Isolation of a bacteriophage specific for CS7-expressing strains of enterotoxigenic *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC) is the most common bacterial cause of childhood diarrhoea in Bangladesh. Among the virulence factors of ETEC, toxins and colonization factors (CFs) play a major role in pathogenesis. Unlike *Vibrio cholerae*, the relationship between ETEC and ETEC-specific phages is poorly understood and the possible role of ETEC phages in the evolution of ETEC strains in the environment is yet to be established. This study was designed specifically to isolate phages that are specific for ETEC virulence factors. Among the 49 phages isolated from 12 different surface water samples, 13 were tested against 211 ETEC strains collected from clinical and environmental sources. One phage, designated IMM-001, showed a significant specificity towards CS7 CF as it attacked all the CS7-expressing ETEC. Electron microscopic analyses showed that the isolated phage possessed an isomeric hexagonal head and a long filamentous tail. An antibody blocking method and phage neutralization assay confirmed that CS7 pilus is required for the phage infection process, indicating the role of CS7 fimbrial protein as a potential receptor for IMM-001. In summary, this study showed the presence of a lytic phage in environmental water that is specific for the CS7 CF of ETEC.

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INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is among the most common of the seven diarrhoeagenic *E. coli* (Clarke, 2001). It is the leading cause of diarrhoea mainly affecting children but also travellers (Black, 1993; Qadri *et al.*, 2005, 2007). Pathogenesis of ETEC strains is mediated by two major virulence factors, enterotoxins and colonization factor antigen (CFA). The ability of ETEC to adhere to the intestinal epithelium of the host is an important virulence determinant, and adhesion is mediated by surface appendages known as colonization factors (CFs) (Qadri *et al.*, 2007). After colonization, ETEC produces one or two enterotoxins called heat-labile toxin (LT) and heat-stable toxin (STp or STh). CFs are mainly fimbrial or fibrillar proteins which mediate adherence to the small intestinal

mucosa, and to date over 25 different CFs have been described for human ETEC (Qadri *et al.*, 2005).

The relationship between the local incidence of cholera, the prevalence of *Vibrio cholerae* in the aquatic environment and cholera phages has been well investigated (Faruque *et al.*, 2005b). In Bangladesh, epidemics caused by ETEC and *V. cholerae* O1 share similar virulence attributes, as well as biannual seasonality (Harris *et al.*, 2008; Qadri *et al.*, 2000a). Phages have been found to play a role in the perpetuation of *V. cholerae* infections in endemic settings (Faruque *et al.*, 2005a). Relatively less information is available about the spread and transmission of ETEC in the environment and the role of bacteriophages in the propagation and spread of the pathogen (Chibani-Chennoufi *et al.*, 2004). Phage typing has been used for detecting and typing LT-positive *E. coli* strains (Jothikumar *et al.*, 2000; Monsur *et al.*, 1989) and may be important for determining an epidemiological link between the prevalence of ETEC in diarrhoeal patients and its spread in the environment; it may also be useful for identifying

Abbreviations: CF, colonization factor; CFA, colonization factor antigen; ETEC, enterotoxigenic *Escherichia coli*; LT, heat-labile toxin; ST, heat-stable toxin.

candidates for phage therapy for clinical work. Although studies have shown that *E. coli* phages can be specific for *E. coli* serogroups (Groisman & Ochman, 1997; Jann *et al.*, 1971; Wallenfels & Jann, 1974) or for the capsular polysaccharide antigen of *E. coli* (Gross *et al.*, 1977; Gupta *et al.*, 1982), no phages specific for ETEC virulence factors have been reported to date.

The aim of the present study was to isolate virulence factor-specific ETEC phages with emphasis on CFs from environmental water samples in Bangladesh. The present report describes the isolation, identification and characterization of a phage specific for the CS7 CF.

