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Limitations of chlorine disinfection of human excreta: implications for Ebola disease control

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Various NGO guidelines suggest that human excreta may be disinfected by the application of concentrated (e.g., 0.5%) chlorine solutions. However, chlorine-based disinfectants are thought to rapidly lose their bactericidal and virucidal properties in contact with high levels of organic matter and chlorine application results in the production of toxic chlororganic compounds. To evaluate the disinfection efficacy of chlorine solutions (HTH, NaDCC and household bleach) against viruses and bacteria within excreta matrices, laboratory-scale disinfection experiments were undertaken. Human excreta matrices containing raw wastewater, with 0%, 10% and 20% (w/v) added faecal sludge, were disinfected with chlorine solutions at a ratio of 1:10 (chlorine solution: excreta matrix). Contact time was set at 30 minutes and bacterial (FC and IE) and viral (SOMPH) indicators were used to measure disinfection efficacy. Results demonstrated that at high levels of solids content, disinfection efficacy was significantly reduced. These results support the need to find a more effective means of disinfecting human excreta in future Ebola outbreaks.

Introduction

The first widespread Ebola virus disease (EVD) outbreak occurred between December 2013 and January 2016, in which a total of 28,638 cases and 11,316 deaths were recorded. Most cases were registered within West Africa, but the disease also spread to countries beyond West Africa, including the United States and some European nations (WHO, 2016a). Ebola is an enveloped, single-stranded RNA virus of the Filoviradae family (CDC, 2016). EVD is a severe illness in humans, having an average fatality rate of around 50 % (with a variance of between 25 % and 90% in past outbreaks). Symptoms are high fever, fatigue, muscle pain, headache and sore throat, followed by vomiting, diarrhoea, rash and, in some cases, internal and external bleeding. EVD is transmitted via direct contact (through broken skin or mucous membranes) with the blood, secretions, organs or other bodily fluids of infected people, and with surfaces and materials (e.g., bedding and clothing) that are contaminated with these fluids (WHO, 2016b).

One of the foci of the WASH response to the 2014 West African Ebola outbreak was to use chlorine disinfection in order to prevent ongoing EVD transmission at Ebola Treatment Centres and Units (ETC and ETU). Doctors without Borders (MSF), the U.S. Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) all recommend the use of 0.05% (500 mg/L) chlorine solution to disinfect living things (hands and people) and 0.5% (5,000 mg/L) solutions to disinfect non-living things (surfaces, personal protective equipment, excreta and dead bodies) (MSF, 2008; CDC, 2015; WHO, 2014). Chlorine source compounds commonly used in ETC are powdered calcium hypochlorite (HTH); granular sodium dichloroisocyanurate (NaDCC or SDIC) and liquid sodium hypochlorite (NaOCl) (domestic bleach). For each of these compounds, disinfection efficacy can vary, based on the concentration of the chlorine solution, contact time, temperature, pH level, and the presence of organic matter (Rutala and Weber, 2004).

Enveloped viruses, including the Ebola virus (EBV), are known to be relatively fragile and less resistant to environmental factors, such as ultraviolet radiation (Sagripanti and Lytle, 2011), than are enteric viruses. Furthermore, EBV was not detected, by nucleic acid amplification or culture assays, in non-bloody stool samples from African hospitals during the 2014 outbreak (CDC, 2014) and there has been no reported EBV

transmission via water or faeces, suggesting that EBV is not normally transmitted via the faeco-oral transmission pathway.

It remains uncertain as to whether EBV may be transmitted via wastewater. Bibby *et al.* (2014) performed experiments with sterilised wastewater that demonstrated that Ebola virus concentrations decreased by approximately 99% within one day. However, infectious particles were shown to persist throughout all eight days of the experiments. Casanova and Weaver (2015), using the enveloped bacteriophage phi 6 as an EBV surrogate, found that, depending on temperature, the phage could undergo a six to seven log inactivation in wastewater within three to seven days. Furthermore, hemorrhagic fevers, such as EBV, commonly have a very low infectious dose (of one to ten organisms by aerosol) (Franz *et al.*, 1997) and low concentrations in wastewater may be sufficient to cause catastrophic effects. Therefore, potential human exposure to infectious Ebola virus, via wastewater and human excreta, emphasises the need for a precautionary approach to the handling of wastewater and human excreta in an epidemic response.

