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Reviving phage therapy for the treatment of Cholera

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Corresponding Author:	Robert Atterbury University of Nottingham Sutton Bonington, Nottingham UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Nottingham
Corresponding Author's Secondary Institution:	
First Author:	Sudhakar Bhandare
First Author Secondary Information:	
Order of Authors:	Sudhakar Bhandare
	Joan Colom
	Abiyad Baig
	Jennifer M Ritchie
	Habib Bokhari
	Muhammad A Shah
	Banwarilal L Sarkar
	Jingliang Su
	Brendan Wren
	Paul A Barrow
	Robert Atterbury
Order of Authors Secondary Information:	
Manuscript Region of Origin:	UNITED KINGDOM
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Reviving phage therapy for the treatment of Cholera

Sudhakar Bhandare[1¶], Joan Colom[1¶], Abiyad Baig[1], Jenny M Ritchie[2], Habib Bokhari[3], Muhammad A. Shah[3], BL Sarkar[4], Jingliang Su[5], Brendan Wren[6], Paul Barrow[1], Robert J. Atterbury[1]*.

[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK.

[2]Department of Microbial Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK.

[3]Department of Biosciences, COMSATS Institute of Information Technology, Chak shahzad campus, Park Road, Islamabad, Pakistan.

[4]National Institute of Cholera & Enteric Diseases (ICMR), WHO Collaborating Centre for Diarrhoeal Diseases Research & Training, P-33, CIT Road, Scheme XM, KOLKATA -700010, India.

[5]Key Laboratory of Animal Epidemiology and Zoonosis of the Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, China.

[6]London School of Hygiene and Tropical Medicine, Keppel Street, London, UK.

* Corresponding author

E-mail: Robert.atterbury@nottingham.ac.uk

[¶] These authors contributed equally to the study.

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Abstract

Cholera remains a major risk in developing countries, particularly after natural or man-made disasters. *Vibrio cholerae* El Tor is the most important cause of these outbreaks, and is becoming increasingly resistant to antibiotics, so alternative therapies are urgently needed. In this study, a single bacteriophage, Phi_1, was used prophylactically and therapeutically to control cholera in an infant rabbit model. In both cases, phage-treated animals showed no clinical signs of disease, compared with 69% of untreated control animals. Bacterial counts in the intestines of phage-treated animals were reduced by up to 4 Log_{10} CFU/g. There was evidence of phage multiplication only in animals which received a *V. cholerae* challenge. No phage-resistant bacterial mutants were isolated from the animals, despite extensive searching. This is the first evidence that a single phage could be effective in the treatment of cholera, without detectable levels of resistance. Clinical trials in human patients should be considered.

Key words: bacteriophage therapy; cholera; phage; infant rabbit; prophylaxis; Vibrio cholerae

Background

V. cholerae has caused seven cholera pandemics since 1817, leading to significant morbidity and mortality [1]. The first six pandemics (1816 – 1923) were caused by the classical O1 biotype, while the seventh (1961 - present) was caused by the El Tor biotype [1]. The current pandemic affects 3-5 million people per annum, causing 21,000 – 143,000 deaths [1,2]. Cholera is contracted from contaminated food and water in developing countries, where sanitation is generally inadequate or has been damaged by wars or natural disasters; then transmitted from person-to-person [3].

Rehydration therapy reduces mortality and, with antibiotics, can diminish the intensity and duration of clinical signs and faecal shedding [4]. However, the World Health Organisation now advises only severe cases of cholera should be treated with antibiotics due to the spread of

antimicrobial resistance (AMR). Alternative approaches to cholera control are urgently needed, both for treatment of primary infections, and prevention of secondary spread. Biological control using bacteriophage (phage) is one alternative, particularly where antibiotic resistance is a problem [5]. Phage have been used to treat experimental infections in a range of animal models including mice, chickens, cattle, pigs and lambs [6–8].

In this study, we show that a phage vB_VcholP_1 (Phi_1) belonging to the *Podoviridae N4virus* genus was highly effective ($p < 0.001$) in preventing clinical symptoms of *V. cholerae* infection in infant rabbits; the most relevant animal model of cholera in humans. Phage-treatment was accompanied by significant reductions ($p < 0.05$) in *V. cholerae* recovered from several intestinal compartments compared with untreated control animals. Notably, we recovered no phage-resistant mutants. This is the first study showing a single phage can prevent clinical symptoms of cholera infection in this model, with no evidence of resistance development. This study demonstrates that phage could be a viable alternative treatment for cholera in humans, and further research to support the application of phage in clinical trials is warranted.

Methods

Bacteriophage isolation

Phage isolation from lake water samples from several locations in eastern China was performed as described previously [9] using host *V. cholerae* O1 strain 2095. Plaques were serially purified a minimum of five times prior to further use. Additional phage isolates Phi_1, Phi_2 and Phi_3 were obtained from Dr. Tom Cheasty, former Head of the Gastrointestinal Infections Reference Unit, Public Health England (PHE), UK. Phages Phi_24 and Phi_X29 were purchased from the Felix d'Herelle Reference Centre for Bacterial Viruses (HER), Quebec, Canada.

Bacteriophage propagation

Liquid lysates (10 mL) were prepared by inoculating mid-exponential cultures of *V. cholerae* with phage at a multiplicity of infection (MOI) of 0.1 and incubating overnight at 37°C in an orbital shaker at 150 rpm. The lysate was centrifuged (10,000 ×g, 10 min), and filtered (0.45 µm pore-size, Sartorius). Phage titres were determined by plating decimal dilutions of lysates onto duplicate LBA plates using the agar overlay method [10]. The top agar from plates showing semi-confluent lysis was transferred to a 250 mL centrifuge tube, to which was added 5 mL of SM buffer per plate. Phage were eluted by incubating at 4°C overnight with gentle shaking, followed by two rounds of centrifugation (4,000 ×*g*, 10 min, 4°C), filtration (0.45 µm pore-size) and storage at 4°C.

Host range profile

Agar overlays of each of the 89 *V. cholerae* strains (S1_Table) were prepared as described above. Aliquots (10 µL) of each phage (10^8 PFU/mL) were spotted onto the lawns and left to dry. The plates were incubated overnight at 37°C, then scored for lysis as previously reported [11].

One-step growth curve

A mid-exponential-phase culture of *V. cholerae* was infected with a single phage (MOI 0.1). Following phage adsorption, the suspension was diluted in LB broth to a final concentration of 10^4 CFU/mL [9]. Samples (1 mL) of the infected culture were collected at 5 min intervals for 90 min and filtered (0.45 µm pore-size). The phage were enumerated on agar overlays as described above and the burst size was calculated [12].

DNA sequencing, assembly and annotation of phage genome

Phage genomic DNA was extracted using a Wizard DNA Clean-Up system (A7280, Promega). Next generation sequencing was performed by Source Biosciences (Nottingham, UK) and NU-OMICS (Newcastle-upon-Tyne, UK) using the Illumina Miseq platform, 2 × 250-bp paired-end run. The sequence data was assembled *de novo*, and single contigs for the phage were generated using the SPAdes v. 3.1.0 assembler [13] with 120× coverage. The data quality was checked using

FastQC (Babraham Bioinformatics) and reads were quality trimmed. Genome annotation was carried out using RAST [14] and Geneious (V6.1.7, Biomatters) software with some manual curation, which provided the translated sequences of protein-coding regions. These sequences were used to interrogate the NCBI database using BLASTp. Conserved protein motifs were identified using a HHpred search of the Pfam database [15]. In the case of BLASTp, proteins were only assigned to a gene sequence where there was $\geq$ 90% identity with protein motifs in the database. The tRNA annotation was performed using tRNAscan-SE [16] and ARAGORN [17]. Post-annotation, the genome was submitted to GenBank (Table S2). The nucleotide sequence alignments were performed by ClustalW (CLUSTAL 2.1) [18]. The maximum likelihood phylogenetic analysis was performed using the generalized time-reversible (GTR) model with FastTree [19] and the phylogeny was visualised using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree).

