Impact of solar irradiation on cholera toxin secretion by different strains of *Vibrio cholerae*

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Cholera toxin is the aetiological agent of cholera – a deadly waterborne disease acquired through the consumption of untreated water contaminated with CTXΦ bacteriophage harbouring strains of *V. cholerae*. Solar disinfection is a re-emerging technique that relies on the ultraviolet component of sunlight to inactivate the growth of *Vibrio cholerae* in water, rendering the water microbiologically safe for consumption. However, studies have shown that DNA damaging agents, such as ultraviolet light, induce the replication of the CTXΦ bacteriophage with subsequent expression of the cholera toxin. In this study we investigated the impact of solar irradiation on the secretion of cholera toxin by toxigenic strains of *V. cholerae* in water. The cholera toxin ELISA assay, qualitative and quantitative real-time PCR as well as growth on solid media were used to determine cholera toxin secretion, DNA integrity and growth of the bacteria after 7 h and 31 h of solar irradiation. Solar irradiation in water reduced the integrity of DNA, inactivated the growth of *V. cholerae* and, most importantly, prevented the secretion of detectable levels of cholera toxin. This finding is encouraging for resource-poor communities that may rely on solar disinfection to alleviate the burden of cholera-related fatalities.

Introduction

Cholera is a life-threatening waterborne disease caused by *Vibrio cholerae,* a motile Gram-negative bacterium that is autochthonous in natural aquatic ecosystems.[1] The disease is a constant threat and continues to ravage resource-poor communities around the world that lack adequate access to safe potable water and sanitation, with subsequent high fatality rates.[2,3] Seven cholera pandemics have been reported worldwide; six were ascribed to the classical biotype of the O1 serogroup of *V. cholerae* while the most recent pandemic was attributed to the El Tor biotype of the same serogroup. Another serogroup of *V. cholerae* (O139) has been implicated in cholera outbreaks in some parts of Asia.[4,5]

It has been hypothesised that only *V. cholerae* serogroups O1 and O139 are capable of causing cholera outbreaks. However, this notion is changing as some non-O1/O139 strains of *V. cholerae* have been reported to harbour the CTXΦ bacteriophage genes[6,7] providing them with the potential to cause cholera outbreaks. The CTXΦ bacteriophage contains the genes responsible for the production of the cholera toxin – the aetiological agent of cholera.[8,9] Cholera toxin is a multi-subunit ADP-ribosylating toxin that binds to the GM1 ganglioside of the intestinal epithelial cells. The toxin is made up of the cholera toxin A subunit, which consists of a single unit, and cholera toxin B, which contains five units.[10] The subunits are produced in the bacterial cytoplasm and are transported to the periplasm where they are folded and assembled into the 84-kDa heterohexameric AB5 toxin complex.[11]

The culturability of a variety of waterborne pathogens has been shown to be inhibited by solar radiation, specifically its ultraviolet component,[12-14] thereby reducing the risk associated with the acquisition of deadly water-related infections.[15,16] The mechanisms through which solar ultraviolet radiation (SUVR) inactivates the growth of water contaminating microorganisms are quite complex. The technological application of solar radiation to inactivate microorganisms in water has been termed solar disinfection (SODIS). SODIS treatment involves the exposure of water in transparent colourless vessels to direct sunlight for at least 7 h on clear sunny days or 2 days in cloudy weather.[17,18] Ultraviolet(UV)-A, which is the more abundant, active component of solar radiation, has been reported to directly and indirectly target various vital microbial cell components and processes such as transcription and translation, transport systems, metabolism, chaperones and catalase (responsible for counteracting dangerous oxygen radicals), thereby inducing microbial cellular death.[19,20] UVA has also been reported to cause single-strand breaks in DNA, and conditions that result in such damage have been shown to induce multiplication of CTXΦ and, simultaneously, increase the expression of *ctxAB* genes as a result of the SOS DNA repair response.[21] The DNA repair response increases the repression of the LexA protein on the CTXΦ promoter (P_{rstA}) upstream of the *ctxAB* gene, through the protease activity of the RecA protein,[21] thereby enabling its transcription. Even though the *ctxAB* gene has its own promoter (P_{ctxAB}), reports have shown that it can be transcribed from CTXΦ P_{rstA}. DNA damaging agents, such as mitomycin C and UV light, have been shown to induce multiplication of CTXΦ as well as the expression of CT.[21,22] Sunlight contains a sufficient dose of UV light and can increase the bacteriophage titre, thereby enabling its transmission to potential recipient strains of *V. cholerae*.[23] The transmission of CTXΦ is, however, dependent on its stability which relies on factors such as the viability of the bacteria, duration of UV exposure and environmental factors such as temperature, salinity and pH.[23]

In this study we investigated the potential for sunlight to induce the secretion of cholera toxin by toxigenic strains of *V. cholerae* by means of solar irradiating the microorganisms in water.

