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Evaluation of *Escherichia coli* as Indicator of Point-of-Use Chlorination Efficiency of Drinking Water

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ABSTRACT: In this study, the relevance of the presence of *Escherichia coli* in drinking water as an indicator of point-of-use chlorination efficiency is examined. The survival of clinical isolates of human enteric pathogenic bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Vibrio cholerae*) as well as *E. coli* was monitored as a function of effective germicidal concentration and contact time. The inactivation kinetics indicated that the minimum effective dose for three-log units (99.9%) inactivation of *E. coli* ($C \cdot T_{99.9\%}$ = 10 mgl^{-1}-min) can sufficiently eliminate the other pathogens ($C \cdot T_{99.9\%}$ ranged from 5.6–10.5 mgl^{-1}-min); the exception being *K. pneumoniae*, which required more than 1.4-times higher dose. In general, the results implied that the branded hypochlorite solution should effectively inactivate almost all vegetative bacteria in household drinking water at the manufacturer's recommended dosage of 0.5 mgl^{-1} after at least 30 minutes contact time. The application of point-of-use chemical disinfectants to drinking water in households will significantly reduce the incidence of water-borne infections particularly in rural communities where central treatment of water is mostly unavailable. © JASEM

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Over one billion people lack access to an improved drinking water supply translating to an average of one in six persons in the world. Water-borne diseases have the greatest health impact worldwide with almost 90% of the 4 billion annual diarrheal disease cases attributed to unsafe water and inadequate sanitation and hygiene (World Health Organization, 2010b). Death resulting from diarrhea is estimated at almost 2 million each year. This burden is mostly felt in the developing world among the poorer urban and rural households of the poorer countries (World Health Organization, 2010a). The World Health Organisation (WHO) also estimates that 94% of these diarrheal cases are preventable through modifications to the environment, including access to safe water and good sanitation practices. Simple techniques for treating water at home, such as chlorination, the use of filters, and solar disinfection (Conroy *et al*., 2001; Rose *et al*., 2006), and storing it in safe containers (Eniola *et al*., 2007; Olayemi *et al*., 2005) could save a huge number of lives each year.

Disinfection with chlorine is of unquestionable importance in the provision of safe drinking water; it is a final barrier in water treatment (The Chlorine Institute, 2004). Chlorine does not only ensure the destruction of microbial pathogens, it also confers, unlike UV radiation or ozonation, some residual disinfectant on the water to reduce the risk of microbial regrowth and the health risk of recontamination. One other advantage of hypochlorite is that while it may compare well with the efficiency of ozone and chlorine dioxide it does not produce bromate like ozone. However, it may be less effective against *Cryptosporidium parvum* and *Giardia lamblia* (Facile *et al*., 2000). Several studies have reported varying efficiency of hypochlorite against bacteria, viruses and fungi (Barbeau *et al*., 2006) and others have been conducted to evaluate the impact of water quality on chlorine chemistry and disinfection efficacy (Barbeau *et al*., 2004) but none has reported acquired microbial resistance to it. Free chlorine residual of 0.2–0.5 mgl^{-1} is usually desirable at the end user's point however, in situations where the source water is practically exposed to feacal contamination the concentration of free chlorine could be increased to > 0.5 mgl^{-1} (World Health Organization, 2008). Chlorine solution, because of its easy of application is by far the most commonly used point-of-use drinking-water disinfectant; it can be easily monitored and controlled (The Chlorine Institute, 2004).

In developing countries, various portable chlorine-based disinfection products, packaged as affordable means of point-of-use water treatment for small households or in emergencies, could be an alternative solution for providing potable water to the populace. One of such safe water purification solutions is branded *Water Guard* and it contains 1% sodium hypochlorite (NaOCl) in solution as the active agent. It is recommended that 1 capful (≈ 5ml) of *Water Guard* solution in 25 L of 'clean and clear' drinking water will inactivate pathogens. However, drinking water sources from surface water and groundwater under the influence of surface water which sometimes

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may have high organic matter content and pH, may require filtration before sodium hypochlorite is applied, to prevent formation of potentially harmful disinfection by products (DBPs) (Adams *et al*., 2005). This form of multi-barrier approach to water treatment is important to enhance disinfection efficiency.

The objective of this work was to evaluate the validity of using *E. coli* as indicator of the efficiency of point-of-use chlorine-based disinfection of drinking water.

