Influence of solar water disinfection on immunity against cholera

Authors: Cornelius Cano Ssemakalu [1], Eunice Ubomba-Jaswa[2], Keolebogile Shirely Motaung [3] and Michael Pillay [*1]

1 Faculty of Applied and Computer Sciences, Vaal University of Technology, Vanderbijlpark 1900, South Africa

2 Council for Scientific and Industrial Research, Natural Resource and the Environment, P.O.Box 395, Pretoria 0001, South Africa

3 Department of Biomedical Sciences, Tshwane University of Technology, 175 Nelson Mandela Drive, Arcadia Campus, Pretoria 0001, South Africa.

***For Correspondence, E-mail: mpillay@vut.ac.za Tel. (+27) 16 950 9614; Fax: +27 16 950 9794**

Abstract

Cholera remains a significant problem in developing countries. This is attributed to the unavailability of proper water treatment and sanitary infrastructure. As a consequence, countries facing a cholera outbreak rely on interventions such as the use of oral rehydration therapy, antibiotics, vaccination and the provision of chlorine tablets to save lives and prevent new cholera infections. These interventions have been accepted but their implementation remains a challenge due to constraints associated with the cost, ease of use and technical knowhow. These challenges have been significantly reduced through the use of solar water disinfection. The success of solar water disinfection in mitigating the risk associated with the consumption of waterborne pathogens has mainly been associated with solar irradiation. This has prompted a lot of focus on the solar component for enhanced disinfection. However the role played by the host immune system following the consumption of solar irradiated water pathogens has not received any significant attention. The mode of inactivation resulting from the exposure of microbiologically contaminated water results in immunologically important microbial states as well as components. In this review, the possible influence that solar water disinfection may have on the immunity against cholera is discussed.

Keywords: Cholera, SODIS, Solar Ultraviolet Radiation, Vaccine, *V. cholerae,* Waterborne disease

Introduction

Cholera is a life threatening waterborne disease characterised by secretory diarrhoea often accompanied by vomiting (Osei & Duker 2008). It is estimated that there are 5 million cases of cholera resulting in approximately 130,000 fatalities per year globally (WHO 2010). Cholera is spread through faecal contamination of water and food and is generally prevalent in resource poor communities due to the lack of basic sanitary infrastructure and limited or no access to potable water. Various measures such as the provision of basic sanitary infrastructure and treated piped water, construction of village hospitals and immunisation have been proposed to prevent cholera outbreaks and epidemics. However, their implementation remains a global challenge (Echeverria*, et al.* 1983, WHO 2011, WHO 2012). During an actual cholera outbreak or epidemic it is almost impossible to implement the previously mentioned prevention measures. But, interventions that result in the prevention of new infections and saving of lives may be required. Such interventions should enable the active participation of all tiers of the affected society.

Currently, the use of Oral Rehydration Therapy (ORS), antibiotics, and to an extent, vaccination has been recommended as interventions to save lives and prevent new infections (WHO 2010, Date*, et al.* 2011). Although these interventions are acceptable their implementation remains a challenge due to constraints such as the cost of execution, ease of use and technical knowhow. ORS requires trained personnel on site to prepare the solution. Alternatively, ORS sachets could be purchased and distributed to the population facing a cholera outbreak or epidemic. Antibiotics could be used to treat patients with cholera. However, this intervention is threatened by the emergence of more virulent strains of *Vibrio cholerae* that may be resistant to the readily available antibiotics (WHO 2010).

Vaccines may have the potential to prevent new infections if they are readily available. The unavailability of vaccines could be attributed to the costs and logistics involved in their preparation, shipping and storage (Date*, et al.* 2011) as well as their multi-dose regimen (Date*, et al.* 2011, William 2011). Furthermore, the vaccines may not be as efficacious in the affected community as previously documented in clinical trials done elsewhere (Shahjahan 2005, Ryan*, et al.* 2006, WHO 2010). Vaccination of infected persons is also complicated by issues concerning the vaccination schedule and whether the affected population should stop using water from their current sources while they wait for subsequent doses of the vaccine.

Clearly an intervention that could significantly reduce the burden associated with cost is required. Such an intervention should also be easy to use, sustainable (McGuigan*, et al.* 2012) and compatible to the life style of the people living in the affected community. Solar water Disinfection (SODIS) is one intervention that satisfies these criteria and could be used in conjunction with the currently available prevention and crisis control interventions.