Material and methods

Vibrio cholerae *strains and culture conditions*

Seven strains of *V. cholerae* – six toxigenic and one non-toxigenic – were used in this study: NCTC 5941 and NCTC 12945 obtained from the national collection of type cultures; BRITS01 isolated from Brits, South Africa; G4222

isolated from a cholera patient in Gauteng, South Africa; ERWATA01 isolated at East Rand Water, Johannesburg, South Africa; ENV1009 isolated at Rand Water (Vereeniging) in South Africa; and UG01 isolated from groundwater in Katojo, Uganda. All strains were stored at -80 °C as bacterial stocks on beads. Each strain was cultured on nutrient agar plates by incubation at 37 °C for 18 h. From each isolate, 2–5 colonies were inoculated in Luria broth (LB, pH 8.5) and incubated at 37 °C on a rotary shaker at 200 rpm overnight until the stationary phase was reached. These cultures were used for (1) cholera toxin analysis by an enzyme-linked immunosorbent assay (ELISA), (2) semi-quantitative and qualitative real-time polymerase chain reaction (qRT-PCR) analysis and (3) solar exposures in water because of their resilience, as recommended by Berney et al.[24]

Preparation of Vibrio cholerae *for solar exposure*

Vibrio cholerae was harvested by centrifugation (at 3000 rpm for 10 min) from overnight stationary phase cultures of the batch cultures grown in LB. The pellet for each strain was washed three times with 1 x phosphate buffered saline (PBS, pH 7.5) to remove all traces of LB and the bacteria were diluted in commercially available still bottled water (Bonaqua, Pretoria, South Africa) to an optical density (OD_{600nm}) of 0.1 that corresponded approximately to 11.5 colony forming units (CFU)/mL before SUVR exposure. The unusually high starting dose was used to increase the possibility of detecting the cholera toxin produced by the toxigenic strains of *V. cholerae*.

Exposure to natural solar radiation

The bacterial suspension (15 mL) was transferred into 25-cm^3 transparent polystyrene unventilated tissue culture flasks. The flasks were exposed to natural sunlight by placing them horizontally on the roof of a building at an elevation of 1400 m in Pretoria, South Africa (25°44'50.40"S, 28°16'50.50"E). Control samples were prepared in a similar manner and placed next to the experimental ones but were protected from direct sunlight by being covered with an opaque ventilated cardboard box. The samples were exposed to SUVR during clear sunny days for either 7 h or 31 h. The 7-h exposure period was used because it is the optimal recommended time for SODIS and the 31-h period was used to assess for any further production of cholera toxin. Following exposures to SUVR, the samples were prepared for enumeration by the plate count method. Cholera toxin production was assayed using an ELISA and qRT-PCR analysis was used to assess the integrity of the DNA.

Enumeration of Vibrio cholerae

Bacterial samples exposed to SUVR were serially diluted in sterile 1 x PBS and plated on nutrient agar using the Miles and Misra drop count technique[25] with slight modifications. Briefly, 10 μL of the appropriate dilution was dropped onto sterile nutrient agar plates in quadruplicate. The plates were incubated at 37 °C for 18–20 h. Plates with fewer than 50 discrete colonies per drop were selected for counting. The total count was divided by the number of drops, multiplied by 100 to convert to 1 mL, and then divided by the dilution factor to give the number of CFU/mL.[14]

Ganglioside GM1 cholera toxin ELISA assay

Materials used for the cholera toxin ELISA were: 96-well ELISA microplates (Greiner Bio-One, LASEC SA Centurion, South Africa); purified choleragenoid rabbit polyclonal IgG horseradish peroxidase (HRP) conjugated antibody (catalogue number PA1-73189) against the B subunit of the cholera toxin (BIOCOM Biotech, Thermo Scientific, Rockford, IL, USA); monosialoganglioside GM1 (G7641-1MG, Sigma, Johannesburg, South Africa) purchased as 1 mg of powder and dissolved in methanol to a final concentration of 1 mg/mL; wash buffer prepared by adding 0.05% Tween 20 to 1 x PBS; blocking buffer consisting of 1% bovine serum albumin (BSA) in wash buffer (1% BSA); and tetramethylbenzidine (TMB) substrate reagent (catalogue number 421101, BioLegend, BIOCOM Biotech, San Diego, CA, USA) prepared and used for colour development according to the manufacturer's instructions. All incubations were done at room temperature (23–25 °C). After each incubation period, the ELISA plate was emptied and washed three times with the wash buffer. To each well in the plate, 100 ng/mL of ganglioside GM1 in 1 x PBS was added and left overnight (about 15 h). The plate was then washed with wash buffer and blocking buffer was added. After 1 h, the plate was emptied, washed and then the supernatants from the solar-irradiated and non-irradiated samples were added. The cholera toxin B subunit and wash buffer were used as positive and negative controls, respectively. After 1.5 h of incubation, the plate was emptied, washed and the polyclonal rabbit HRP conjugated anti-cholera toxin B subunit antibody (1:1000 dilution in blocking buffer) was added and left for 1 h. The plate was emptied, washed and 100 μL of TMB substrate was added to each well. The plate was left at room temperature for 30 min before the stop solution consisting of 100 μL of 1M sulphuric acid was added to the wells. Within 10 min of adding the stop solution, the plate was read at an OD_{450nm} in the BioTek Power Wave HT plate reader (BioTek, Winooski, VT, USA). Prior to reading of the plates, the minimum detection ability of the cholera toxin ELISA was determined by using seven tenfold dilutions (from a concentration of 1 000 000 pg/mL to 1 pg/mL) of the cholera toxin. These results were used to plot a standard curve. All assays were performed in duplicate.