METHODS

Isolation and selection of high-titre phages from water samples. Environmental water samples from ten different ponds and two rivers around Dhaka city were collected in 500 ml sterile bottles. Ten millilitres of each surface water sample was sterilized with 1 ml chloroform using a slightly modified procedure (Monsur *et al.*, 1985). For isolation of ETEC phages, a total of 13 different CF-specific ETEC propagating strains from our laboratory collection were employed: E1392-75 (CS1 plus CS3), 278485 (CS2 plus CS3), 3023 (CS3), E11881/9 (CS4 plus CS6), VM 75688 (CS5 plus CS6), BCE # 243 (CS6), 2255272 (CS7), E34420A (CS8 or CFA/III), 350CIA (CS12 or PCFO159), E7474A (CS14 or PCFO166), E-20738A (CS17), 258909-3 (CFA/I) and E9034A (CS21). Chloroform-treated water specimens were filtered through a 0.22 µm membrane filter. Twenty microlitres of filtered water sample was spotted onto well-dried lawns of indicator ETEC strains on heart infusion agar (HIA; Becton Dickinson) plates (Monsur *et al.*, 1985). The plates were incubated overnight at 37 °C and examined the following day. Phage plaques were observed on many of the inoculated spots in the plates. The phages that showed the highest stability were selected for further studies. The stability was judged by no loss of titre on rechecking at different time intervals. Each of the selected phages was purified by successively picking (three times) a single plaque from the propagating strain by the agar layer method (Monsur *et al.*, 1985).

Only phages that lysed ETEC strains expressing one or more specific CFs and that caused confluent lysis of the propagating strains were selected and tested against other ETEC strains. Phage stability, indicated by clear plaque formation and high titre, was the criterion for the 13 selected phages in this test. For preparation of high-titre phage, 2 ml 0.7% melted soft agar (T1N1), 0.1 ml of a young host bacterial culture and 0.1 ml of a serial tenfold dilution of the phage lysate was mixed and poured over the surface of a HIA plate. The plates were incubated at 37 °C overnight. The nearly confluent lysed plates were frozen overnight at -20 °C. Upon thawing the next day, about 4 ml clear fluid was obtained per plate. The fluid was then passed through a 0.22 µm membrane filter, and usually contained about 10⁹ p.f.u. phage ml⁻¹.

Isolation and detection of ETEC strains from environmental and clinical specimens. The environmental water specimens were cultured to isolate ETEC strains for the present study. In addition, diarrhoeal stool specimens were also tested to isolate ETEC strains around the same time period in which the environmental water specimens were being analysed in order to have a collection of ETEC strains that were prevalent at that particular time period. This was carried out to expand the potential host range of the phages tested to determine the susceptibility of the diarrhoea-causing ETEC strains to phage infections.

To isolate ETEC strains, a 150 ml aliquot of each water sample was filtered through a 0.22 µm Millipore nitrocellulose membrane filter. The filtrate was washed with 1 ml MacConkey broth (BD) and used to inoculate 9.0 ml MacConkey broth and incubated at 37 °C for 4 h. Subsequently, 100 µl of these cultures was spread onto MacConkey agar plates and incubated overnight at 37 °C. For isolation of ETEC strains from clinical samples, stools from the diarrhoeal patients were streaked onto MacConkey agar plates and incubated at 37 °C overnight. For both water and stool samples, lactose-fermenting *E. coli* were tested for the LT gene using primers 5'-ACGGCGTTA-CTATCCTCTC-3' (forward) and 5'-TGGTCTCGGTCAGATATG-TG-3' (reverse) and for the STp/STh gene using primers 5'-TCTT-TCCCCCTCTTTTAGTCAG-3' or 5'-AGTGGTCCTGAAAGCATG-3' (forward) and 5'-ACAGGCAGGATTACAACAAAG-3' or 5'-TAC-AAGCAGGATTACAACAC-3' (reverse) (Sjoling *et al.*, 2007). A multiplex PCR amplification method was used whereby colonies were taken from MacConkey agar plates and suspended in an Eppendorf tube containing 100 µl PBS. The tube was boiled for 10 min and held on ice for a further 10 min. The suspension was then centrifuged at 16000 g for 10 min. A sample of the resulting supernatant (2 µl) containing template DNA was used for the PCR. Three specific primer sets were used for detection of the *eltB* (LT B subunit), *estA* (STh) and *stI* (STp) genes in the *E. coli* strains (Sjoling *et al.*, 2007). PCR was performed in a PTC-200 thermal cycler (MJ Research) for 45 cycles as described by Sjoling *et al.* (2007). The PCR products were separated in a 2% agarose gel, stained with ethidium bromide and visualized under UV light.

