFs) of phage PBST08**

| Predicted function         | ORF number | Description  | Related bacteriophage          | Coverage (%) | Identity (%) |
|----------------------------|------------|--|--------------------------------|--------------|--------------|
| Structural protein         | ORF122     | o-spanin [Salmonella phage PIZ SAE-01E2]                 | Salmonella phage PIZ SAE-01E2  | 95%          | 99.19%       |
|                            | ORF123     | head outer capsid protein [Escherichia phage buks]       | Escherichia phage buks         | 95%          | 96.55%       |
|                            | ORF124     | head-tail joining protein [Salmonella phage STP03]       | Salmonella phage STP03         | 100%         | 98.39%       |
|                            | ORF125     | head-tail joining protein [Salmonella phage sidste]      | Salmonella phage sidste        | 100%         | 94.67%       |
|                            | ORF126     | HK97 gp10 family phage protein [Salmonella enterica]     | Salmonella enterica            | 100%         | 100.00%      |
|                            | ORF132     | tail fiber protein [Salmonella phage TS3]                | Salmonella phage TS3           | 100%         | 99.77%       |
|                            | ORF133     | tailspike protein [Salmonella phage L13]                 | Salmonella phage L13           | 100%         | 99.85%       |
|                            | ORF153     | tail protein [Salmonella phage L13]                      | Salmonella phage L13           | 100%         | 99.34%       |
|                            | ORF154     | o-spanin [Salmonella phage BPS11Q3]                      | Salmonella phage BPS11Q3       | 81%          | 85.06%       |
|                            | ORF155     | scaffold protein [Salmonella phage fmb-p1]               | Salmonella phage fmb-p1        | 99%          | 98.28%       |
|                            | ORF156     | capsid protein [Salmonella phage MA12]                   | Salmonella phage MA12          | 100%         | 100.00%      |
|                            | ORF157     | capsid and scaffold protein [Salmonella phage SE-W109]   | Salmonella phage SE-W109       | 86%          | 97.87%       |
|                            | ORF163     | tail assembly chaperone [Escherichia phage vB_EcoS_XY1]  | Escherichia phage vB_EcoS_XY1  | 100%         | 84.03%       |
|                            | ORF188     | structural protein [Salmonella phage LPSE1]              | Salmonella phage LPSE1         | 100%         | 98.98%       |
|                            | ORF190     | head morphogenesis protein [Salmonella phage 5sent1]     | Salmonella phage 5sent1        | 90%          | 95.80%       |
|                            | ORF193     | tail protein [Salmonella phage sidste]                   | Salmonella phage sidste        | 100%         | 96.40%       |
|                            | ORF194     | tail assembly chaperone [Salmonella phage vB_SenS-EnJE1] | Salmonella phage vB_SenS-EnJE1 | 100%         | 97.83%       |
|                            | ORF196     | tape measure protein [Salmonella phage BPS11Q3]          | Salmonella phage BPS11Q3       | 99%          | 98.20%       |
| Replication and regulation | ORF2       | DNA polymerase [Salmonella phage BPS11Q3]                | Salmonella phage BPS11Q3       | 100%         | 99.52%       |
|                            | ORF3       | DNA polymerase I [Salmonella phage MA12]                 | Salmonella phage MA12          | 100%         | 99.03%       |
|                            | ORF5       | DNA helicase [Salmonella phage SE40]                     | Salmonella phage SE40          | 72%          | 52.86%       |