Solar water disinfection

SODIS is a process in which the quality of drinking water is improved through exposure to natural sunlight in transparent vessels for a period of 6 to 8 hours on clear days and for two days during cloudy weather (Heaselgrave*, et al.* 2006, Boyle*, et al.* 2008, Navntoft*, et al.* 2008, Ubomba-Jaswa*, et al.* 2008). The process by which the disinfection occurs seems quite easy and straight forward although the underlying mechanisms are complex. Effective bacterial inactivation is judged by the inability of the microorganisms to form colonies after SODIS treatment (Smith*, et al.* 2000). Downes and Blunt (1877) were the first to present empirical evidence of the bactericidal effect of sunlight; however, its use to sanitise water

87 can be traced as far back as 2000BC. Presently, Downes and Blunt's (1877) observations
88 regarding the bactericidal effect of solar radiation have been refined and tested in the field
89 by various research teams with subsequent implementation in various countries
90 (Eawag/Sandec 2008). Studies by Acra et al. (1989) and Conroy et al. (1996) showed that the
91 bactericidal effect resulting from solar radiation was due primarily to the ultraviolet
92 component of sunlight.

93 Ultra Violet A (UVA) the most abundant component of Solar Ultra Radiation (SUVR) reaching
94 the earth's surface enables the formation of reactive oxygen species such as superoxide
95 radicals, hydroxyl radicals, hydrogen peroxide and singlet oxygen. These reactive molecules
96 also known as photosensitisers are formed through a process known as photo-oxidation
97 (Elasri & Miller 1999, Sinton*, et al.* 1999, Qiu*, et al.* 2004, Navntoft*, et al.* 2008). During
98 SODIS, the interaction between the photosensitisers and the actively growing
99 microorganism results in irreversible damage to the microbial catalyase systems rendering
100 them susceptible to damage from peroxide formation (Bailey*, et al.* 1983, Alonso-Sáez*, et al.*
101 2006). Furthermore, UVA through photo-oxidation blocks the electron transport chain
102 incapacitating ATP synthesis; induces damage to the cell membrane thus inactivating
103 transport systems; interferes with metabolic energy production and causes single strand
104 breaks in DNA (Berney*, et al.* 2006, Bosshard*, et al.* 2010a, Bosshard*, et al.* 2010b). On the
105 whole, UVA causes indirect multi-target damage to the microbial cellular components such
106 as DNA, protein and lipids through the formation of photosensitisers (Joux*, et al.* 1999).

107 Despite the fact that biological systems exposed to SUVR causes reduced functionality and
108 destruction, there are protective mechanisms in cells that are capable of reversing some of
109 this damage especially at the DNA level. A number of different DNA repair mechanisms

relevant to SUVR damage have been established including photo-reactivation repair, nucleotide excision repair and post replication repair and SOS repair (Diffey 1991, Arrage*, et al.* 1993, Joux*, et al.* 1999). However, these repair mechanisms are all dependent on the dose of SUVR (Bosshard*, et al.* 2010a), the environment of exposure (Faruque*, et al.* 2006, Quinones*, et al.* 2006, Ssemakalu 2011) as well as cellular targets.

Impact of SODIS on the spread of waterborne diseases

The consumption of SODIS water in sub-Saharan African and various East Asian countries has reduced the percentage of individuals acquiring water borne diseases such as dysentery typhoid and cholera (Conroy*, et al.* 1996, Conroy*, et al.* 2001, Du Preez*, et al.* 2010). This has been attributed mainly to the ability for SUVR to inhibit the growth of the contaminating microorganisms, viruses such as poliovirus and giardia cysts (Heaselgrave*, et al.* 2006, Heaselgrave & Kilvington 2012). The effect of SUVR on the pathogens is not dependent on their antibiotic status. Furthermore, sunlight the primary source of SUVR is readily available in waterborne disease endemic regions.

The epidemiological benefits of consuming SODIS water go beyond the technique and biology of microbial inactivation. Therefore it is important to consider the immunological effects that may arise from the consumption of SODIS water as an integral aspect of the overall benefits. The nature of the microbial constituents in water following SODIS is ambiguous (Bosshard*, et al.* 2009, Bosshard*, et al.* 2010b, Ssemakalu 2011) but may present an assortment of microbial antigenic determinants or epitopes. The consumption of SODIS water may result in an immune reaction and/or an immune response depending on how the microbial epitopes are received and processed by the cells of the immune system.

The effect of SODIS water on human mucosal immunity

The consumption of SODIS water is of great relevance to the intestinal mucosa. In this environment, a thin layer of epithelial cells separates the inner corpus from the surrounding environment. The antigen-antibody effect of SODIS occurs in the intestinal mucosal environment. The prospective antigens in SODIS water are acquired by Antigen Presenting Cells (APCs) and transported to the mesenteric lymph nodes as well as the numerous small isolated lymphoid follicles along the wall of the intestine for presentation to T-cells. Following the presentation of the antigens by the APC, the T cells are then activated with subsequent migration to all the non-lymphoid tissues (Lefrancois & Puddington 2006). An even more important component of the immune system of intestinal mucosal environment is the lamina propia (LP) tissue. The LP is a connective tissue beneath the basement membrane supporting the overlying epithelial cells of the small and large intestine. This tissue is rich in various cells of both the innate and adaptive immune system such as APCs as well as T-cells (Rescigno*, et al.* 1998, Guermonprez*, et al.* 2002, Trombetta & Mellman 2005, Lefrancois & Puddington 2006). In the presence of any foreign material arising from the consumption of SODIS water; it is highly probable that this material may be engaged by the cells of the immune system. But the extent of this engagement still remains unknown.