Qualitative and quantitative real-time PCR analysis

Prior to DNA extraction, overnight bacterial cultures in LB were diluted to a uniform OD_{600nm} of 1. Genomic DNA was extracted using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. RT-PCR was performed using 5 μL of the supernatant from each extraction. Two genes – the outer membrane protein gene (*ompW*) specific to *V. cholerae* and the *ctx* gene (complex A and B) involved in cholera toxin production – were targeted in the RT-PCR. Two primer sets[26-28] were used to amplify the *ompW* and *ctxAB* gene segments. The classical and El Tor variants of the *tcpA* gene were amplified with primers designed by Mukhopadhyay et al.[29] To distinguish the O1 and O139 serogroups, the O1 and O139 *rfb* genes were targeted with primers published by Hoshino et al.[30] All the primers used were synthesised at Inqaba Biotech (Pretoria, South Africa) and are shown in Table 1.

Each single-plex RT-PCR amplification reaction contained: 1 x SensiMix HRM reaction buffer (containing dNTPs and 6 mM $MgCl_2$); 0.2 μM primer; heat-activated DNA polymerase; EvaGreen dye (Quantace, London, UK); and 5 μL of DNA. Nuclease-free water (Applied Biosystems, Carlsbad, CA, USA) was used to make up the reaction to a final volume of 25 μL. Amplification was performed in a RotorGene 6000 rotary thermal cycler (5-plex) with high-resolution melting (HRM) capability (Qiagen, Hilden, Germany). The heat-activation step of the DNA polymerase was performed at 95 °C for 10 min, followed by 45 cycles of DNA denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 30 s. A final extension step was performed at 72 °C for 5 min.

To differentiate and identify amplification products, HRM curve analysis was performed by lowering the temperature to 60 °C for 5 min, followed by increasing the temperature to 90 °C in increments of 0.1 °C/s. Fluorescence was measured continuously and melting temperature peaks were calculated based on the initial fluorescence curve (F/T) by plotting the negative derivative of fluorescence over temperature versus temperature (-dF/dT versus T).

Statistical analysis

The measure of the significance of the difference observed between the solar irradiated and non-irradiated samples was determined using the Student's *t*-test; $p < 0.05$ was considered statistically significant.

Table 1: PCR primer sequences used to characterise *Vibrio cholerae* strains

Target gene	Primer	Primer sequences (5'-3')	Reference
ompW	ompW1	CACCAAGAAGGTGACTTTAATTGTG	Nandi et al. [26]
	ompW3	GGTTTGTCGAATTAGCTTCACC	
ctxAB	ctxAB-F	GCCGGGTTGTGGGAATGCTCCAAG	Goel et al. [27]
	ctxAB-R	GCCATACTAATTGCGGCAATCGCATG	
tcpA	tcpA-F(Cla)	CACGATAAGAAAACCGGTCAAGAG	Mukhopadhyay et al. [29]
	tcpA-R(Cla)	ACCAAATGCAACGCCGAATGGAGC	
	tcpA-F(Elt)	GAAGAAGTTTGTAAAAGAAGAACAC	
	tcpA-R(Elt)	GAAAGGACCTTCTTTCACGTTG	
Rfb complex (O1)	O1F2-1	GTTTCACTGAACAGATGGG	Hoshino et al. [30]
	O1F2-2	GGTCATCTGTAAGTACAAC	
Rfb complex (O139)	O139F2	AGCCTCTTTATTACGGGTGG	Hoshino et al. [30]
	O139R2	GTCAAACCCGATCGTAAAGG	

Cla, classical; Elt, El Tor

Results

Characterisation of the Vibrio cholerae *strains*

The seven strains of *V. cholerae* used in this study were characterised by RT-PCR of four different gene targets: the *ompW*, *ctxAB*, *tcpA* (classical and El Tor biotype) and the *rfb* complex (specific for the O1 and O139 serogroup). All the *V. cholerae* strains were positive for the *ompW* gene; only the 1009 strain was negative for the *ctxAB* gene (Table 2). However, not all the *ctxAB* gene positive *V. cholerae* strains belonged to the same serogroup or biotype; three of them – NCTC 5941, G4222, and ERWATA01 – belonged to the O1 serogroup while only one strain, NCTC 12945, was in the O139 serogroup. The other two strains (UG01, BRITS01) were found to be *ctxAB* positive but did not belong to either the O1 or O139 serogroups; the BRITS01 strain did not harbour any of the *tcpA* genes while the UG01 strain harboured a hybrid (classical and El Tor) *tcpA* gene.