Transmission Electron Microscopy (TEM)

High titre phage lysates were purified by ultracentrifugation using a CsCl gradient [20]. A 3 µL sample of CsCl-purified phage was applied to a hydrophilic (glow-discharged) carbon and Pioloform-coated 300 square mesh copper grid (Agar Scientific Ltd). Following adsorption (2 min), excess sample was removed with filter paper. The grid was rinsed twice with 5 µL distilled de-ionised water and the excess was removed before staining with 1% uranyl acetate. Once dry, the grids were observed on a JEOL JEM-1400 TEM with an accelerating voltage of 100 kV. Digital images were recorded using a SIS Megaview III digital camera with iTEM software.

Infant rabbit trials

All experimental protocols involving animals were approved by the local Animal Welfare and Ethical Review Body under UK Home Office project license 70-7495 and performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and EU Directive 2010/63/EU. The infant rabbit cholera model was used to test the effectiveness of phage treatment [21].

123 Time-mated New Zealand White rabbits were obtained from Harlan Laboratories (UK). Following
124 parturition, litters were housed as a group with the lactating doe for the duration of the study.
125 Two hours prior to infection with bacteria, 2-day-old rabbits were pre-treated intraperitoneally
126 with ranitidine (5 mg/kg body weight; GlaxoSmithKline, UK). Oral inoculations of bacteria (0.5
127 mL volume) and/or phage (1 mL volume) were delivered using separate size 5 French catheters
128 (Arrow international, USA). The bacterial inoculum was prepared from stationary phase cultures
129 of pathogenic *V. cholerae* O1 (biotype classical) 1051 Sm^R (from the National Institute of Cholera
130 & Enteric Diseases, Kolkata, India). These cultures were resuspended in a sodium bicarbonate
131 solution (2.5 g in 100 mL, pH 9) with a final concentration of approximately 5×10^8 CFU/animal.
132 Phage Phi_1 was administered either 6 h before or 6 h after bacterial challenge for prophylactic
133 and treatment therapies respectively (Table 1). Phage kinetics in the intestinal tract were studied
134 by dosing rabbits with phage only and collecting samples for analyses at time points
135 corresponding to 24 h post bacterial infection (i.e. at 18 h to mimic treatment and 30 h to mimic
136 prophylaxis).
137
138 Diarrhoea was scored using the following scale: none (no signs of faecal contamination or
139 wetness on their ventral surfaces; upon dissection, the colon contained digesta that appeared
140 normal (dark green, hard and formed)); mild (soft yellow stools and/or limited areas of wetness
141 on the rabbits' fur; upon dissection, digesta was missing from the colon or appeared yellow, soft
142 and unformed; some fluid accumulation in the caecum), and severe (extensive areas of wetness
143 on their tails and ventral surfaces; upon dissection no digesta was found in the colon and the
144 cecum and small intestine contained large quantities of clear fluid). Control and treatment group
145 litters were housed separately to avoid cross-contamination and at least 3 litters were used for
146 each treatment strategy.
147 Animals were anaesthetised 24 h post infection using inhalation isoflurane (Isofol®, Abbott, UK)
148 and euthanized with intracardiac KCl (15% w/v, MercuryPharma, Ireland) at 2.5 mL/100 g body

weight. Tissue segments (1 cm) were collected from the upper (I1), middle (I2) and lower (I3) small intestine and caecal fluid (CF) was collected by gravity. The tissue samples were mechanically homogenised between sterile glass slides in 2 mL sterile phosphate buffered saline (PBS). Caecal fluid accumulation ratios (FAR) were calculated as previously reported [21]. When no CF was collected, caecal content was used instead to report numbers of bacteria. For bacterial enumeration, samples were decimally diluted and triplicate 10 µL aliquots spotted onto TSA containing streptomycin (200 µg/mL). In addition, 100 µL of the original sample, and in some instances, a 5x concentration of this volume, was spread onto the same media to enable lower numbers of cells to be detected. Phage enumeration was performed by spotting 10 µL volumes of filtered (0.45 µm syringe filters) intestinal content on to lawns of the host strain. All plates were incubated at 37°C for 24 h before examining for colonies or plaques.

Phage-resistance

Presumptive *V. cholerae* isolates recovered from phage-treated and control animal groups were confirmed by PCR [22] and streaked on both LB agar plates, with and without supplementation with Phi_1 (1×10^9 PFU/mL). The plates were incubated at 37^{o}C for 24 h before examining for colonies.

Statistical analysis

Rabbit disease scores and caecal FARs were analysed using Fisher's exact test and one-way ANOVA, respectively. All bacterial and phage count data were $\log_{10}$-transformed prior to statistical analysis. Bacterial count data were analysed using the Krushal-Wallis test with Dunn's *post hoc* multi-comparison test (Graphpad Prism, version 5.02). Differences in phage count data were analysed using the two-sample Mann Whitney U test (using Minitab, v. 17.2.1, Pennsylvania, USA).

Results

Phage isolation, morphological characterization and selection for use as a therapeutic

Seven phage were isolated from samples of lake water collected from China. A further five phage were obtained from existing collections. The morphological characteristics of each phage were used to determine a provisional taxonomic classification (Table 2).

The host range and burst size of each phage were determined using a collection of 89 V. cholerae O1, O139 and non-O1/O139 strains (S1 Table) in order to identify candidates best suited for therapeutic application. The three *Myoviridae* phage (Phi_2, Phi_24 and Phi_X29) exhibited much narrower host ranges (1.1 to 4.4%) than *Podoviridae* or *Siphoviridae* phages (Table 1). The different phage families did not cluster according to latent period or burst size.

In addition to exhibiting a broad host range and large burst size, phage therapy candidates should not possess genes associated with virulence or lysogeny. Therefore, we sequenced the phage and examined their genomes for proteins of known function. The Genbank accession numbers for all phage genomes are provided in S2 Table. Sequencing revealed that none of the phage genomes contained known virulence genes. However, all of the phage, exceptPhi_1 and Phi_3, contained integrase sequences, suggesting they may be temperate phage and unsuitable for therapeutic applications. Given that Phi_1 exhibited a slightly broader host range than Phi_3, we focused our efforts on Phi_1 (an electron micrograph of Phi_1 presented in Fig 1). The Phi_1 genome is 66.7 kb and contains 110 genes (S3 Table). Amongst these, 12 were listed as early or middle genes associated with metabolism and replication, 6 could be grouped into the late genes related to head morphogenesis and host cell lysis and the remaining 92 genes encoded hypothetical proteins. BlastN analysis revealed that phage Phi_1 was most closely

related to two N4-like viruses, *Vibrio* phage JA1 (Genebank: NC_021540.1) and VCO139 (Genebank: KC438283.1), with 97 % pairwise identity and similar G+C content (34.5% versus 34.6%). No tRNA sequences were detected in the Phi_1 genome in contrast to a single tRNA in each of JA1 and VCO139. To further resolve the taxonomic placement of Phi_1, phylogenetic analysis was performed comparing the genome sequence of Phi_1 with the available published genome sequences of phage in the genus *N4virus*. Phylogeny showed that Phi_1 grouped with VCO139 and JA-1, with the only classified species of the genus *N4virus*, *Escherichia* phage N4, located in a distant clade (Fig 2). Thus, we have identified a previously undescribed *Podoviridae* N4likevirus with characteristics that are favourable for phage therapy including being effective against a range of clinical *V. cholerae* strains grown under laboratory conditions.

Effectiveness of Phi_1 to control experimental cholera in infant rabbits

To assess whether phage Phi_1 could be used to control experimental cholera, therapeutic and prophylactic studies were performed using the infant rabbit cholera model [23]. For the therapeutic trials, groups of infant rabbits were orally infected with approximately 8×10^8 CFU of Sm^R *V. cholerae* O1 strain 1051 and treated with phage (10^9 PFU) 6 h after infection. Control animals receiving only *V. cholerae* developed signs of disease as reported previously for rabbits infected with *V. cholerae* O1 [23]. Signs included the production of watery diarrhoea, loose stool and/or notable caecal fluid accumulation occurring in the majority (11 of 17) of infected animals (Table 3). In marked contrast, none of the phage-treated animals (0 of 19) showed signs of disease at 24 h post infection. Caecal fluid accumulation ratios (FAR) were 6-fold higher in diseased control animals compared to phage-treated animals (mean ± standard error: 0.39 ± 0.08 versus 0.06 ± 0.01; $p < 0.001$), consistent with the lack of disease.