MATERIALS AND METHODS

Bacteria: Clinical isolates of human enteric pathogenic bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Vibrio cholerae* used in this study were obtained from the stock collection culture centre of the Medical Microbiology and Parasitology Laboratory, University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. The purity of the cultures was ascertained by series of streaking on nutrient agar and confirmatory identification done by carrying out some specific morphological and biochemical tests on the isolates (Cruickshank *et al*., 1980; Holt *et al*., 1994). Axenic bacterial cultures were grown in separate nutrient broth flasks placed on a rotary shaker (100 rpm) for 20–24 h at 30^{o}C until they were well into the stationary phase. Cells were harvested by centrifugation at 10,000 *g* for 10 minutes; supernatant was discarded and cell filtrate re-suspended in sterile sodium phosphate buffer and washout repeated thrice as to remove remnants of broth nutrients. Thereafter, harvested cells were re-suspended in sterile buffered water and filtered through Whatmann's filter paper (1.0-µm-pore Nucleopore polycarbonate membrane) to remove aggregated groups of bacteria. The concentration of the filtrate was standardized using McFarland's technique with a UV/vis spectrophotometer at 600 nm in a 1-cm quartz cell (Spectronic 20 model). The standardised filtrate (containing *ca.* 2 x 10^5 CFU/ml) was used in the hypochlorite inactivation studies. This bacterial concentration forms an average of the range of populations of microorganisms previously reported in untreated drinking water sources such as shallow wells and boreholes in Ilorin Metropolis, Nigeria (Igunnugbemi *et al*., 2004).

Demand-free water and glassware: Chlorine demand-free water (water for injection) pre-treated through ultra-filtration (UF) and reverse osmosis (RO) systems was supplied by Intravenous Infusion Manufacturing Company in Ilorin, Nigeria and collected in sterile, 1.0L chlorine demand-free Erlenmeyer flasks. The treatment procedure ensured that the test water has negligible chlorine demand. Demand-free glassware was prepared by exposing glassware to water containing 10 mg l^{-1} chlorine for 3 h and rinsing with demand-free water before use (Pernitsky *et al*., 1995). The choice of this treated water (double-filtered and deionised) and glassware was to ensure that chemical constituents that may react or interfere with the hypochlorite were removed (Barbeau *et al*., 2006). In our laboratory, the water was further heat-sterilized by autoclaving at 121^{o}C for 15 minutes before the disinfection assay experiment was carried out. Sterility was confirmed by inoculating 1 ml of the water in 9 ml of trypticase soy broth (TSB) (in triplicates) and incubating at 35^{o}C for 5–6 days. The TSB culture was observed daily for turbidity while aliquots from the broth were later plated on nutrient agar incubated at 35^{o}C for a further 2 days.

Disinfection assay: Stock solutions of disinfection assays were prepared by adding appropriate drops of the original branded hypochlorite solution to sterile test water to give free chlorine concentrations ranging between 0.2 and 2.0 mgl^{-1}. Any stock solution was used within 2 days of preparation to ensure that there was no substantial degradation of the hypochlorite. An initial experiment was conducted to determine the disappearance of free chlorine residual (FCR) in disinfected water samples over a 30-min duration. Concentration of FCR was quantified by DPD colorimetric method according to Method 4500-Cl G (APHA *et al*., 1999). Recovery of free chlorine residual was »97% in all the replicate water samples analysed.

Inactivation experiments: The disinfection assays for each test organism consisted of a set of eight demand-free 100-ml glass flasks; one reaction vessel was used for each contact time. Each hypochlorite-treated water reactor contained 44 ml of sterile test water, 5 ml of the bacterial filtrate and 1 ml of disinfection assay having appropriate hypochlorite concentration. The reactor vessels were placed in a thermostat incubator preset to maintain constant temperature of 30^{o}C, and away from direct sunlight; the pH of the disinfected water assays ranged between 6.85 and 7.10. These experimental conditions simulated environmental conditions for water storage in most of the tropical rural households particularly during the dry seasons when water scarcity persists. After every one minute contact time and subsequently for 7 minutes, populations of surviving microorganisms were determined by plating-counting technique. One of the flasks was taken and immediately de-chlorinated with 1 ml of a 1% (w/w) sodium thiosulfate solution to quench the activity of the hypochlorite. Subsequently, aliquots (1 ml) of appropriate serial dilutions of the de-chlorinated water samples were inoculated (in triplicate) on nutrient agar (Oxoid Laboratories Inc.) using a spread plate procedure. However, *E. coli* was plated on EMB agar (Fluka Inc.).