Table 2: Real-time PCR characterisation of the seven *Vibrio cholerae* strains used in this study

Strain	*ompW*	*ctxAB*	*rfb* O1	*rfb* O139	*tcpA* classical	*tcpA* El Tor
NCTC 5941	+	+	+	-	+	-
NCTC 12945	+	+	-	+	-	+
BRITS01	+	+	-	-	-	-
G4222	+	+	+	-	-	+
UG01	+	+	-	-	+*	+*
ERWATA01	+	+	+	-	-	+
1009	+	-	-	-	-	-

**Alternative variant*

Exposure of Vibrio cholerae *to solar ultraviolet radiation*

Exposure of the seven *V. cholerae* strains to SUVR was done on a sunny day in June (South African winter). The average (±s.d.) minimum and maximum ambient temperatures at the point of exposure were 19.96±0.65 °C and 27.25±1.06 °C, respectively, whereas the water in which the bacteria were irradiated had a temperature of 23.40±2.00 °C. There was a mean reduction in the culturability of all the exposed strains of *V. cholerae* of 7.5±0.81 $\log_{10}$ CFU/mL from an average initial level of 11.02±0.69 $\log_{10}$ CFU/mL after 7 h of exposure. The two toxigenic strains (NCTC 5941 and 12945) were non-culturable after exposure for a further 24 h (Figure 1). The lack of total inactivation was expected because an unusually high dose of the microorganism (11 log CFU/mL) was used initially to increase the odds of detecting any cholera toxin secreted.

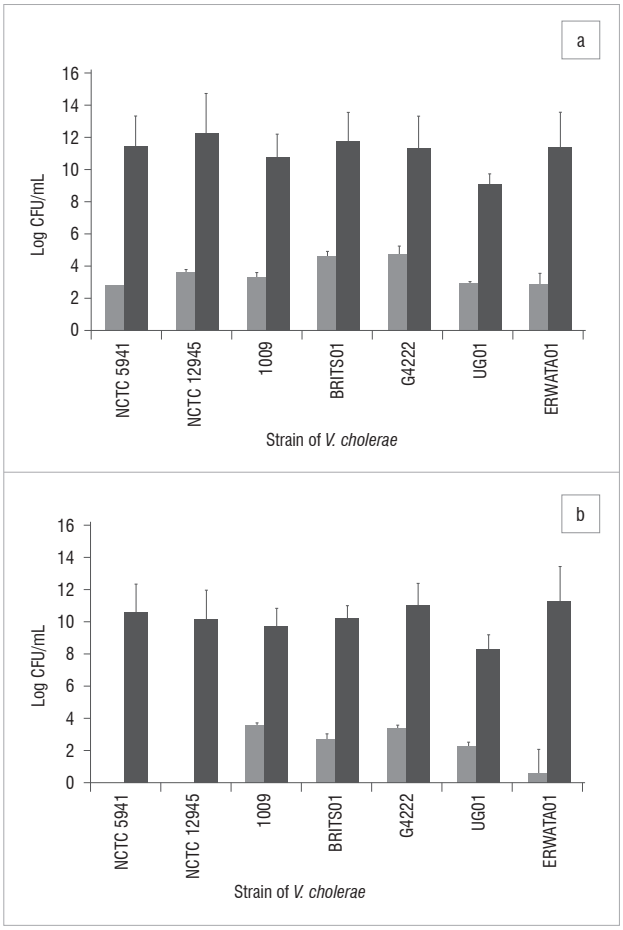

Figure 1: Log CFU/mL counts of solar-irradiated (■) and non-irradiated (■) strains of *Vibrio cholerae* after (a) 7 h and (b) 31 h. Error bars indicate the standard deviation of triplicate experiments.

Relative quantification of the ctxAB *gene*

A quantitative RT-PCR of the *ctxAB* gene relative to the *ompW* housekeeping gene was done for each of the *ctxAB*-positive *V. cholerae* strains (those that were cultured at 37 °C for 15 h in LB). On average, the crossing point for the *ompW* gene amplification of all the strains was 22.06 with the difference between the lowest and highest CT being 3.6 cycles. To control for possible cell copy number differences in each qRT-PCR, CT values of the *ctxAB* gene for each strain were normalised using the *ompW* CT values for the same strain (using the same DNA extraction from overnight cultures, diluted to an OD_{600nm} of 1). The average crossing point for the *ctxAB* gene for all the *ctxAB*-positive strains was 14.58 with a difference of 10.2 cycles between the lowest and highest CT value (Table 3). The *ctxAB* gene in the isolate from BRITS required an additional 9.8 cycles of amplification compared to all the other toxigenic strains. HRM analysis showed that the *ctxAB* amplicon from the BRITS01 strain was very similar to that of the other toxigenic strains (unpublished data). The solar-irradiated samples showed higher CT values in comparison with the non-solar exposed ones (Table 3). Following 7 h of exposure, the *ctxAB* gene crossing point for all the toxigenic strains occurred on average 0.6 cycles ($p=0.027$) earlier for the controls than for the experimental samples. The same was observed for the *ompW* gene but with a slight decrease in the number of cycles (0.47 cycles, $p=0.182$). This result translated into an approximate 20% reduction in amplifiable DNA after 7 h of solar exposure relative to the non-solar exposed samples. After 31 h of exposure the crossing point for the *ctxAB* and *ompW* genes occurred 1.75 ($p=0.0012$) and 1.27 ($p=0.021$) cycles earlier, respectively, for the controls compared with the exposed samples, which translated into a 50% reduction in amplifiable DNA in the solar-irradiated samples when compared with the non-irradiated ones. The percentage reduction in amplifiable DNA was based on results obtained through qRT-PCR of serial dilutions of *V. cholerae* based on the *ompW* gene (unpublished data).