Furthermore, phage treatment was associated with a significant reduction in the number of *V. cholerae* recovered from the intestine compared to the control group, with no detectable

colonies recovered in more than half the animals (Figs 2A-C). Median reductions of 2-4 Log_{10} CFU/g *V. cholerae* were recorded in different intestinal compartments, including in caecal content (Fig 3D). This, together with the low volumes of fluid evident in the intestine, would lead to a marked reduction in the number of organisms shed from the host.

We also assessed the ability of Phi_1 to be used prophylactically. In these studies, infant rabbits were administered 10^9 PFU phage 6 h prior to infection with approximately 5×10^8 CFU/animal *V. cholerae*. Reflecting the therapeutic trials, phage-treated animals showed no symptoms of disease, and exhibited significant reductions in recoverable *V*. cholerae and intestinal fluid compared with untreated control animals (Table 2 and Figs 2A-D). Overall, these data indicate that Phi_1 is effective at killing *V. cholerae* in several intestinal compartments both prior to and following challenge with virulent *V. cholerae*.

Phage Phi_1 amplifies in the intestine and did not give rise to phage-resistant mutants

When administered 6 h after *V. cholerae* infection, approximately 10^6-10^7 PFU/g of phage were recovered in the intestine of the animals, approximately 100-fold higher than in animals given phage only (range 10^4 – 10^6 PFU/g) (Table 3). Slightly lower levels of phage were recovered during the prophylaxis experiments, most likely reflecting the increased time for transit through the intestine prior to bacterial inoculation (18h and 30h, respectively). However, in both cases, significant amplification of phage was recorded in most intestinal compartments, leading to a multiplicity of infection (MOI) throughout the intestinal tract of about 1-2 phage per *V. cholerae* cell (Table 4). Finally, this data suggests that significant numbers of phage (10^4-10^5 PFU/g) were recoverable from the intestine up to 30 h post administration, even in the absence of *V. cholerae*.

V. cholerae colonies recovered from all the *in vivo* experiments were tested for their susceptibility to phage Phi_1 to determine levels of phage-resistance. Somewhat surprisingly,

247 none of the colonies grew in LB media supplemented with 10^9 PFU of phage Phi_1, indicating
248 that they remained sensitive to the phage. Moreover, attempts to generate phage-resistant
249 mutants *in vitro* using plate-based methods were not successful suggesting that the as-yet-
250 uncharacterised phage Phi_1 receptor is important for *V. cholerae* viability under these
251 conditions.
252

253 ## Discussion

254 Here, we show for the first time, that oral administration of a single *Podoviridae* phage could
255 prevent clinical cholera symptoms in infant rabbits without the development of phage
256 resistance. Our findings provide further evidence that phage can both reduce the severity of
257 disease and limit spread of the organism to the environment. Given the well-documented
258 challenges associated with the emergence of antibiotic-resistant bacteria, phage may yet
259 provide a viable alternative to antibiotics.

260 The strain of *V. cholerae* used has been shown experimentally by this group to result in cholera
261 using the infant rabbit model [21], with fluid accumulation in the small intestine, perianal
262 staining and dehydration resulting in death if humane termination is not carried out. The infant
263 rabbit model combines sensitivity with a greater convenience than other whole animal models
264 such as the ligated intestinal loop model in adult rabbits [24] or mouse models [21].

265 Given that animals receiving only bacteriophage had detectable levels of phage in their
266 intestines for at least 24 h; prophylaxis experiments with a longer interval between phage and
267 bacteria administration would be worth assessing. However, as the rabbits are in an enclosed
268 environment, environmental contamination with phage may occur with the ingestion and re-
269 ingestion of phage from the mother's skin or the bedding.

Camilli and colleagues published a study describing the prophylactic use of a 3-phage (ICP) cocktail to treat cholera [25]. They recorded a marked reduction in disease and V. cholerae recovered from rabbits given the phage cocktail. However, in contrast to the present study, they also recovered phage-resistant mutants. Susceptibility profiling of the *in vivo* passaged *V. cholerae* against the individual phage present in the ICP cocktail revealed that resistance differed depending on the animal host as well as over time. The phage used in the Camilli study were all members of the *Podoviridae*, a genus previously identified as containing phage that make 'better' *in vivo* therapeutic agents [26]. Rational and systematic evaluation of phage characteristics according to morphology, genomics and a number of cultural phenotypes including latent period, burst size and host-range, appears to be critical in the selection of phage as therapeutic agents. Latent period, burst size and the presence of a DNA-dependent RNA polymerase (DdRp) have all been found to correlate with *in vivo* efficacy in controlling experimental *E. coli* infections [26]. Both phage Phi_1 and ICP3 encode a specific RNA polymerase which could improve their effectivity *in vivo*. However, it could also be that phage Phi_1 uses a crucial receptor for *V. cholerae* survival in the intestinal tract, such as the O1 lipopolysaccharide antigen. It is well known that phase variable mutants of O1 receptor are protected from phage infection, but become attenuated [27]. Selecting phage which target surface virulence determinants can be an effective approach, as phage-resistant mutants are often attenuated. In one study, using *E. coli* phage targeting the K1 capsule resulted in the recovery of acapsular but attenuated mutants [8]. The potential development of resistance is a concern if phage are applied in the field, since oral administration to patients may result in extensive shedding of bacteria and phage in the environment, potentially resulting in recirculation of phage-resistant mutants. In some circumstances, this could be avoided by limiting phage administration to cases in clinics and composting the evacuated faeces. Alternatively, the impact of phage recirculation could be minimised by using different phage

preparations that target different receptors, or combinations of receptors, in order to limit the emergence of resistant strains.

Two previous studies of phage therapy to treat cholera in small groups of human patients found either little clinical effect [28], or the requirement for large phage doses (>10^{15} PFU) [29]. However, the phage used were not well-characterised, and some appeared to be temperate and ill-suited for therapy. Additionally, neither study neutralised stomach acid prior to phage administration, which may significantly affect the results. Both studies used phage cocktails which, if combined carefully, may offer some protection against the emergence of resistant mutants. However, the performance of phage cocktails may be no better than individual phages [30], and could be worse. The use of cocktails requires a balance to be struck between the practical limitations of preparing lysates of many different phage, and the need to include sufficient phage to minimise the emergence of resistant mutants. Principally, this should be done through genomic and phenotypic analysis to combine compatible phage which target different receptors.

Characterisation of the interaction of Phi_1 and its receptor(s) may provide some clues as to why phage-resistant mutants were not recovered. Prophylactic and therapeutic trials with Phi_1 need to be performed in human volunteers to determine if this treatment is viable. Should this prove successful, bacteriophage therapy could be deployed relatively easily to remote and underserved communities in developing countries due to the ease and speed with which phage can be prepared, using basic laboratory equipment. Alternatively, preparations of phage can be made using lyophilisation, spray-drying, emulsification and microencapsulation, which remain stable for years (recently reviewed in [31]). Phage therapy has significant potential to save hundreds or thousands of lives during outbreaks of cholera which follow natural and man-made disasters; an aim strongly worth pursuing.

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Figure 1. Electron micrograph of *Podoviridae* phage Phi_1.

Figure 2. The maximum likelihood phylogenetic comparison of vB_VcholP_1 with the published genome sequences of phage species from genus N4virus. The maximum likelihood phylogenetic analysis was carried out using the generalized time-reversible (GTR) model with FastTree and the phylogeny was visualised using FigTree.

Figure 3. Efficacy of phage Phi-1 in reducing *V. cholerae* O1 colonisation of the infant rabbit intestine. Rabbits were administered 1×10^9 PFU phage Phi-1 orally, 6 h pre- or post- infection with $5 – 8 \times 10^8$ CFU *V. cholerae* O1. Viable *V. cholerae* were recovered from the upper (A), mid (B) and distal (C) small intestine and in caecal fluid (D) at 24 h post bacterial infection following tissue homogenisation and plating on selective media. Symbols represent individual animals, with open symbols representing samples where the number of recoverable colonies was below the limit of detection. The number of animals in each group was 17, 22 and 19 respectively, and each group was derived from 3 independent litters. Bars represent the median and interquartile

419 range. Data were compared using the Kruskal Wallis test followed by Dunn's post hoc multiple
420 comparisons test.