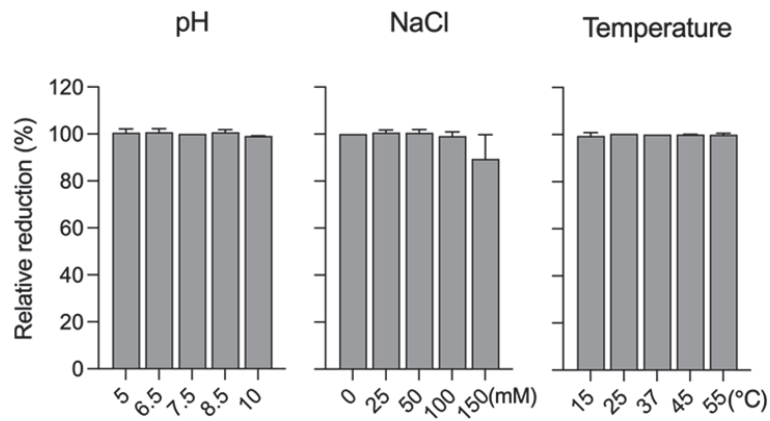
|               |        |  |                               |      |         |
|---------------|--------|--|-------------------------------|------|---------|
|               | ORF34  | replicative DNA helicase/primase [Salmonella phage TS3]          | Salmonella phage TS3          | 100% | 99.86%  |
|               | ORF42  | HNH endonuclease [Salmonella phage L13]                          | Salmonella phage L13          | 100% | 100%    |
|               | ORF53  | phosphodiesterase [Escherichia phage Shashou]                    | Escherichia phage Shashou     | 70%  | 89.33%  |
|               | ORF54  | putative DNA-binding protein [Salmonella phage L13]              | Salmonella phage L13          | 100% | 100%    |
|               | ORF75  | replication protein [Escherichia phage vB_EcoS_XY1]              | Escherichia phage vB_EcoS_XY1 | 100% | 67%     |
|               | ORF82  | DEAD/DEAH box helicase [Salmonella enterica]                     | Salmonella enterica           | 100% | 99%     |
|               | ORF90  | metallophosphoesterase [Salmonella enterica]                     | Salmonella enterica           | 100% | 100%    |
|               | ORF99  | putative helicase [Salmonella phage fmb-p1]                      | Salmonella phage fmb-p1       | 59%  | 92%     |
|               | ORF140 | helix-turn-helix domain-containing protein [Salmonella enterica] | Salmonella enterica           | 100% | 100%    |
|               | ORF151 | DNA-binding transcriptional regulator [Salmonella phage 5sent1]  | Salmonella phage 5sent1       | 96%  | 88%     |
|               | ORF152 | terminase small subunit [Salmonella phage dunkel]                | Salmonella phage dunkel       | 99%  | 99%     |
|               | ORF160 | site-specific recombinase [Salmonella phage ST3]                 | Salmonella phage ST3          | 100% | 99%     |
|               | ORF171 | putative intein containing helicase [Salmonella phage fmb-p1]    | Salmonella phage fmb-p1       | 92%  | 88%     |
|               | ORF180 | DNA-binding protein [Raoultella phage RP180]                     | Raoultella phage RP180        | 33%  | 77%     |
|               | ORF187 | terminase large subunit [Salmonella phage ST3]                   | Salmonella phage ST3          | 100% | 100%    |
| Lysis         | ORF62  | amidase [Salmonella phage ST4]                                   | Salmonella phage ST4          | 100% | 96.76%  |
|               | ORF114 | lysozyme [Salmonella phage vB_SenS_ER21]                         | Salmonella phage vB_SenS_ER21 | 100% | 98.15%  |
|               | ORF147 | putative holin [Salmonella phage Jersey]                         | Salmonella phage Jersey       | 100% | 95.24%  |
|               | ORF164 | peptidoglycan endopeptidase [Escherichia phage phiWAO78-1]       | Escherichia phage phiWAO78-1  | 100% | 76.86%  |
|               | ORF184 | holin class I [Salmonella phage Shelanagig]                      | Salmonella phage Shelanagig   | 85%  | 94.74%  |
| DNA packaging | ORF81  | restriction endonuclease [Salmonella phage BPS11Q3]              | Salmonella phage BPS11Q3      | 100% | 95.79%  |
|               | ORF116 | NinH-like protein [Salmonella virus VSe103]                      | Salmonella virus VSe103       | 100% | 98.65%  |
|               | ORF119 | portal protein [Salmonella phage templet]                        | Salmonella phage templet      | 63%  | 63.27%  |
|               | ORF185 | Nin protein [Salmonella phage ST3]                               | Salmonella phage ST3          | 100% | 100.00% |



**Figure S2. Analysis of purified ST01 and CecA::ST01.** SDS-PAGE analysis of the purified endolysins, which were purified by Fast Protein Liquid Chromatography (FPLC) using His column and SP column.



**Figure S3. Antibacterial activity of CecA::ST01 against gram-negative pathogens.** Antimicrobial activity of CecA::ST01 was tested by CFU reduction assay using *P. aeruginosa* PA01, *A. baumannii* ATCC 17978, *K. pneumonia* KCTC 2208, *E. coli* ATCC 8739, *E. aerogenes* F276, *E. cloacae* ATCC 13047. Bacterial cells at the mid-exponential phase ( $OD_{600}=0.8$ ) were harvested by centrifugation at 15,000 x g for 1 min and adjusted to  $1 \times 10^6$  CFU/ml in 20 mM Tris-HCl pH 7.5 after washing, followed by the treatment with 0, 0.125, 0.25, 0.5, 1  $\mu$ M of CecA::ST01.



**Figure S4. Effect of pH, NaCl, temperature on the antibacterial activity of CecA::ST01.** CFU reduction assay was performed with 0.0625  $\mu$ M of CecA::ST01 using freshly grown *A. baumannii* ATCC 17978 cells in the buffer containing different concentration of NaCl. To determine pH, temperature stability, CecA::ST01 was pre-incubated in the buffer with different pH or at different temperature for 1 h then used for the assay. Relative activity (%) was calculated by comparing the lytic activity under specific condition with the maximal activity (20 mM Tris-HCl, pH 7.5 at 37°C).