Secretion of cholera toxin

The standard curve for the cholera toxin ELISA is shown in Figure 2. On average none of the negative controls (wash buffer) yielded an OD_{450nm} of more than 0.052± 0.002 (mean±s.d.). The mean±s.d. OD_{450nm} for cholera toxin at a concentration of 1 pg/mL for this assay was found to be 0.056±0.003, while the mean±s.d OD_{450nm} for a concentration of 10 pg/mL was 0.081±0.009. On this basis, an OD of 0.081 corresponding to a cholera toxin (sample : negative control) ratio of 1.45 or greater was selected as the cut-off value for what was considered positive for cholera toxin secretion.[31] The six toxigenic and one non-toxigenic strains of *V. cholerae* were tested for their ability to secrete cholera toxin in the culture medium. After 15 h of incubation at 37 °C, the biomass (determined by the OD_{600nm} reading) increased on average from 0.001 to 1.481±0.12 OD_{600nm} units with visible biofilm formation in *V. cholerae* isolates obtained from Uganda (UG01) and South Africa (ENV1009, G4222, BRITS01). Of all the isolates assessed in this study, only three (G4222, UG01 and NCTC 129450) produced cholera toxin in the culture medium under laboratory conditions (Table 4).

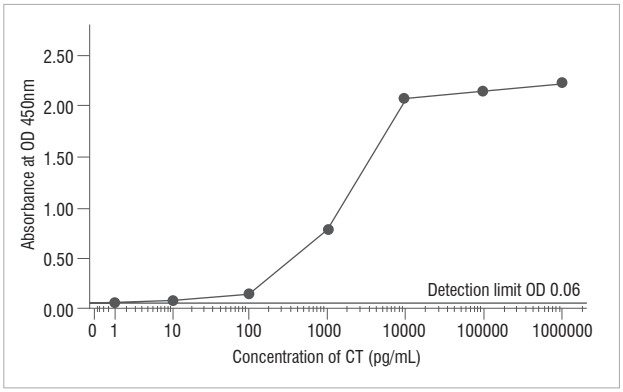

Figure 2: Standard curve of CT-B subunit determined by ELISA. Each point is a mean of duplicate experiments.

Table 4: Cholera toxin ELISA ratio (sample : negative control)

Strain	Luria broth	7 h		31 h	
		Exposed	Control	Exposed	Control
NCTC 5941	1.147	1.029	0.933	0.990	0.960
NCTC 12945	12.094	1.038	0.971	0.904	1.040
BRITS01	0.918	0.942	0.981	0.865	0.940
UG01	3.145	1.077	0.942	1.125	1.020
G4222	41.147	0.856	0.856	0.952	0.910
ERWATA01	1.004	1.154	1.029	0.942	1.030
1009	0.908	1.038	0.913	1.000	0.940

Note: Control samples were not solar irradiated.

The other strains had a cholera toxin ELISA (sample : negative control) ratio of below 1.45 units and hence were considered negative for cholera toxin secretion. Following exposure to SUVR, the secretion of CT by exposed and non-exposed samples was assessed after 7 h and after 31 h. There were no detectable levels of cholera toxin in either the exposed or non-exposed samples at both time points, with none of the samples showing a sample : negative control ratio of 1.45 or more.

Discussion and conclusions

As a means of mitigating the contraction of waterborne diseases or deaths resulting from the consumption of untreated water, the use of SUVR through a technique known as SODIS has been recommended. We investigated the effect of SUVR on the secretion of cholera toxin by *V. cholerae*.

Table 3: Crossing point values of *ctxAB* and *ompW* genes of *Vibrio cholerae* strains after 7 h or 31 h of exposure to solar UV radiation

Strain	*ctxAB** (in Luria broth)	7 h				31 h			
		Exposed *ctxAB*	Control *ctxAb*	Exposed *ompW*	Control *ompW*	Exposed *ctxAB*	Control *ctxAb*	Exposed *ompW*	Control *ompW*
NCTC 5941	11.2	16.5	16.0	23.5	23.5	17.0	15.0	24.0	23.0
NCTC 12945	11.5	18.0	17.0	27.0	25.0	20.0	17.0	27.0	24.0
BRITS01	21.4	28.0	26.5	26.0	24.0	27.5	26.0	25.0	24.0
G4222	11.9	21.0	21.0	27.0	27.0	19.5	18.5	26.5	25.0
UG01	11.6	15.0	15.0	23.0	23.0	16.0	14.0	23.2	23.0
ERWATA01	11.6	21.4	20.8	27.0	28.0	20.0	19.0	26.0	25.8

*Note: Control samples were not solar irradiated. All exposures were in water except those indicated by *.*