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Footnotes

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Conflict of interests: The authors declare that they have no conflicts of interest.

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Corresponding author contact information:

Dr. Robert Atterbury

School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK.

Email: robert.atterbury@nottingham.ac.uk

Supporting information

S1 Table. Data regarding the origin, serotype/biotype, geographical source, year of isolation and sample type of the Vibrio cholerae strains used in this project. The sensitivity of each strain to the phage used in the therapeutic trials (Phi_1) is given as '+' (sensitive) or '-' (not sensitive).

S2 Table. Phage genomes accession numbers.

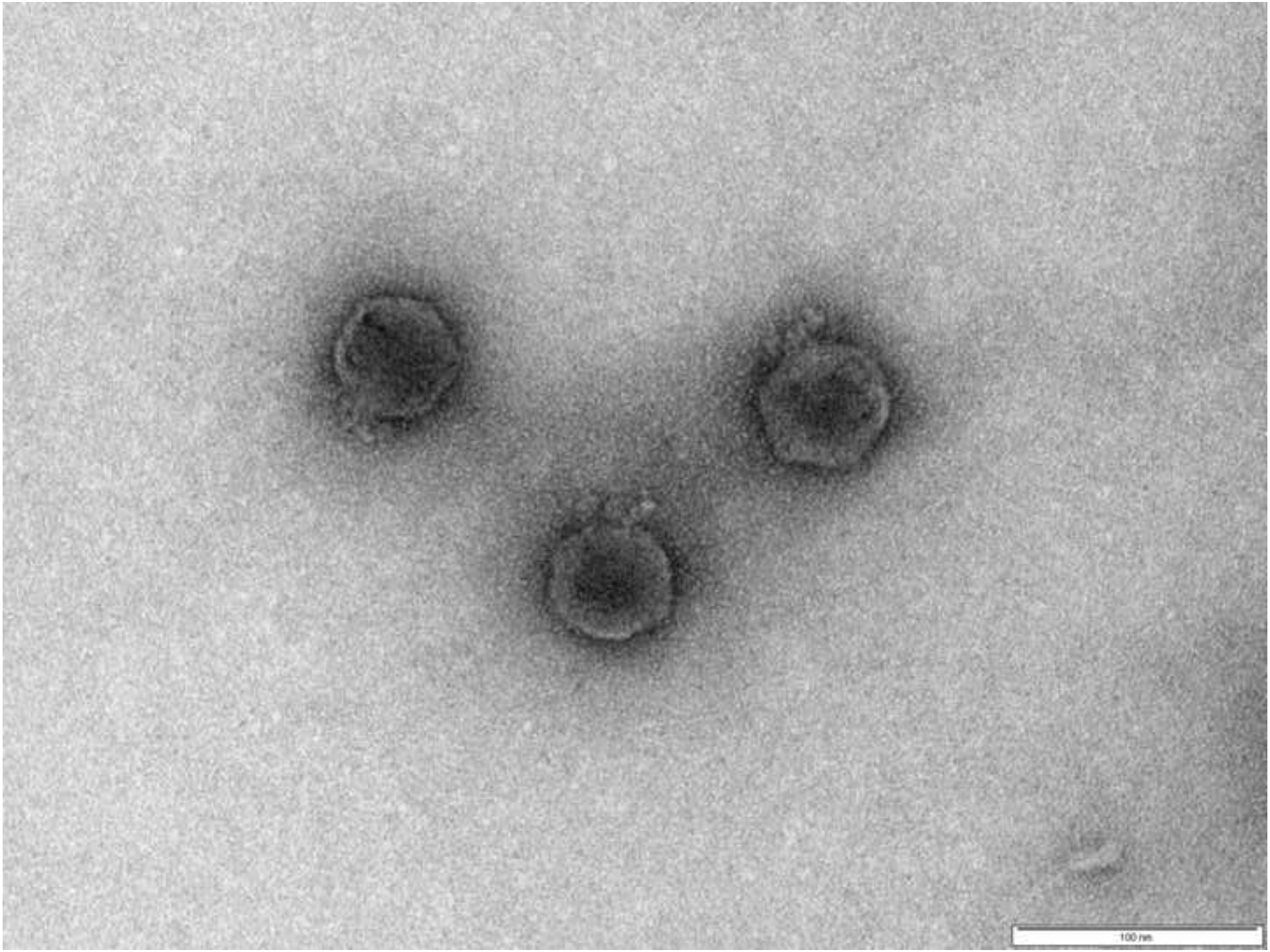
444 S3 Table. The conserved coding sequences (CDSs) and functional annotation of V. cholerae
445 phage Phi_1 genome.