Detection of ETEC toxin types and CFs. The production of LT and ST was confirmed by previously described ganglioside GM1 ELISAs (Svennerholm & Wiklund, 1983; Svennerholm *et al.*, 1986). Briefly, *E. coli* colonies were inoculated into separate wells of GM1-coated microtitre plates containing Luria-Bertani broth with lincomycin and glucose, and the plates were incubated overnight at 37 °C with agitation. After incubation, the broth culture was transferred to fresh GM1-coated plates and tested for ST by an inhibition ELISA procedure with the ST cholera toxin B subunit conjugate as described previously (Svennerholm *et al.*, 1986). The original plate used for culture was analysed for GM1-bound LT by using a mouse monoclonal antibody (mAb) specific to the toxin (Begum *et al.*, 2007; Svennerholm & Wiklund, 1983). After the addition of rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase (Dako), the enzyme substrate, hydrogen peroxide (0.1%), together with *o*-phenylenediamine (1 mg ml⁻¹) were added and the A₄₅₀ was measured after 20 min. ETEC strains 286C2 and ST 64111 were used in the assays as LT- and ST-positive controls, respectively. The toxin-positive colonies were also plated onto CFA agar plates with and without bile salts (Evans *et al.*, 1977; McConnell *et al.*, 1989) and incubated overnight at 37 °C. Enterotoxin-positive *E. coli* colonies from CFA agar plates were tested for the expression of CFA/I, CS1, CS2, CS3, CS4 and CS6, and colonies from CFA agar plus bile plates were tested for the expression of CS5, CS7, CS17, CFA/III (CS8), CS12 (PCFO159) and CS14 (PCFO166) by mAb-based dot-blot assays (Binsztin *et al.*, 1991; Lopez-Vidal & Svennerholm, 1990; Lopez-Vidal *et al.*, 1988; Viboud *et al.*, 1993). The mAbs against CFs, except for anti-CS21, were produced at the Department of Medical Microbiology and Immunology at Göteborg University, Sweden. The CS21 antibody was produced at the International Centre for Diarrhoeal Disease Research, Bangladesh (Qadri *et al.*, 2000b).

Detecting the host specificity of phages. To determine the phage susceptibility and specificity patterns for ETEC strains, plates with fresh cultures of the test strain were spotted with different phages, each at a concentration of 10² and 10³ routine test dilutions. The plaque morphology of each phage was studied on HIA after serial tenfold dilutions. Each of the high-titre phages was then used to determine the routine test dilution by the soft agar layer method. The

routine test dilution was defined as the highest dilution that failed to give confluent or complete lysis.

Single colonies of the test strains were inoculated into 3 ml heart infusion broth (HIB) and incubated at 37 °C for 4 h. The fresh culture was then spread on a well-dried HIA plate containing 20 ml agar per Petri dish. The plate was dried at 37 °C for 1 h and then incubated at 37 °C for 18–20 h. Medium not containing culture was used as a negative control. The sensitivity of a strain to a phage was recorded as 1–15 plaques (+), >15 plaques but less than confluent lysis (++), and semi-confluent to confluent lysis (+++). The presence or absence of bacterial lysis by the phage was recorded for each ETEC strain tested. Lysogenization and induction of lysogens was not tested in this study.

Serogrouping. ETEC colonies were grown overnight at 37 °C on 5% sheep blood agar plates. Cells were scraped aseptically from the plate and suspended in 1 ml saline, boiled for 1 h and centrifuged at 3000 g for 15 min, and the sediment was resuspended in saline. Serogrouping was carried out using standard techniques (Edwards & Ewing, 1972) using eight commercially available polyvalent and 43 monovalent antisera (Denka Seiken).

Preparation of specimens for electron microscopy. Morphological studies on bacteriophage were carried out by negative staining with 2% uranyl acetate and examination under a FEI Tecnai 12 BioTWIN transmission electron microscope (Ghosh *et al.*, 1989).

Phage inactivation by purified CS7 fimbrial protein (plaque inhibition assay). The phage-neutralizing capacity of purified CS7 CF was determined by incubating 10^7 p.f.u. phage with various concentrations of CS7 protein (Qadri *et al.*, 2006). One hundred microlitres of purified CS7 fimbrial protein, diluted to concentrations of 100–500 µg ml⁻¹, and CFA/I (500 µg ml⁻¹) were incubated with purified phage particles (10^7 p.f.u. ml⁻¹) for 2 h at 37 °C. The phage-CS7 mixtures (20 µl) were spotted onto a lawn that was made with CS7-expressing ETEC on a well-dried HIA plate. Plaques were counted after overnight incubation at 37 °C.