As mentioned, precautionary action has previously been taken in some situations to disinfect human excreta/wastewater using concentrated chlorine solutions. EBV appears to be susceptible to several disinfectants used in health care facilities (Rutala and Weber, 2004) and a recent study by Cook *et al.* (2015) suggests that sodium hypochlorite solutions effectively decontaminate EBV (Mak variant) suspended in a simulated organic load on personal protective equipment (PPE) and stainless steel surfaces that are common to clinical settings. However, it has been posited that addition of chlorine compounds may be an ineffective way to disinfect media containing large amounts of solid and dissolved organic matter, because they lose their bactericidal and virucidal properties when they react with organic matter, forming combined chlorine residuals with relatively low disinfecting power. Additionally, there is a lack of information about how effective chlorine solutions may be at disinfecting human excreta and human wastewater from ETC and ETU according to those practices recommended by certain humanitarian agencies.

The research described here attempted to assess the efficacy of the human excreta disinfection protocols recommended by certain NGO for use in emergency settings. Laboratory-scale disinfection experiments were performed on simulated human excreta matrices containing varying levels of suspended and dissolved solids and employing chlorine solutions prepared from various commercially available chlorine compounds.

Methods

Information as to how to disinfect the excreta of Ebola patients in ETU and ETC safely and effectively is limited, but it has been suggested (MSF, 2008) that 0.5% chlorine solutions (5,000 mg/l) may be used. Accordingly, the disinfection processes should take place within twelve-litre capped buckets and contact time between the disinfectant and excreta should be at least fifteen, and possibly thirty minutes, before the bucket contents are disposed of to a sewerage system or the environment (MSF, 2008; CDC, 2015; WHO, 2014).

In an attempt to replicate these chlorine disinfection practices for human excreta, a series of laboratory-scale experiments was performed at the laboratories of the Environment & Public Health Research Group (EPHReG) of the University of Brighton (UK). In order to produce representative human excreta matrices, raw wastewater and dewatered faecal sludge were collected from Hailsham North Wastewater Treatment Works (UK) and kept in the fridge for no more than one week prior to experimental runs. Experiments took place in 50 ml polypropylene centrifuge tubes (Corning®, NY). Three tubes contained only the three excreta matrices (control) and nine tubes contained 45 ml of excreta matrix (x3) plus 4.5 ml of a chlorine solution (x3). Faecal indicator bacteria (faecal coliforms (FC) and intestinal enterococci (IE)) and a viral indicator (somatic coliphages (SOMPH)) were used to assess the efficacy of the disinfection processes.

Production of excreta matrices

A literature review was undertaken to elucidate the likely concentration of human excreta from healthy persons and EVD patients. This information was subsequently used to inform the production of human excreta simulants. Human excreta consist of faeces and urine and their physical and chemical characteristics depend on the amount and type of food consumed, as well as the health of the person excreting (Feachem et al., 1983). A healthy person usually excretes an average of 300 grammes of faeces and 1200 grammes of urine per day (Schouw et al. 2002; Torodel, 2010; Feachem et al., 1983), in other words a 1:4 dry/wet ratio. However, this ratio may vary radically in excreta from ETC/ETU, since most patients will have diarrhoea. According to Wenzl et al. (1995), the water content in patients with diarrhoea varies between 81.7 and 91.2 %, compared with 72 to 75.3 % in a healthy person. Furthermore, the total faecal weight per day in patients with diarrhoea can be two to three times higher than that of a healthy person. Chertow et al. (2014) observed

that EVD patients exhibit large volumes of watery diarrhoea, estimated at five or more litres per day, which could persist for up to seven days.

In order to produce appropriately simulated excreta matrices, raw wastewater and faecal sludge, respectively, were used as the liquid and solid fractions in the laboratory experiments described here. Three excreta matrices containing varying amounts of solid and dissolved organic matter were produced, representing a daily human excreta load (urine + faeces). Matrix 20% was composed of 80% raw wastewater (36 ml) plus 20% of faecal sludge (9 grammes) and represented excreta from a healthy person; Matrix 10% was composed of 90% raw wastewater (40.5 ml) plus 10% of faecal sludge (4.5 grammes) and represented excreta from a person suffering from mild diarrhoea; Matrix 0% was composed of 100% raw wastewater (45 ml) and represented excreta from a person suffering from severe diarrhoea.

Production of chlorine solutions

There is insufficient published information regarding the volume of chlorine solution to be used in the disinfection of the excreta of Ebola patients. Only the Filovirus Hemorrhagic Fever Guidelines (MSF, 2008) appear to provide information on the chlorine solution volumes to be applied to excreta. These state: "Collect waste in a bucket with 2 cm of 0.5 % chlorine solution. Add 0.5 % chlorine with a cup sufficient to cover the waste in a bucket for 15 minutes." From these MSF recommendations and assuming that buckets used in ETC have a radius measuring 11 cm, as is the case with 'OXFAM buckets', it was concluded that at least 759.88 (± 760.00) ml of chlorine solution should be used per bucket for excreta disinfection.