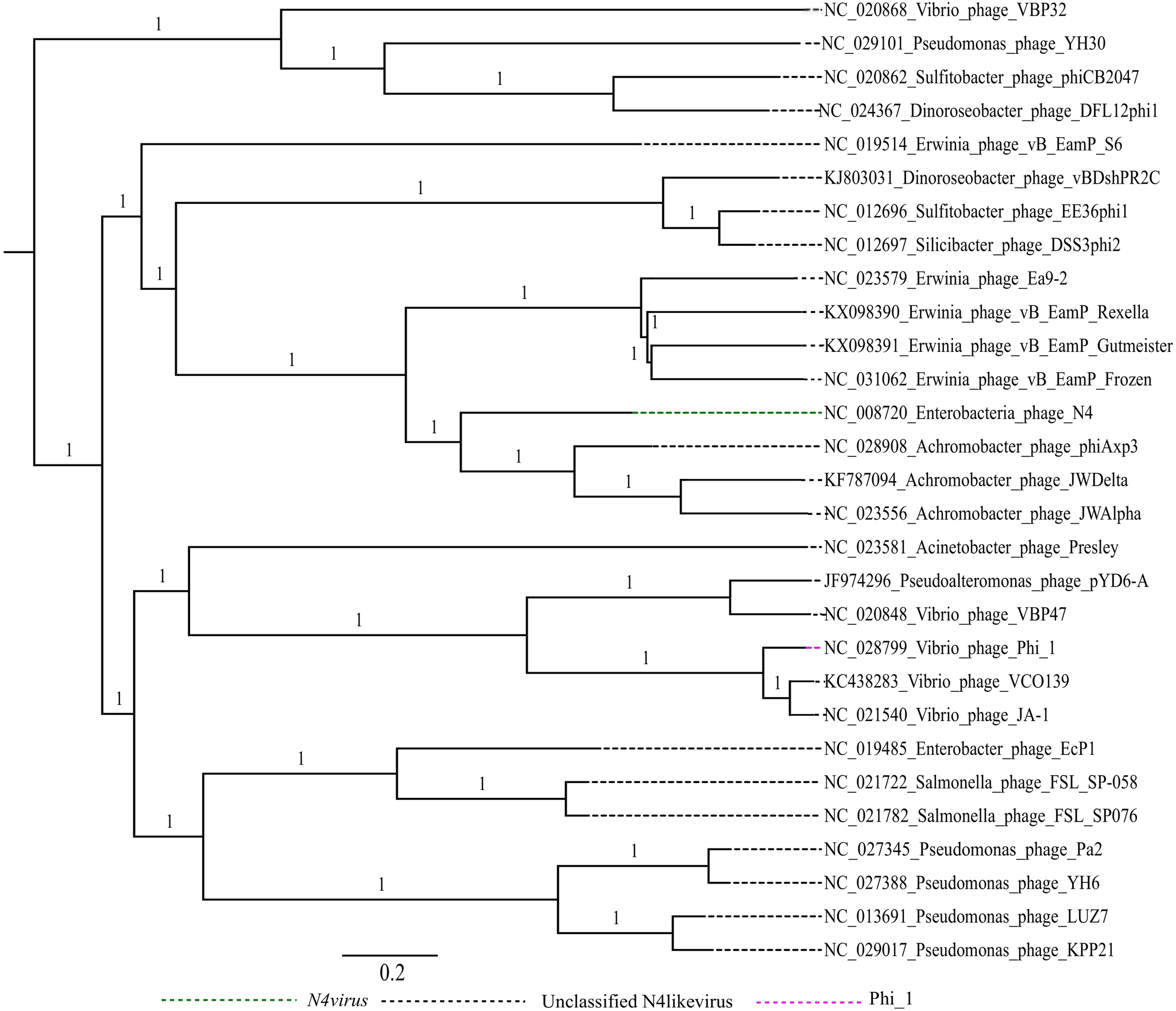
446

447

Figure 1

Click here to access/download;Figure;Fig_1.tif





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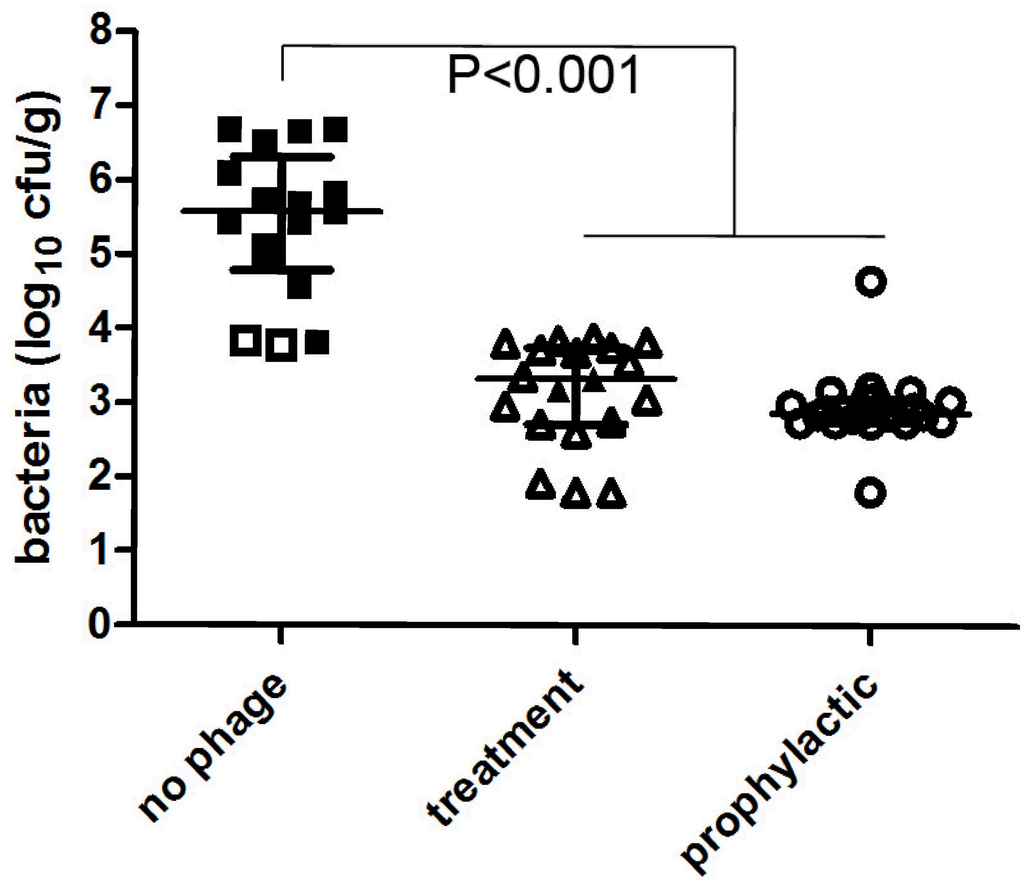

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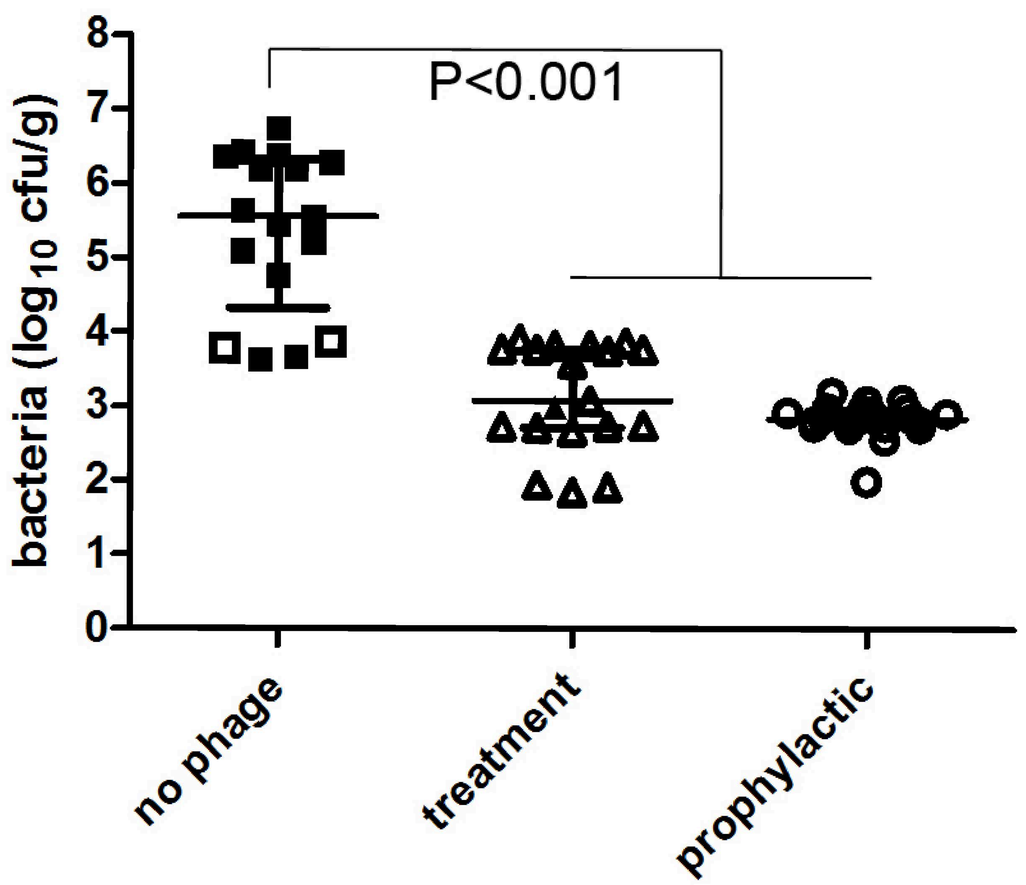

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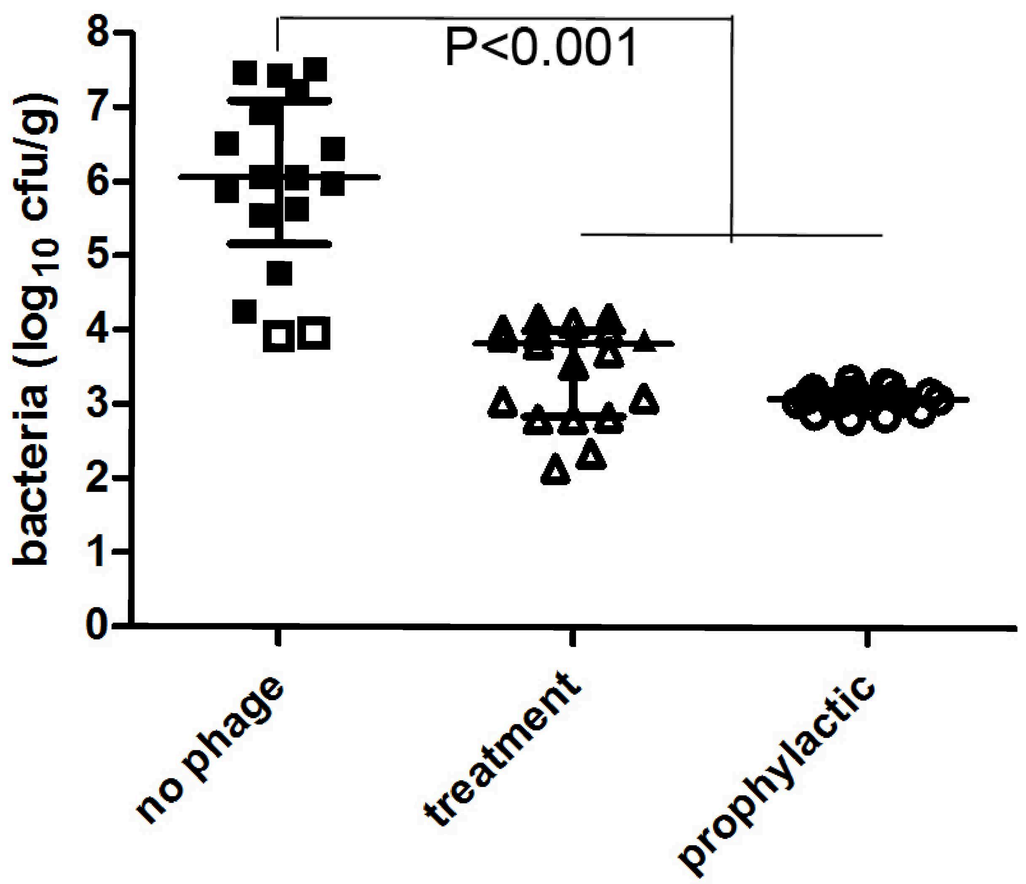