Inhibition of phage infection with CF-specific antibodies. To test the inhibition of phage infection, 100 µl of different dilutions of purified CS7-specific mouse mAb (Qadri *et al.*, 2000a) in HIB (1:10–1:50 dilutions) was incubated for 3 h at room temperature with a CS7-expressing ETEC strain (2255272; 10^8 c.f.u. in 100 µl HIB). To test for non-specific responses of the antibody, we used mouse mAb specific for CFA/I (Lopez-Vidal & Svennerholm, 1990) at the same dilutions as the CS7 mAb to test for inhibition of phage infection. HIB not containing antibody was used as a control. Each antibody dilution was tested in triplicate. The results were expressed as the percentage of phage activity remaining in comparison with control without antibody.

The recipient CS7-expressing cells pre-incubated with different dilutions of CS7 antibody, control antibody or medium were plated and incubated at 37 °C for 1 h prior to the addition of 20 µl phage solution (10^7 p.f.u. ml⁻¹ in HIB). The plaques were counted the following day after an overnight incubation.

Effect of selected physical conditions on the survival of phage. Phage suspensions in HIB were incubated at different temperatures (30, 37, 42, 50, 55, 60 and 65 °C). Survival of the phage was determined at 1, 3 and 6 h and after overnight treatment to evaluate the number of p.f.u. in the phage suspension using the drop method (Sezzano *et al.*, 1985). Phage suspensions in HIB at different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and salt (sodium chloride) concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 M) were incubated at 37 °C. Survival of the phage was determined at 3 and 6 h and after overnight treatment to evaluate the number of p.f.u. in the

phage suspension using the drop method (Monsur *et al.*, 1985; Sezzano *et al.*, 1985).

PCR and oligonucleotide primers. To verify further the type of phage, PCR was used to detect T phage-specific genes in the phage genome. PCRs were performed in a final volume of 50 µl with 1 µl specific phage lysate (10^7 p.f.u. ml⁻¹) as template and heated at 95 °C for 20 min. For amplification of the T4 gene 32, we used the primers FR60 (5'-CGTAAATCTACTGCTGAACCTCGC-3') and FR61 (5'-GAATGCATCCAAATCATCAGCCAC-3') as described by Monod *et al.* (1997). The conditions involved in this PCR were 35 cycles consisting of a 30 s denaturation period at 96 °C, 2 min annealing at 54 °C and 5 min extension at 72 °C.

RESULTS AND DISCUSSION

Isolation of phage from environmental samples

Forty-nine phages were isolated from different surface water samples (ten ponds and two rivers) around Dhaka city using 13 different CF-specific ETEC strains. Among the 49 phages, 13 were selected for further study (Table 1) on the basis of their stability and high titre.

Distribution of different toxins, CFs and serogroups among the isolated ETEC strains

A total of 211 ETEC strains were isolated from water ($n=56$) and stool ($n=155$) specimens. Among the 211 ETEC strains, 84 and 55 strains were found to express LT and ST, respectively, whereas the remaining 72 expressed both LT and ST (Table 1) as shown by both PCR and ELISA. Only 12/13 different CFs tested were detected either individually or in combination with others. Eleven different O antigens were identified among the ETEC strains. Of the 211 ETEC strains, 35 did not express any of the 13 CFs tested for. Most CF-positive ETEC strains studied here belonged to only one O serogroup (Table 1). Only strains of the CS5 plus CS6 and the CFA/I-positive strains could be grouped into more than one 'O' group. Strains harbouring CS5 plus CS6 together were of the O115 or O167 serogroup. The CFA/I type strains belonged to the O126 or O128 serogroup (Table 1).

Determination of phage specificity for ETEC virulence factors

The 13 selected lytic phages were tested against 211 ETEC strains to identify the host range and specificity to CFs and toxins. Among these 13 phages, only one, designated IMM-001, was successful as far as the virulence specificity was concerned (Table 1). The remaining 12 phages were not specific for either toxin or for any of the 13 CFs tested. The IMM-001 phage showed a significant specificity towards ETEC strains that expressed LT toxin and CS7 CF. All the CS7 ETEC strains that were lysed belonged to the O114 serogroup (Table 1). However, non-CS7 and LT-negative strains belonging to O114 types were not susceptible to IMM-001. Moreover, the LT-positive ETEC strains car-