The pathogenicity of *V. cholerae* has been linked to only two key virulence factors – toxin co-regulated pili (TCP) and cholera toxin.[32] We used seven strains of *V. cholerae,* of which six harboured the genes responsible for cholera toxin production (Table 2). Four of the *ctxAB*-positive strains of *V. cholerae* contained the *tcpA* gene while the other two strains (UG01, Brits) were negative for both the *rfbO1* and *rfbO139* genes (Table 2). The latter were then categorised as non-O1/O139 toxigenic strains of *V. cholerae*. The UG01 strain could not be characterised as either El Tor or classical as it was found to be positive for both *tcpA* genes. It is possible that this strain of *V. cholerae* is an El Tor/classical hybrid strain. El Tor variants of the O1/O139 *V. cholerae* have been isolated from Asia and Africa[33,34] but there is no report on the existence of altered El Tor variants that are non-O1/O139. The other non-O1/O139 toxigenic strain (BRITS01) did not contain the *tcpA* gene, meaning that CTXΦ may have found an alternative route into this strain. Besides horizontal gene transfer, the current theory is that the TCP was the only route through which CTXΦ could gain access into the *V. cholerae* cell. However, recent studies by Campos et al.[35,36] have shown that two novel filamentous bacteriophages (VEJΦ and VGJΦ) could also be used to transduce CTXΦ amongst *V. cholerae* strains that express the mannose-sensitive haemagglutinin (MSHA) receptor. The MSHA receptor is ubiquitously expressed on the surface of many *V. cholerae* strains and the identification of these novel bacteriophages may explain the existence of non-O1/O139 strains of *V. cholerae* containing CTXΦ. Furthermore, this may also explain the sporadic cholera outbreaks that have recently been attributed to non-O1/O139 strains of *V. cholerae*.[37,38] Therefore the developments of affordable early detection systems for *V. cholerae* in the environment are necessary to prevent cholera outbreaks.

Exposure of the toxigenic strains of *V. cholerae* to SUVR showed that the bacteria did not produce detectable amounts of the cholera toxin in the water. Furthermore, the RT-PCR results confirmed that the integrity of the DNA significantly deteriorated as the quality of amplifiable DNA decreased (Table 3). These findings are contrary to what has been observed by other researchers in regard to cholera toxin secretion. Quinones et al.[21,22] reported that the SOS DNA repair resulting from DNA damaging agents such as UV light induced the multiplication of CTXΦ and increased the expression of cholera toxin. Similarly, Faruque et al.[23] showed that the exposure of *V. cholerae* to sunlight resulted in an increase of viral titres, which has also been linked to cholera toxin production.[21,22] Solar radiation contains UV light which is a DNA damaging agent[19,20] and hence could initiate the SOS DNA repair thereby increasing multiplication of CTXΦ as well as expression of cholera toxin. However, both these studies exposed *V. cholerae* to conditions containing a DNA damaging agent in a nutrient-rich environment and for shorter periods. It is also known that a nutrient-rich environment is capable of counteracting the oxygen radicals responsible for the deleterious effects on the microbial cell membrane, which thereby reduces the impact of SUVR on cellular targets.[39] These findings suggest that cells that have undergone cellular damage as a result of SUVR in a nutrient-rich environment are capable of repairing themselves. In our study, all *V. cholerae* strains were solar irradiated for longer periods in water that could be regarded as a nutrient-poor environment. Therefore the bacterial cells received a full dosage of SUVR and were afforded no protection in comparison with cells growing in a nutrient-rich medium.

In conclusion, we have shown that the exposure of toxigenic strains of *V. cholerae* to SUVR, as recommended for SODIS, reduces the quantity of amplifiable DNA and inactivates the culturability of these microorganisms.[14] However, the major finding from this study was the inability of the pathogenic strains of *V. cholerae* to secrete cholera toxin above the baseline detection limit following SUVR. The detection limit used in this study (10 pg/mL) was lower than the lethal dose (LD_{50}) of 250 μg of cholera toxin needed to cause cholera in mice.[40] This finding is encouraging to communities that may rely on SODIS for drinking water as a short-term intervention, especially in places where cholera outbreaks are frequent.

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Authors' contributions

M.P. was the project leader and made conceptual contributions. C.C.S. and W.J. were responsible for the design and execution of the experiments. C.C.S. conceptualised the study and wrote the manuscript.