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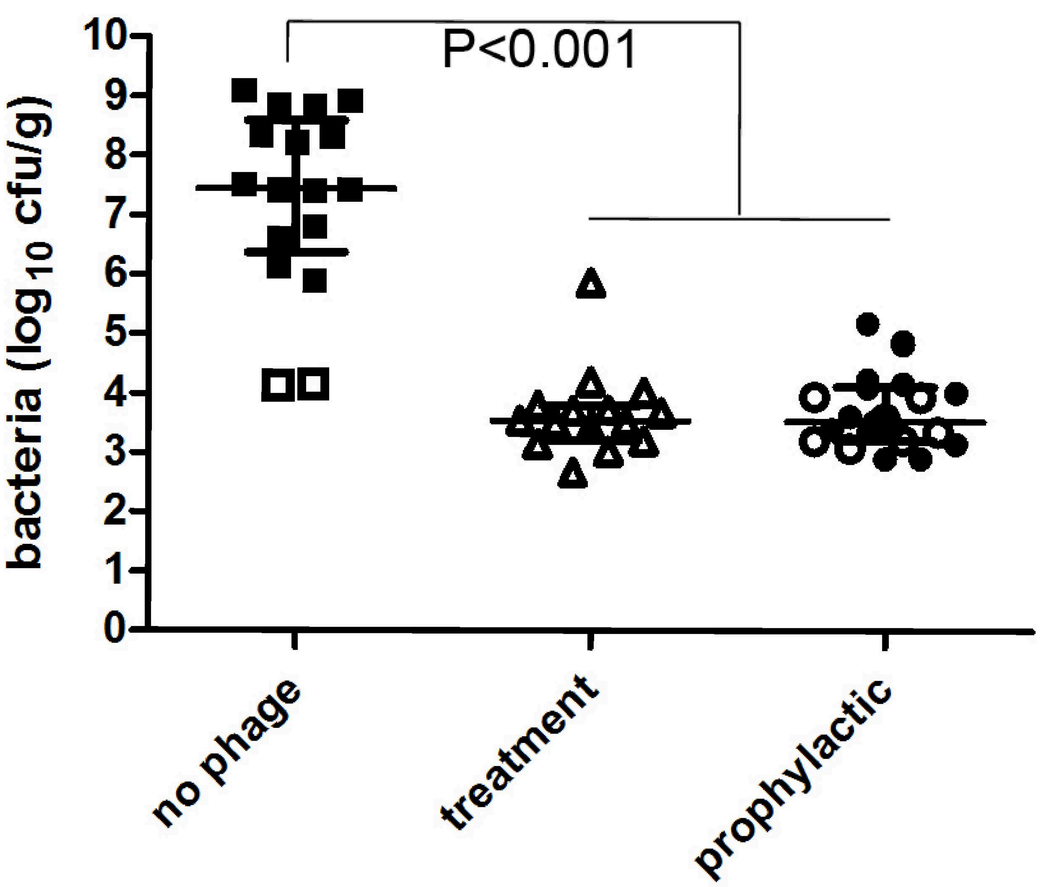

Table 1

Table 1. Litter size, bacterial and phage dose and treatment inoculation schedule of rabbit experiments.

Group	Number of animals	*V. cholerae* inoculum (CFU/animal)	Phage inoculum (PFU/animal)	Treatment schedule
Control 1	6	7×10^8	-	-
Control 2	5	2×10^8	-	
Control 4	7	6×10^8	-	
Therapeutic 1	10	1×10^9	1×10^9	6-8 h post-bacterial infection
Therapeutic 2	6	4×10^8	1×10^9	
Therapeutic 3	4	1×10^9	1×10^9	
Prophylactic 1	10	5×10^8	1×10^9	6 h prior-bacterial infection
Prophylactic 2	6	5×10^8	1×10^9	
Prophylactic 3	6	8×10^8	1×10^9	
Therapeutic control	8	-	1×10^9	6-8 h post-bacterial infection
Prophylactic control	6	-	1×10^9	6 h prior-bacterial infection

Table 2

Table 2. Bacteriophage source and characterization including host range and one-step growth curve.

Phage full name	Short name	Phage source	Genus	Head diameter (nm) [a]	Tail length (nm) [a]	Host range (%)	Latent period (min) [a]	Burst size (PFU) [a]
vB_VcholP_QH	Phi_QH	Qing He river (Beijing)	*Podoviridae*	51 ± 0.1	12 ± 0.0	84.6	12 ± 4.0	92 ± 09
vB_VcholP_CJY	Phi_CJY	Cui Jia Yao river (Beijing)	*Podoviridae*	54 ± 0.1	10 ± 0.0	16.5	13 ± 4.3	182 ± 62
vB_VcholP_H1	Phi_H1	Fu Jia Wan lake (Hubei)	*Podoviridae*	56 ± 0.1	11 ± 0.0	37.3	6 ± 2.3	89 ± 32
vB_VcholP_H2	Phi_H2	Ye Zhi Hu lake (Hubei)	*Podoviridae*	57 ± 0.1	12 ± 0.0	18.7	15 ± 1.6	63 ± 13
vB_VcholP_H3	Phi_H3	Nan Hu lake (Hubei)	*Podoviridae*	55 ± 0.1	12 ± 0.0	70.3	7 ± 3.5	126 ± 18
vB_VcholP_J2	Phi_J2	Yudai He river (Jiangxi)	*Podoviridae*	54 ± 0.1	12 ± 0.0	16.5	5 ± 0.6	34 ± 13
vB_VcholP_J3	Phi_J3	Yudai He river (Jiangxi)	*Podoviridae*	52 ± 0.1	11 ± 0.0	76.9	14 ± 1.5	56 ± 17
vB_VcholP_1	Phi_1	PHE	*Podoviridae*	34 ± 0.2	13 ± 0.1	67.0	12 ± 0.0	43 ± 05
vB_VcholM_2	Phi_2	PHE	*Myoviridae*	53 ± 0.2	118 ± 0.4	4.4	14 ± 1.6	6 ± 01
vB_VcholS_3	Phi_3	PHE	*Siphoviridae*	75 ± 0.1	156 ± 0.2	62.6	13 ± 4.1	54 ± 26
vB_VcholM_24	Phi_24	HER	*Myoviridae*	64 ± 0.1	69 ± 0.1	1.1	4 ± 0.0	87 ± 26

vB_VcholM_X29	Phi_X29	HER	*Myoviridae*	64 ± 0.1	95 ± 0.3	2.2	16 ± 0.0	77 ± 16

[a]Mean of three independent measurements ± Standard Error.

Public Health England (PHE), Felix d'Herelle Reference Centre for Bacterial Viruses (HER).

Table 3

Table 3. Disease status and fluid accumulation ratios (FAR) in infant rabbits treated with phage Phi_1 pre- and post- infection with *V. cholerae* O1.