Table 1. Screening of 13 selected phages against 211 ETEC strains

No. of strains tested	No. of sensitive strains*	CF	Serogroup	Toxin type†		Phage tested‡												
				LT	ST	1	2	3	4	5	6	7§	8	9	10	11	12	13
5	3	CS1 + CS3	O6	—	+	S	N	S	N	N	N	N	N	N	S	N	N	N
10	7	CS1 + CS3	O6	+	+	S	N	N	N	N	S	N	N	S	N	N	N	S
10	6	CS2 + CS3	O6	+	+	N	S	S	N	S	N	N	N	N	N	N	N	N
5	2	CS3	O6	—	+	S	N	S	N	N	N	N	N	N	N	N	N	N
8	5	CS4 + CS6	O25	+	+	N	N	S	S	N	N	N	N	N	N	N	S	N
7	5	CS4 + CS6	O25	—	+	N	N	N	N	S	N	N	N	N	N	N	N	N
5	4	CS5 + CS6	O115, O167	—	+	S	N	N	N	S	N	N	N	N	N	N	N	N
10	7	CS5 + CS6	O115	+	+	N	N	N	N	S	S	N	N	N	N	N	N	N
4	3	CS6	O115	+	—	N	N	N	S	N	N	N	N	N	N	N	N	N
6	4	CS6	O115	—	+	N	N	N	S	N	N	N	S	N	N	S	N	N
5	4	CS6 + CS8	O25	+	—	N	S	N	N	N	N	N	S	N	N	S	N	N
50	50	CS7	O114	+	—	N	N	N	N	N	N	S	N	N	N	N	N	N
10	6	CS12	O159, NT	+	+	N	N	N	N	N	N	N	N	N	N	S	N	N
7	5	CS14	O166, NT	—	+	S	N	N	N	N	N	N	S	S	N	N	N	N
9	6	CS14	O15, NT	+	+	S	S	S	N	N	N	N	N	N	N	N	S	N
10	8	CS17	O128, NT	+	—	S	N	S	N	N	S	N	N	S	N	N	N	S
10	6	CFA/I	O126, O128	—	+	S	N	S	N	N	N	N	N	S	N	N	N	N
5	4	CFA/I	O126	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N
10	7	CF-negative	114, NT	—	+	S	N	S	S	S	N	N	S	N	N	N	S	N
15	9	CF-negative	O15, O8, NT	+	—	S	S	N	N	S	N	N	N	S	N	N	S	N
10	4	CF-negative	NT	+	+	N	N	N	S	N	N	N	N	N	S	N	S	S

NT, Not typable.

*The number of ETEC strains susceptible to phages.

†Positive (+) or negative (—) for toxin expression.

‡Single strains or multiple strains of each type tested were susceptible to the phages tested. S, Susceptible; N, not susceptible.

§The IMM-001 phage, which infected and lysed all CS7-expressing ETEC.

rying CFs other than CS7 were immune to IMM-001 infection. The specificity pattern of IMM-001 led us to assume that this phage is specific for CS7 CFs. The CS7-specific phage produced clear and distinct plaques. It was interesting that other common enteric bacteria were not lysed by IMM-001 (Table 2), indicating that IMM-001 only had the lytic property specific for the ETEC strains carrying the CS7 CF.

Possible role of CS7 antigen in IMM-001 infection

The requirement for the CS7 antigen during infection of IMM-001 was demonstrated by a CS7-specific mAb blocking assay. In this assay, it was evident that blocking the CS7 pili in ETEC with specific mAb hampered the infectious capability of phage IMM-001. Altered infectious activity of the phage was identified by a reduced number of plaques on the plate. The plaque number increased in a dose-dependent manner with the increase in CS7-specific mAb dilution (Fig. 1a). Thus, when the CS7-specific mAb dilution was at its lowest (1:10 dilution), only one viable plaque produced by IMM-001 was observed on the plates and the number of plaques increased up to the highest dilution (1:50). The CFA/I-specific mAb had no effect on

decreasing the plaque titre, even at a dilution of 1:10 (Fig. 1a). Thus, this experiment provided evidence that unoccupied or free CS7 antigen may be required for the IMM-001 phage to attach itself to the host bacterium. Interestingly, there is evidence indicating that the *V. cholerae* bacteriophage (CTXΦ) utilizes the *V. cholerae* type