The nature of antigens derived from SODIS water

Given the complex nature of the constituents of SODIS water and the possible influence it may have on the immune system, it is important to consider three crucial factors discussed by Pradeu and Edgardo (2006). The first factor requires consideration of the quantity of the antigens. In this regard it is widely known that a low antigen dose would not trigger a

154 sufficient immune response simply because the generation of antigen specific regulatory
155 cells is favoured (Faria & Weiner 2005). This could be the case with SODIS users during
156 periods of an absence of outbreaks and epidemics. During such periods the concentration of
157 *V. cholera* in the water that a community utilises is often low (Ryan & Calderwood 2000). On
158 the other hand, during outbreaks or epidemics the bacterial load in untreated water is high
159 enough to cause a waterborne disease. For instance, it would take between 9 and 11 logs of
160 *V. cholerae* cells to infect a healthy individual whereas in individuals with hypochlorhydria
161 between 4 and 6 logs are required to cause cholera. The infected individuals excrete almost
162 13 logs of *V. cholerae* cells in their stool per day (Ryan & Calderwood 2000). This results in a
163 rapid dissemination of the infection in the population because of the unavailability of
164 adequate sanitary facilities. Solar irradiation has been shown to effectively inactivate a
165 significant amount of *V. cholerae* cells from a bacterial dose comparable to that required to
166 cause a cholera infection (Ssemakalu*, et al.* 2012). Therefore it is possible that individuals
167 that rely on SODIS to decontaminate their water during a cholera outbreak or epidemic,
168 access a high antigen dose of *V. cholerae*. This may result in the generation of a proper
169 immune response. Alternatively such a high antigen dose may result in the
170 unresponsiveness in T cell function through anergy/deletion (Faria & Weiner 2005).

171 The second factor considers the degree of molecular difference between the new antigen
172 and the antigens with which the immune receptors constantly interact (Avci & Kasper 2009).
173 In developing countries, the consumption of waterborne disease causing microorganisms is
174 apparent. Communities that regularly consume waterborne pathogens such as *V. cholerae*
175 probably develop tolerance towards these pathogens (Svennerholm*, et al.* 1980). The
176 development of tolerance towards waterborne pathogens could make vaccines generated

177 from common pathogenic entities less effective amongst the SODIS users as well as
178 individuals in water borne endemic areas. Alternatively, SODIS treatment of water
179 containing pathogens may possibly result in beneficial alteration, accessibility and
180 preservation of the integrity of the possible epitopes. These epitopes may include proteins
181 such as the chitin binding protein A, outer membrane protein U and unsheathed flagella
182 (William 2011). Furthermore, the consumption of SODIS water during a waterborne disease
183 outbreak such as cholera, if at all immunogenic, derives its epitopes from the current status
184 of the microbial strain and hence may provide a relevant immune response.

185 The third factor to consider is the speed of appearance of the infrequent antigenic
186 determinants. SODIS may induce slow or extreme rapid modifications of the antigenic
187 epitopes thereby preventing the ability to prompt an immune response. It is also possible
188 that SODIS may provide the right conditions for the generation of critical modifications on
189 epitopes that could result in the induction of an immune response rather than an immune
190 reaction.

191 Considering the above factors, the consumption of SODIS water may result in three major
192 consequences discussed by Faria and Weiner (2005): i) a non-inflammatory response
193 marked by anti-inflammatory cytokine secretion, ii) the priming of a systematic immune
194 response involving the production of serum antibodies as well as proinflammatory
195 cytokines, and iii) a state of systemic and or local immunological tolerance.

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198 **Summary**

199 The views expressed in this manuscript do not aim to under look the relevance of SODIS in
200 underprivileged communities simply because there is a higher infection rate within non
201 SODIS users (Firth*, et al.* 2010, Graf*, et al.* 2010). Nonetheless it is imperative to substantiate
202 the role that SODIS water consumption may have on the immune system. Could it be
203 possible that the consumption of SODIS water may confer significant desirable
204 immunological effects onto the consumers? This may be true considering the benefits of
205 SODIS in the current literature. However, empirical evidence is required to substantiate all
206 the hypotheses put forward since the extent of protection that may be conferred onto the
207 SODIS water consumers remains unknown. There is almost no knowledge on how the
208 bacterial states following solar irradiation in water may influence antigen processing or
209 development of the antigen presenting cells. In our laboratory we are investigating some of
210 these hypotheses through studying the influence that antigens and bacterial states
211 generated through solar irradiation of *V. cholerae* may have on the immune system.

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