Three different chlorine solutions (0.5 % or 5,000 mg/L) were produced from calcium hypochlorite (HTH 65%, Mistral®), sodium dichloroisocyanurate (NaDCC 65%, Mistral) and sodium hypochlorite (NaOCl) from 'thin' Sainsburys® domestic bleach (1.5 %). On the day of each experiment, chlorine compounds were mixed with distilled water in 100 ml Schott bottles, which were tightly closed and agitated manually to dissolve the chlorine compounds. There was an interval of one hour before the Hach iodometric titration method 8209 (Digital Titration) (Hach Company, Loveland, CO) was employed to test whether the chlorine levels were adequate.

Production of sodium thiosulphate solution ($Na_2S_2O_3$)

Sodium thiosulphate pentahydrate ($Na_2S_2O_{35}H_2O$, BDH Chemicals) was used to prepare a dechlorination/neutralisation solution. This solution aimed not just to stop the disinfection reaction at the desired time but also to avoid residual chlorine from interfering during the enumeration of bacteria and bacteriophages. Hach free chlorine method 8021 (DPD) was performed to elucidate the residual chlorine in the excreta matrices and consequently to calculate the minimum sodium thiosulphate dose required to neutralise the samples.

Disinfection experiments

Experiments were performed at room temperature (22 ±1ºC) at a chlorine solution/excreta matrix ratio of 1:10. There were a total of four disinfection experiments, each using faecal material collected on different occasions. Excreta matrices were distributed among twelve tubes. Duplicate 1 ml samples from the three control tubes were taken to assess the initial levels of bacterial and viral indicators in the matrices. Subsequently, using sterile pipettes, 4.5 ml of chlorine solutions were transferred to the remaining nine tubes. The tubes were then closed with screw caps and inverted twice to allow the excreta matrix and chlorine solution to mix. The disinfection processes lasted for 30 minutes (contact time), after which time 5 ml of the neutralising agent sodium thiosulphate ($Na_2S_2O_3$) were added to the mix. Duplicate samples (1 ml) were then taken from all twelve tubes and bacterial and viral indicators were enumerated.

Enumeration of faecal indicator bacteria and bacteriophages

Excreta matrix samples were serially diluted (4-fold) in ¼ strength Ringer's solution. To measure levels of bacteria and bacteriophages in the "Initial" and "Control" samples, 10^{-3} and 10^{-4} dilutions were used; while for disinfected samples, 10^{0} and 10^{-1} dilutions were used. Enumeration of faecal coliforms (FC) and intestinal enterococci (IE) followed the ISO 9308/1:1999 and ISO 7899/2:2000 membrane filtration methods, respectively. Results were also expressed as colony-forming units per ml of excreta matrix (CFU/1ml). The procedures for enumerating somatic coliphages (SOMPH) were based on the double-agar-layer ISO 10705-2:2012 standardised method and results were expressed as plaque-forming units per ml of excreta matrix (PFU/1ml).

Results and discussion

Mean levels and the average (%) reduction of bacteria and bacteriophage concentrations before and after chlorine disinfection during all four experiments and in all excreta matrices are summarised in Table 1. The mean average percentage reduction of all microorganisms was also computed in order to provide an indication of the general levels of disinfection for each chlorine compound in the three excreta matrices. Post-chlorination control samples demonstrated similar microbial levels to the initial (pre-chlorination) control samples.

Table 1. Mean levels and percentage reduction of bacterial and viral indicators in excreta matrices pre- and post-chlorine disinfection

Excreta matrices	Mean levels (CFU or PFU/ml)			Mean reduction (%)			
	FC	IE	SOMPH	FC	IE	SOMPH	All
Initial Matrix 0%	15,150	14,763	8,138	-	-	-	-
Matrix 0% + HTH	0	0	9	100.0	100.0	99.6	99.9
Matrix 0% + NaDCC	11	4	7	99.9	100.0	99.7	99.9
Matrix 0% + Bleach	11	13	6	99.9	99.9	99.9	99.9
Matrix 0% + average all chlorine solutions	8	5	7	99.93	99.96	99.73	-
Initial matrix 10 %	37,500	14,738	12,313	-	-	-	-
Matrix 10% + HTH	1,232	173	190	98.4	99.1	98.3	98.6
Matrix 10% + NaDCC	792	95	146	98.9	99.2	98.7	99.0
Matrix 10% + Bleach	221	112	173	99.7	99.4	98.2	99.1
Matrix 10% + average all chlorine solutions	748	127	170	99	99.23	98.4	-
Initial matrix 20 %	59,250	36,350	16,488	-	-	-	-
Matrix 20% + HTH	1,660	637	1,290	98.2	97.5	95.3	97.0
Matrix 20% + NaDCC	1,275	1,374	425	98.2	93.3	96.1	95.9
Matrix 20% + Bleach	2,860	1,491	438	92.9	92.1	96.0	93.7
Matrix 20% + average all chlorine solutions	1,932	1,167	718	96.43	94.3	95.8	-
Average initial levels of all matrices	37,300	21,950	12,313	98.4	97.8	98.0	-