References

1. Blow NS, Salomon RN, Garrity K, Reveillaud I, Kopin A, Jackson FR, et al. *Vibrio cholerae* infection of *Drosophila melanogaster* mimics the human disease cholera. PLoS Pathog. 2005;1(1):e8. http://dx.doi.org/10.1371/journal.ppat.0010008
2. World Health Organization (WHO). Progress on drinking water and sanitation: 2012 update. Geneva/New York: WHO/United Nations Children's Fund; 2012. p. 58. Available from: http://www.who.int/water_sanitation_health/publications/2012/jmp_report/en/index.html
3. World Health Organization (WHO). Outbreak bulletin. Geneva: WHO; 2011. p. 5. Available from: http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/outbreak-news/3066-outbreak-bulletin-vol-1-issue-2-july-19-2011.html
4. Siddique AK, Akram K, Zaman K, Mutsuddy P, Eusof A, Sack RB. *Vibrio cholerae* O139: How great is the threat of a pandemic? Trop Med Int Health. 1996;1(3):393–398. http://dx.doi.org/10.1046/j.1365-3156.1996.d01-54.x
5. World Health Organisation (WHO). 1998-Cholera-*Vibrio cholerae* O139 strain. Geneva: WHO; 1998. Available from: http://www.who.int/csr/don/1998_09_22/en/index.htmL
6. Nasreldin E, Layla B, Mohammed A, Burhan I, Faisal A, Obeid ET, et al. Molecular characterization of *Vibrio cholerae* non-O1/non-O139 isolated from coastal water of Arabian Gulf. Afr J Microbiol Res. 2012;6(2):447–452.
7. Sheikh AF, Goodariza H, Aslani S. Identification of *Vibrio cholerae* pathogenicity island (ctxA, OmpW and tcpA) in non-O139 and non-O1 *V. cholerae* strains isolated from Karun River in Ahvaz, Iran. Afr J Microbiol Res. 2012;6(6):1185–1189.
8. Aliabad NH, Bakhshi B, Pourshafie MR, Sharifnia A, Ghorbani M. Molecular diversity of CTX prophage in *Vibrio cholerae*. Lett Appl Microbiol. 2012;55(1):27–32. http://dx.doi.org/10.1111/j.1472-765X.2012.03253.x
9. Mekalanos JJ. Duplication and amplification of toxin genes in *Vibrio cholerae*. Cell. 1983;35(1):253–263. http://dx.doi.org/10.1016/0092-8674(83)90228-3
10. Sanchez J, Holmgren J. Cholera toxin structure, gene regulation and pathophysiological and immunological aspects. Cell Mol Life Sci. 2008;65(9):1347–1360. http://dx.doi.org/10.1007/s00018-008-7496-5
11. Aoki H, Wu H, Nakano T, Ooi Y, Daikoku E, Kohno T, et al. Nanotransportation system for cholera toxin in *Vibrio cholerae* O1. Med Mol Morphol. 2009;42(1):40–46. http://dx.doi.org/10.1007/s00795-008-0431-x
12. Berney M, Weilenmann HU, Simonetti A, Egli T. Efficacy of solar disinfection of *Escherichia coli, Shigella flexneri, Salmonella Typhimurium* and *Vibrio cholerae*. J Appl Microbiol. 2006;101(4):828–836. http://dx.doi.org/10.1111/j.1365-2672.2006.02983.x
13. Bosshard F, Berney M, Scheifele M, Weilenmann H-U, Egli T. Solar disinfection (SODIS) and subsequent dark storage of *Salmonella typhimurium* and *Shigella flexneri* monitored by flow cytometry. Microbiology. 2009;155(4):1310–1317. http://dx.doi.org/10.1099/mic.0.024794-0
14. Ssemakalu CC, Pillay M, Barros E. The effect of solar ultraviolet radiation and ambient temperature on the culturability of toxigenic and non-toxigenic *Vibrio cholerae* in Pretoria, South Africa. Afr J Microbiol Res. 2012;6(30):5957–5964. http://dx.doi.org/10.5897/AJMR12.601
15. Conroy RM, Meegan ME, Joyce T, McGuigan K, Barnes J. Solar disinfection of drinking water protects against cholera in children under 6 years of age. Arch Dis Child. 2001;85(4):293–295. http://dx.doi.org/10.1136/adc.85.4.293
16. Du Preez M, McGuigan KG, Conroy RM. Solar disinfection of drinking water in the prevention of dysentery in South African children aged under 5 years: The role of participant motivation. Environ Sci Technol. 2010;44(22):8744–8749. http://dx.doi.org/10.1021/es103328j

17. Boyle M, Sichel C, Fernández-Ibáñez P, Arias-Quiroz GB, Iriarte-Puña M, Mercado A, et al. Bactericidal effect of solar water disinfection under real sunlight conditions. Appl Environ Microbiol. 2008;74(10):2997–3001. http://dx.doi.org/10.1128/AEM.02415-07

18. Ubomba-Jaswa E, Boyle MAR, McGuigan KG. Inactivation of enteropathogenic *E. coli* by solar disinfection (SODIS) under simulated sunlight conditions. J Phys Conf Ser. 2008;101:012003. http://dx.doi.org/10.1088/1742-6596/101/1/012003

19. Bosshard F, Bucheli M, Meur Y, Egli T. The respiratory chain is the cell's Achilles' heel during UVA inactivation in *Escherichia coli*. Microbiology 2010;156(7):2006–2015. http://dx.doi.org/10.1099/mic.0.038471-0

20. Bosshard F, Riedel K, Schneider T, Geiser C, Bucheli M, Egli T. Protein oxidation and aggregation in UVA-irradiated *Escherichia coli* cells as signs of accelerated cellular senescence. Environ Microbiol. 2010;12(11):2931–2945. http://dx.doi.org/10.1111/j.1462-2920.2010.02268.x

21. Quinones M, Davis BM, Waldor MK. Activation of the *Vibrio cholerae* SOS response Is not required for intestinal cholera toxin production or colonization. Infect Immun. 2006;74(2):927–930. http://dx.doi.org/10.1128/IAI.74.2.927-930.2006