Plating and incubation and microbial enumeration: All other culture plates were incubated at 35°C for 24–48 h while *E. coli* plates were incubated at 45°C. Preparation of culture media, inoculation and incubation procedures, and enumeration of standard plates were in accordance to Standard Methods for microbiological examinations (APHA *et al*., 1999). Culture plates were enumerated after 48 h and average value of the replicates recorded as CFU ml^{-1}. These values were then log normalized.

Data transformation and statistical analysis: Generally chlorine disinfection reaction follows a first-order chemical reaction:

$$\frac{dN}{dT} = -kN, \qquad (1)$$

where T is time and k is the first-order constant (Chick, 1908).

The reaction rate is dependent on the relative concentrations of microorganisms and disinfectant, and has been described by the Chick-Watson kinetic model:

$$\frac{\log Ns}{No} = -kCnT \qquad (2)$$

where N_o is the initial concentration of bacteria, N_s is the concentration of surviving bacteria at time t, k is the pseudo first-order reaction rate constant, C is the concentration of disinfectant and n the coefficient of dilution, an empirical factor frequently assumed to be unity (Pernitsky *et al*., 1995).

Survival data were obtained according to a simplified Chick–Watson model given as log N_s/N_o versus dose, where N_o is the initial number of organisms and N_s is the number of surviving organisms at contact time T (Cho *et al*. 2003). The dose ($C{\cdot}T$) is the product of the disinfectant concentration, C in mgl^{-1} and contact time, t in minutes. In this study, the effective lethal dose ($C{\cdot}T_{99.9\%}$), is defined as the dose level for 99.9% bacterial inactivation. The dose–survival experiments were standardized at 30°C and water pH of 6.85–7.10.

RESULTS AND DISCUSSION

The survival curves of *Pseudomonas aeruginosa* and *Escherichia coli* are very similar, and require about the same dose for 3 log units of inactivation (Figures 1 and 2). Also, the survival curve of *Shigella dysenteriae* was relatively similar to that of *E. coli* at the lower dose levels but the curves diverged at the higher dose levels. However, there was significant divergence in the curves of *Salmonella typhi* and *E. coli* as the dose level increases (Figures 1 and 2). The survival curves of *Staphylococcus aureus* and *Vibrio cholerae* were significantly different from that of *E. coli* (Figures 1 and 2). However, these curves were quite similar to that of *Salmonella typhi*. The survival curve of *Streptococcus faecalis* followed that of *E. coli* though a slight divergence was observed at the higher dose levels greater than 10 mgl^{-1}-min. The survival curves of *Klebsiella pneumoniae* and *E. coli* were significantly different (Figures 1 and 2) with *Klebsiella pneumoniae* exhibiting the greater resistance.