Table 2. Phage susceptibility test results for different bacteria

Enteric pathogen tested	No. of isolates tested	No. of isolates susceptible
ETEC (CS7)	50	50
<i>E. coli</i> (non-toxigenic)	25	0
Enteropathogenic <i>E. coli</i>	5	0
<i>Shigella flexneri</i>	10	0
<i>Shigella sonnei</i>	9	0
<i>Shigella boydii</i>	8	0
<i>Shigella dysenteriae</i>	5	0
<i>Klebsiella</i> spp.	10	0
<i>Salmonella</i> spp.	6	0
<i>Citrobacter</i> spp.	2	0
<i>Proteus</i> spp.	8	0

Fig. 1. Inhibition of phage IMM-001 infection by CS7-specific mAb (a) and purified CS7 fimbrial antigen (b). (a) Reduction in plaque formation with different dilutions of mAb. The bars indicate the percentage infection mediated by IMM-001 on a HIA plate containing a lawn of CS7-expressing ETEC. (b) Neutralization capacity of purified CS7 fimbrial antigen on plaque formation after incubation with IMM-001 phage. The varying concentrations of CS7 antigen are shown ($\mu\text{g ml}^{-1}$). The light-grey bar indicates the percentage infection when treated with CFA/I mAb (1 : 10) and CFA/I antigen (500 $\mu\text{g ml}^{-1}$) in (a) and (b), respectively. In (a) and (b), the infection percentage for the control (no mAb and no antigen, respectively) is indicated by dark-grey bars.

IV pilus (known as toxin co-regulated pilus), an essential intestinal CF, as its receptor (Waldor & Mekalanos, 1996). Thus, the role of intestinal CFs of pathogenic bacteria as a bacteriophage receptor is not an unusual phenomenon as has been shown for the toxin co-regulated pilus.

Determination of the phage neutralization capacity of the CS7 antigen

The role of CS7 in IMM-001 infection was further reinforced by the phage neutralization capacity of the purified CS7 antigen. Purified CS7 antigen could neutralize IMM-001 when incubated with the phage particle prior to infection. The concentration of the CS7 antigen was found to be inversely proportional to the number of plaques (Fig. 1b). When IMM-001 phage particles were pre-incubated with a relatively high concentration of CS7 antigen (500 $\mu\text{g ml}^{-1}$), the plaque number was significantly reduced, whereas phage pre-incubated with a lower concentration of CS7 antigen (100 $\mu\text{g ml}^{-1}$) produced a greater number of plaques on the plates. A possible explanation for the CS7 neutralizing capacity may be that the purified CS7 protein binds to the IMM-001 tail and thus prevents the phage particle binding with the CS7 fimbrial protein present on the surface of ETEC strains.

Resistance of phage IMM-001 to selected physical conditions

The titre of the phage remained unchanged in liquid medium for over a year and produced at least 10^9 p.f.u. ml^{-1} . The pure phage was viable in different salt concentrations (0.5–5.0 M sodium chloride). We did not grow the *E. coli* host in the medium containing different

salt concentrations. The phage was tolerant to temperatures ranging from 30 to 65 °C after overnight incubation and also survived in liquid medium at a pH ranging from 4 to 10.

Morphology of phage IMM-001

Electron microscopic images of phage IMM-001 revealed some important morphological features such as an isometric head and a long filamentous tail. The head diameter and tail length of IMM-001 were determined as 50 and 152 nm by electron microscopy (Fig. 2). *E. coli* λ phages have this type of morphological appearance (Hershey & Dove, 1983). IMM-001 also lacked the T-phage-specific gene (gene 32) characteristic of λ phages. However, we did not have enough evidence to conclude that the CS7-specific ETEC phage is a λ phage. Additional studies are needed to determine the lysogenic properties of this phage and also to screen for genes conserved in the λ phage family to characterize IMM-001 further.

Diarrhoeal pathogen-specific phages are interesting with respect to their role in therapy and also for an understanding of their natural diversity in the environment. This information is important for a better understanding of the presence of ETEC in endemic countries and the relationships that phages have in perpetuating or terminating infections. In a narrower context, the presence of CF-specific ETEC phages offers an opportunity for the development of novel antimicrobial drugs that can be directed against ETEC infection to hinder the binding of CS7 to the intestinal mucosa. The phage IMM-001 isolated here, which is specific for CS7 CF, may have epidemiological applications in tracing the presence of an ETEC burden in environmental water.

Fig. 2. Electron micrograph of the CS7 ETEC-specific phage IMM-001, showing an isometric hexagonal head and a long filamentous tail.

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