22. Quinones M, Kimsey HH, Ross W, Gourse RL, Waldor MK. LexA represses CTXΦ transcription by blocking access of the C-terminal domain of RNA polymerase to promoter DNA. J Biol Chem. 2006;281(51):39407–39412. http://dx.doi.org/10.1074/jbc.M609694200

23. Faruque SM, Asadulghani, Rahman MM, Waldor MK, Sack DA. Sunlight-induced propagation of the lysogenic phage encoding cholera toxin. Infect Immun. 2000;68(8):4795–4801. http://dx.doi.org/10.1128/IAI.68.8.4795-4801.2000

24. Berney M, Weilenmann H-U, Egli T. Flow-cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS). Microbiology. 2006;152(6):1719–1729. http://dx.doi.org/10.1099/mic.0.28617-0

25. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. J Hyg (Lond). 1938;38(6):732–749. http://dx.doi.org/10.1017/S002217240001158X

26. Nandi B, Nandy RK, Mukhopadhyay S, Nair GB, Shimada T, Ghose AC. Rapid method for species-specific identification of *Vibrio cholerae* using primers targeted to the gene of outer membrane protein OmpW. J Clin Microbiol. 2000;38(11):4145–4151.

27. Goel AK, Tamraker A, Nema V, Kamboj D, Singh L. Detection of viable toxigenic *Vibrio cholerae* from environmental water sources by direct cell duplex PCR assay. World J Microbiol Biotechnol. 2005;21(6–7):973–976. http://dx.doi.org/10.1007/s11274-004-7317-4

28. Le Roux WJ, Van Blerk GN. Use of a high resolution melt real-time polymerase chain reaction (PCR) assay for the environmental monitoring of *Vibrio cholerae*. Afr J Microbiol Res. 2011;5(21):3520–3526.

29. Mukhopadhyay AK, Chakraborty S, Takeda Y, Nair GB, Berg DE. Characterization of VPI pathogenicity island and CTXphi prophage in environmental strains of *Vibrio cholerae*. J Bacteriol. 2001;183(16):4737–4746. http://dx.doi.org/10.1128/JB.183.16.4737-4746.2001

30. Hoshino K, Yamasaki S, Mukhopadhyay AK, Chakraborty S, Basu A, Bhattacharya SK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. FEMS Immunol Med Microbiol. 1998;20(3):201–207. http://dx.doi.org/10.1111/j.1574-695X.1998.tb01128.x

31. Centers for Disease Control and Prevention (CDC). Laboratory methods for the diaganosis of *Vibrio cholerae*. Atlanta, GA: CDC; 2012. Available from: http://www.cdc.gov/cholera/laboratory.htmL

32. Davis BM, Waldor MK. Filamentous phages linked to virulence of *Vibrio cholerae*. Curr Opin Microbiol. 2003;6(1):35–42. http://dx.doi.org/10.1016/S1369-5274(02)00005-X

33. Hasan NA, Choi SY, Eppinger M, Clark PW, Chen A, Alam M, et al. Genomic diversity of 2010 Haitian cholera outbreak strains. Proc Natl Acad Sci USA. 2012;109(29):E2010–E2017. http://dx.doi.org/10.1073/pnas.1207359109

34. Taneja N, Mishra A, Sangar G, Singh G, Sharma M. Outbreaks caused by new variants of *Vibrio cholerae* O1 El Tor, India. Emerg Infect Dis. 2009;15(2):352–354. http://dx.doi.org/10.3201/eid1502.080943

35. Campos J, Martinez E, Marrero K, Silva Y, Rodriguez BL, Suzarte E, et al. Novel type of specialized transduction for CTXΦ or its satellite phage RS1 mediated by filamentous phage VGJΦ in *Vibrio cholerae*. J Bacteriol. 2003;185(24):7231–7240. http://dx.doi.org/10.1128/JB.185.24.7231-7240.2003

36. Campos J, Martinez E, Izquierdo Y, Fando R. VEJΦ, a novel filamentous phage of *Vibrio cholerae* able to transduce the cholera toxin genes. Microbiology. 2010;156(1):108–115. http://dx.doi.org/10.1099/mic.0.032235-0

37. Kirschner AKT, Schlesinger J, Farnleitner AH, Hornek R, Süß B, Golda B, et al. Rapid growth of planktonic *Vibrio cholerae* non-O1/non-O139 strains in a large alkaline lake in Austria: Dependence on temperature and dissolved organic carbon quality. Appl Environ Microbiol. 2008;74(7):2004–2015. http://dx.doi.org/10.1128/AEM.01739-07

38. Nasreldin E, Layla B, Mohammed A, Burhan I, Faisal A, Obeid ET, et al. Molecular characterization of *Vibrio cholerae* non-O1/non-O139 isolated from coastal water of Arabian Gulf. Afr J Microbiol Res. 2012;6(2):447–452.

39. Navntoft C, Ubomba-Jaswa E, McGuigan KG, Fernández-Ibáñez P. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions: Natural well-water and solar light. J Photochem Photobiol B Biol. 2008;93(3):155–161. http://dx.doi.org/10.1016/j.jphotobiol.2008.08.002

40. Gill DM. Bacterial toxins: A table of lethal amounts. Microbiol Rev. 1982;46(1):86–94.