Treatment		None	Phage administration[a]	
			Therapeutic	Prophylactic
Disease (%)		69	0[c]	0[c]
Disease score[b]				
	Severe	1	0	0
	Mild	10	0	0
	None	6	19	22
Total no. animals		17	19	22
FAR[d]		0.39 ± 0.31	0.06 ± 0.05[d]	0.04 ± 0.02[d]

[a]Phage Phi_1 was orally administered 6 h before (prophylactic) or 6 h after (therapeutic) the bacteria.

[b]Number of rabbits with disease as described in the text.

[c]Fisher's exact test was used to compare the disease in animals given phage Phi-1, pre- or post-infection with *V. cholerae* O1. $p < 0.001$.

[d]Fluid accumulation ratio (FAR) is calculated from the weight of the caecal fluid to the tissue for each animal.

[e]One way ANOVA followed by Dunnett's multiple comparison test was used to analyse the values. $p < 0.001$.

Table 4

Table 4. Bacteriophage concentration, production and MOI during prophylactic and therapeutic treatments.

Sample[e]	Therapeutic treatment phage concentration (Log_{10} PFU/g)[a]				Prophylactic treatment phage concentration (Log_{10} PFU/g)[a]			
	Control[†]	Treatment	Phage production	MOI	Control[‡]	Treatment	Phage production	MOI
I1	4.2 ± 1.8	6.4 ± 0.3[d]	2.2	1.9	4.7 ± 0.4	5.1 ± 0.2	0.4	1,8
I2	5.3 ± 0.9	6.6 ± 0.2[c]	1.3	2.0	4.6 ± 0.3	5.1 ± 0.1	0.5	1,8
I3	4.8 ± 0.8	6.8 ± 0.3[d]	2.0	1.7	5.0 ± 0.6	5.7 ± 0.2[b]	0.7	1,8
MC	6.6 ± 0.5	7.6 ± 0.7[c]	1.0	2.0	5.7 ± 0.6	6.7 ± 0.2[c]	0.9	2,1
CF	6.1 ± 2.5	7.8 ± 0.3[b]	1.7	2.3	5.4 ± 0.7	7.1 ± 0.1[d]	1.7	2,0

[a]Mean concentration ± Standard error. Phage Phi_1 was orally administered following the therapeutic or prophylactic schedule. Prophylaxis, control n=6, treatment n=22. Therapeutic control n=8, treatment n=18. Mann Whitney U test was used to compare values between groups. [b] ($p < 0.05$), [c] ($p < 0.01$) and [d] ($p < 0.001$).

[e]Values from Upper small intestine (I1), mid small intestine (I2), low small intestine (I3), mid colon (MC) and caecal fluid (CF) are shown.

[†]Animals given only *Vibrio cholerae* 1051.

[‡]Animals given only phage Phi_1.

Supplementary Table 1

Strain	Biotype	Collection	Source	Year of Isolation	Sample Type	Phi_1
10	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Sewage	-
238	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Khewra, Punjab - Pakistan	2011	Salt mine	-
404	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Unknown	-
406	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Unknown	-
709	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
719	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
722	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
729	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
732	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
736	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
739	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	-
742	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Murree, Punjab - Pakistan	2011	Clinical	+
750	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Chamman, Baluchistan - Pakistan	2011	Clinical	-
751	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Larkana, Sindh - Pakistan	2011	Clinical	+
752	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Muzaffargarh, Punjab - Pakistan	2011	Clinical	+
753	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Zoab, Baluchistan - Pakistan	2011	Clinical	+
754	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Badin, Sindh - Pakistan	2011	Clinical	+
755	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rahimyar Khan, Punjab - Pakistan	2011	Clinical	+
756	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Dadu, Sindh - Pakistan	2011	Clinical	+
757	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Bahawalnagar, Punjab - Pakistan	2011	Clinical	+
758	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Sanghar, Sindh - Pakistan	2011	Clinical	+
759	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Larkana, Sindh - Pakistan	2011	Clinical	+
760	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Larkana, Sindh - Pakistan	2011	Clinical	-
761	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Qasoor, Punjab - Pakistan	2011	Clinical	+
762	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Muzaffarabad, Azad Jammu and Kashmir - Pakistan	2011	Clinical	+
763	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Sukkur, Sindh - Pakistan	2011	Clinical	-
764	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Clinical	+
765	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Chakwal, Punjab - Pakistan	2011	Clinical	-
767	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Clinical	+
768	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Clinical	+
769	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Peshawar, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
770	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Swabi, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
771	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Multan, Punjab - Pakistan	2011	Clinical	+
772	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Multan, Punjab - Pakistan	2011	Clinical	+
773	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Sialkot, Punjab - Pakistan	2011	Clinical	+
774	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Clinical	+
775	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Kamalia, Punjab- Pakistan	2011	Clinical	-
776	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Okara, Punjab - Pakistan	2011	Clinical	-
1051	Vibrio cholerae O1 Classical	Felix d'Herelle reference center for bacterial virues	National Institute of Cholera and Enteric Diseases, Kolkata - India	1979	Clinical	+
2095	Vibrio cholerae O1 El Tor Inaba	Dr. Jingliang Su, CAU, China	Beijing, China	2011	Clinical	-
A-4	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Attock, Punjab - Pakistan	2011	Clinical	+
BW-5	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Chiniot, Punjab - Pakistan	2011	Clinical	+
CS-1	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Charsada, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
CS-12	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Charsada, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
CS-15	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Charsada, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
CS-16	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Charsada, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
CS-18	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Charsada, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
CW-1	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Bahawalpur, Punjab - Pakistan	2011	Fish	-
D-1	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	-
D-13	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	-
D-25	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	+
D-30	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	+
D-56	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	+
D-59	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	D I Khan, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	+
DN-4	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2010	Clinical	+
F-5	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Clinical	+
F-6	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Clinical	+
FB-O1	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Drinking water	-
FN-2	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Sewage	-
FN-4	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Sewage	-
FN-5	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Clinical	-
GB-39	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Gilgit, Gilgit Baltistan - Pakistan	2011	Clinical	+
HH-1	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Hyderabad, Sindh - Pakistan	2010	Clinical	+
HH-14	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Hyderabad, Sindh - Pakistan	2010	Clinical	+
HH-15	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Hyderabad, Sindh - Pakistan	2010	Clinical	+
HH-4	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Hyderabad, Sindh - Pakistan	2010	Clinical	+
Ht-10	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Drinking water	-
Ht-10A	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Drinking water	-
J-1	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Islamabad, Federal Capital - Pakistan	2011	Sewage	-
KCH-18	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Karachi, Sindh - Pakistan	2010	Clinical	+
KPD-3	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Khairpur, Sindh - Pakistan	2010	Clinical	+
KtH-4	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Jamshoro, Sindh - Pakistan	2010	Clinical	+
KTH-7	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Jamshoro, Sindh - Pakistan	2010	Clinical	+
M14	Vibrio cholerae O1 El Tor Inaba	Neil Williams, University of Bristol	Unknown	Unknown	Clinical	+
N-10	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	-
N-5	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
N-7	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
NP-14	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	-
NP-3	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
NP-5	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
NP-6	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	-
NP-7	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Nowshera, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
O395NT	Vibrio cholerae O1 El Tor Ogawa	Neil Williams, University of Bristol	Unknown	Unknown	Unknown	+
O5	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2011	Sewage	-
P-1	Vibrio cholerae non-O1/non-O139	Professor Brendan Wren and Muhammad Ali, LSHTM	Peshawar, Khyber Pakhtunkhwa - Pakistan	2011	Sewage	-
PS-18	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Peshawar, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	-
PS-25	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Peshawar, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
PS-7	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Peshawar, Khyber Pakhtunkhwa - Pakistan	2011	Clinical	+
RG-6	Vibrio cholerae O1 El Tor Ogawa	Professor Brendan Wren and Muhammad Ali, LSHTM	Rawalpindi, Punjab - Pakistan	2010	Clinical	+

Supplementary Table 2

***V. cholerae* phage**	**GenBank accession number**
vB_VcholP_QH	KM612259
vB_VcholP_CJY	KM612260
vB_VcholP_H1	KM612261
vB_VcholP_H2	KM612262
vB_VcholP_H3	KM612263
vB_VcholP_J2	KM612264
vB_VcholP_J3	KM612265
vB_VcholP_1	KP280062
vB_VcholM_2	KJ545483.2
vB_VcholS_3	KP280063
vB_VcholM_24	KJ572844.2