FC were demonstrated to be the most abundant microorganism analysed in the excreta matrices, followed by IE and SOMPH, respectively. Matrix 20% exhibited the highest concentration of all microorganisms, followed by matrix 10% and Matrix 0%. Chlorine solutions effectively disinfected matrix 0% (raw wastewater), but the level of disinfection efficacy decreased as the amounts of suspended and dissolved solids increased (Matrices 10% and 20% respectively).These results were expected, as various constituents of the excreta matrices (e.g., organic compounds, including humic materials, ammonia, nitrite, nitrate, iron and manganese) can exert a chlorine demand, therefore reducing the disinfection efficacy of the chlorine solutions (Metcalf & Eddy, 2003). Overall disinfection levels (% reduction) in all three matrices are presented in Figure 1. Average reduction levels for each microorganism ranged from 100% (in Matrix 0% disinfected by HTH or NaDCC solutions) to 92.1% (in Matrix 20% disinfected by bleach solution). Overall microorganism disinfection levels ranged from 99.9% (for all chlorine compounds in Matrix 0%) to 93.7% (in Matrix 20% disinfected by domestic bleach). IE appeared to be the most resistant microorganism to chlorination (97.8% reduction) of those tested, followed by somatic coliphages (98 %) and faecal coliforms

(98.4%). At first glance, it may appear that chlorine solutions effectively disinfect human faecal matrices. However, when the data are analysed in detail, it becomes evident that a considerable quantity of bacteria and viruses (phages) are capable of surviving in excreta Matrices 10% and 20% following the disinfection protocol (e.g., an average of 1,932 indicator bacteria/ml and 718 bacteriophages/ml in the disinfected Matrix 20%).

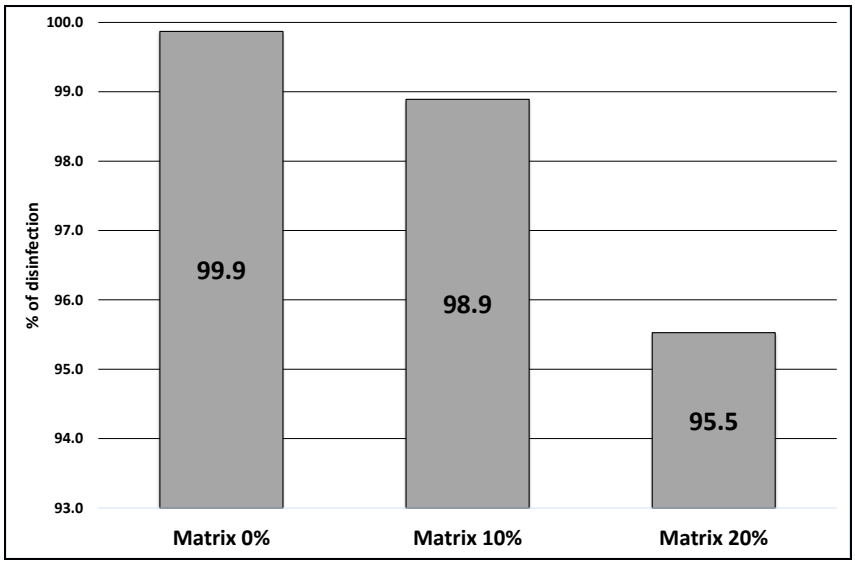

Figure 1. Mean levels (%) of disinfection (bacteria and virus) achieved by all chlorine solutions in each excreta matrix

Conclusions

This study demonstrated that whereas concentrated chlorine solutions have the potential to disinfect human faecal excreta, their efficacy decreases markedly as levels of dissolved and suspended solids in the excreta matrix increase. This observation supports recent concerns regarding the use of chlorine solutions to disinfect human excreta in Ebola treatment settings and all disease outbreak settings. There is a concerted international effort to find an alternative disinfectant for human excreta for use in emergencies. To this effect, ongoing laboratory experiments are being conducted by the authors of this study. These focus on testing a physicochemical (hydrated lime slurry) disinfection process (WHO, 2014b) that was previously effectively used during the emergency situation in Haiti following its 2010 cholera outbreak (Sozzi *et al.* 2015). Furthermore, this alternative disinfectant is being compared with existing chlorine disinfection protocols in larger "bucket-scale" experiments and results of these studies will soon be made available.

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