Supplementary Table 3

Locus tag	Function	Start:End of CDS	Orientation	Size (bp)
AVV30_gp001	hypothetical protein	183:389	forward	207
AVV30_gp002	hypothetical protein	389:601	forward	213
AVV30_gp003	hypothetical protein	598:990	forward	393
AVV30_gp004	hypothetical protein	1071:1235	forward	165
AVV30_gp005	hypothetical protein	1373:1624	forward	252
AVV30_gp006	hypothetical protein	1661:1903	forward	243
AVV30_gp007	hypothetical protein	1900:2121	forward	222
AVV30_gp008	hypothetical protein	2172:2309	forward	138
AVV30_gp009	hypothetical protein	2372:2629	forward	258
AVV30_gp010	hypothetical protein	2801:2986	forward	186
AVV30_gp011	hypothetical protein	3100:3333	forward	234
AVV30_gp012	hypothetical protein	3389:3655	forward	267
AVV30_gp013	hypothetical protein	3658:3897	forward	240
AVV30_gp014	hypothetical protein	3902:4141	forward	240
AVV30_gp015	hypothetical protein	4202:4408	forward	207
AVV30_gp016	DNA-directed RNA polymerase RNAP1	4427:5368	forward	942
AVV30_gp017	hypothetical protein	5368:5583	forward	216
AVV30_gp018	hypothetical protein	5602:6051	forward	450
AVV30_gp019	hypothetical protein	6193:6750	forward	558
AVV30_gp020	DNA-directed RNA polymerase RNAP2	6918:7703	forward	786
AVV30_gp021	hypothetical protein	7755:7937	forward	183
AVV30_gp022	hypothetical protein	7938:8147	forward	210
AVV30_gp023	hypothetical protein	8180:8368	forward	189
AVV30_gp024	hypothetical protein	8382:8585	forward	204
AVV30_gp025	hypothetical protein	8590:8718	forward	129
AVV30_gp026	hypothetical protein	8727:8972	forward	246
AVV30_gp027	hypothetical protein	8945:9217	forward	273

AVV30_gp028	N-acetylmuramoyl-L-alanine amidase	9203:9727	reverse	525
AVV30_gp029	hypothetical protein	9751:10047	forward	297
AVV30_gp030	hypothetical protein	10049:10246	forward	198
AVV30_gp031	hypothetical protein	10248:10409	forward	162
AVV30_gp032	hypothetical protein	10411:10725	forward	315
AVV30_gp033	PF10947 family protein	10735:11154	forward	420
AVV30_gp034	putative lipoprotein	11151:11453	forward	303
AVV30_gp035	hypothetical protein	11450:11644	forward	195
AVV30_gp036	thymidylate synthase	11641:12492	forward	852
AVV30_gp037	hypothetical protein	12502:12678	forward	177
AVV30_gp038	hypothetical protein	12688:12810	forward	123
AVV30_gp039	hypothetical protein	12905:13105	forward	201
AVV30_gp040	hypothetical protein	13112:13327	forward	216
AVV30_gp041	hypothetical protein	13634:13960	forward	327
AVV30_gp042	hypothetical protein	14631:14792	forward	162
AVV30_gp043	hypothetical protein	14812:15123	forward	312
AVV30_gp044	hypothetical protein	15125:15601	forward	477
AVV30_gp045	hypothetical protein	15914:16054	forward	141
AVV30_gp046	hypothetical protein	16101:16556	forward	456
AVV30_gp047	PF11753 family protein	16553:16846	forward	294
AVV30_gp048	hypothetical protein	16849:17031	forward	183
AVV30_gp049	hypothetical protein	17045:17683	forward	639
AVV30_gp050	hypothetical protein	17744:18199	forward	456
AVV30_gp051	hypothetical protein	18827:19027	forward	201
AVV30_gp052	hypothetical protein	19129:19728	forward	600
AVV30_gp053	hypothetical protein	19959:20843	forward	885
AVV30_gp054	metallopeptidase domain protein	20900:22126	forward	1227
AVV30_gp055	hypothetical protein	22178:22372	forward	195

AVV30_gp056	hypothetical protein	22344:22535	forward	192
AVV30_gp057	hypothetical protein	22522:22701	forward	180
AVV30_gp058	DNA helicase	22698:23858	forward	1161
AVV30_gp059	hypothetical protein	23860:24309	forward	450
AVV30_gp060	DNA polymerase	24424:25992	forward	1569
AVV30_gp061	hypothetical protein	26109:26663	forward	555
AVV30_gp062	putative HNH homing endonuclease	27085:27618	forward	534
AVV30_gp063	DNA polymerase	27878:28735	forward	858
AVV30_gp064	hypothetical protein	28811:29419	forward	609
AVV30_gp065	phosphoribosyl-ATP diphosphatase	29469:29918	forward	450
AVV30_gp066	hypothetical protein	29918:30931	forward	1014
AVV30_gp067	hypothetical protein	30939:33104	forward	2166
AVV30_gp068	hypothetical protein	33108:33383	forward	276
AVV30_gp069	AAA domain protein	33399:34136	forward	738
AVV30_gp070	ssDNA binding protein	34198:34920	forward	723
AVV30_gp071	crossover junction endodeoxyribonuclease RusA	34920:35717	forward	798
AVV30_gp072	hypothetical protein	35717:35914	forward	198
AVV30_gp073	hypothetical protein	35945:36148	forward	204
AVV30_gp074	viron-encapsulated RNA polymerase	36190:45912	reverse	9723
AVV30_gp075	hypothetical protein	45912:47180	reverse	1269
AVV30_gp076	hypothetical protein	47183:47590	reverse	408
AVV30_gp077	hypothetical protein	47600:49804	reverse	2205
AVV30_gp078	hypothetical protein	49827:50303	reverse	477
AVV30_gp079	hypothetical protein	50306:50890	reverse	585
AVV30_gp080	major capsid protein	50947:52260	reverse	1314
AVV30_gp081	hypothetical protein	52273:53367	reverse	1095
AVV30_gp082	hypothetical protein	53371:53682	reverse	312

AVV30_gp083	portal protein	53682:55799	reverse	2118
AVV30_gp084	hypothetical protein	55869:56156	forward	288
AVV30_gp085	hypothetical protein	56226:57311	reverse	1086
AVV30_gp086	hypothetical protein	57313:58980	reverse	1668
AVV30_gp087	hypothetical protein	58977:59663	reverse	687
AVV30_gp088	terminase large subunit	59671:61269	reverse	1599
AVV30_gp089	hypothetical protein	61262:61939	reverse	678
AVV30_gp090	hypothetical protein	61990:62178	forward	189
AVV30_gp091	hypothetical protein	62179:62310	forward	132
AVV30_gp092	hypothetical protein	62297:62434	forward	138
AVV30_gp093	hypothetical protein	62421:62564	forward	144
AVV30_gp094	hypothetical protein	62545:62679	forward	135
AVV30_gp095	hypothetical protein	62666:62779	forward	114
AVV30_gp096	hypothetical protein	62779:62895	forward	117
AVV30_gp097	hypothetical protein	63083:63274	forward	192
AVV30_gp098	hypothetical protein	63261:63386	forward	126
AVV30_gp099	hypothetical protein	63453:63611	forward	159
AVV30_gp100	hypothetical protein	63694:63870	forward	177
AVV30_gp101	hypothetical protein	63867:63998	forward	132
AVV30_gp102	hypothetical protein	63985:64116	forward	132
AVV30_gp103	hypothetical protein	64332:64472	forward	141
AVV30_gp104	hypothetical protein	64459:64599	forward	141
AVV30_gp105	hypothetical protein	64596:64820	forward	225
AVV30_gp106	hypothetical protein	64811:65005	forward	195
AVV30_gp107	hypothetical protein	64996:65208	forward	213
AVV30_gp108	hypothetical protein	65199:65384	forward	186
AVV30_gp109	hypothetical protein	65581:65802	forward	222
AVV30_gp110	hypothetical protein	65896:66393	reverse	498