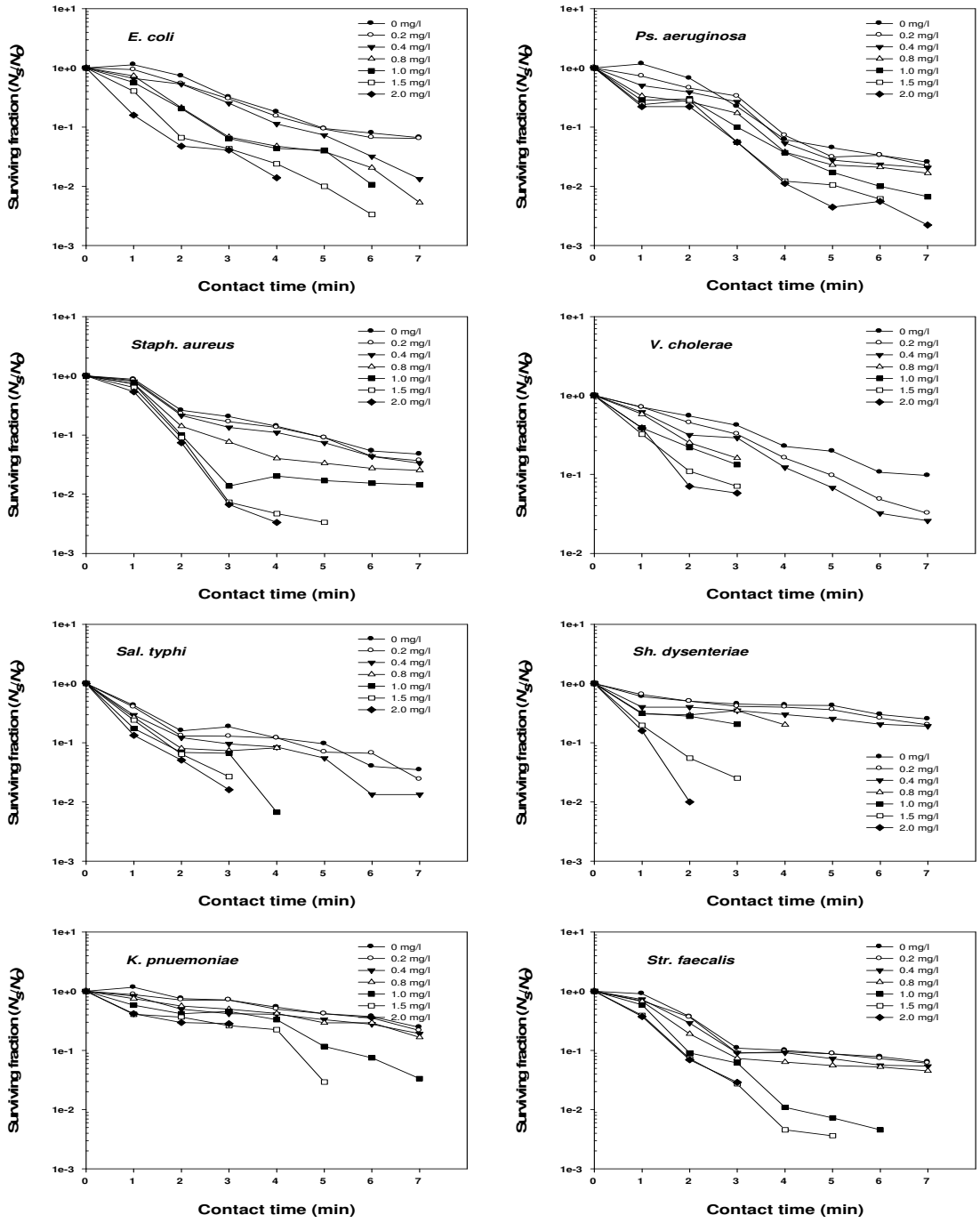

Fig. 1: Hypochlorite inactivation of test organisms in point-of-use water

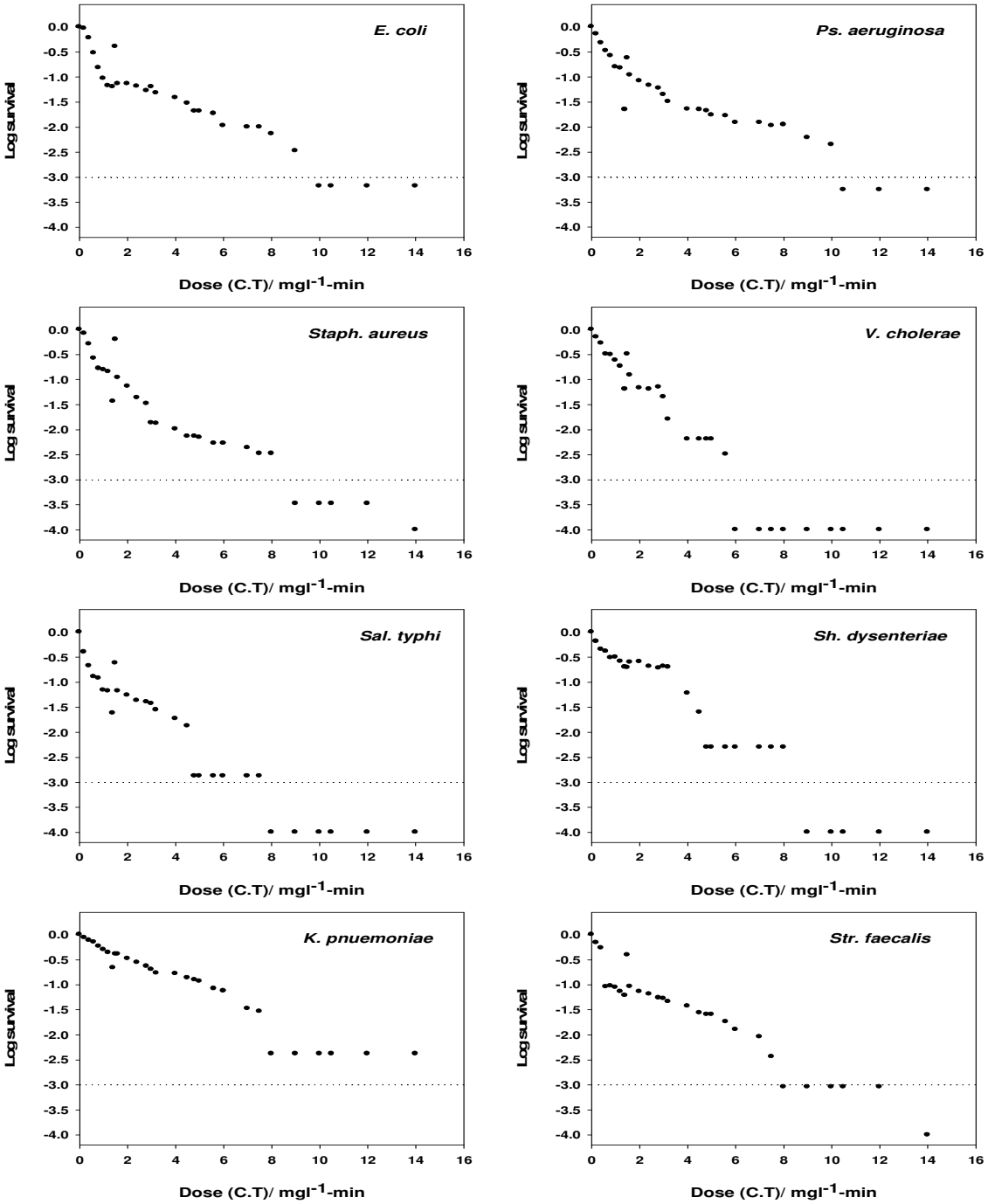

Fig. 2: Survival versus hypochlorite dose (C*T*) for the test organisms

The vegetative bacteria that were studied exhibited comparable resistance. The similarity in resistance to hypochlorite disinfection exhibited by many of the vegetative bacteria used in this study including *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, and *Vibrio Cholerae* which were lower than that of *E. coli* indicated that the organisms are more sensitive and require lesser dose for complete inactivation of the indicator organism. These organisms have also been reported to be less sensitive than *E. coli* to UV light (Chang *et al*., 1985) though another study said that at neutral pH, *S. typhi* strains are somewhat more resistant to HOCl than is *E. coli* (Angulo *et al*., 1997). *Pseudomonas aeruginosa* and *Streptococcus faecalis* exhibited similar resistance pattern to hypochlorite and about the same effective

lethal dose for 3 log units (99.9%) of inactivation as *Escherichia coli*. The comparable resistance of *Streptococcus faecalis* and *Pseudomonas aeruginosa* to that of *E. coli* could be due to the cell wall chemistry of these organisms. Usually, these organisms are encapsulated with cellulosic materials that can increase resistance to chemicals (LeChevallier *et al*., 1996; LeChevallier *et al*., 1988). Chang *et al*. (1985) have reported that *Streptococcus faecalis* has relatively higher resistance to UV radiation. The exception was *Klebsiella pneumoniae*, which required about 1.4 times higher effective lethal dose for 3 log units of inactivation. These data agree reasonably well with those of Chang *et al*. (1985).

The concept of dosage in chlorine disinfection may not be all that straightforward because the concentration and contact time could be varied depending on the treatment protocol employed. A review by Berry *et al*. (2006) indicated that several reports favoured lower concentration with longer contact time. Chlorination dosage can also be influenced by temperature and pH, and turbidity of water (The Chlorine Institute 2004). Effective disinfection requires that turbidity is less than 5 NTU. Aside reducing turbidity thus lowering the chlorine demand, in rural communities, filtration possibly using clean cloth could be an essential requirement to remove pathogenic helminths, cysts or oocysts of protozoa which cannot be completely inactivated by chlorination (WHO 1997; 2002; 2010b).

The relative resistances of the pathogens to the hypochlorite lethal doses compared with that of *E. coli* are presented in Figure 3. The doses of hypochlorite require for complete inactivation of the vegetative pathogenic and indicator bacteria were comparable. *Pseudomonas aeruginosa* and the indicator organism exhibited about the same resistance to hypochlorite. *Salmonella typhi* and *Vibrio cholerae* were the least resistant to the branded hypochlorite solution while *Klebsiella pneumoniae* is the only organism that was significantly more resistant than *E. coli*.

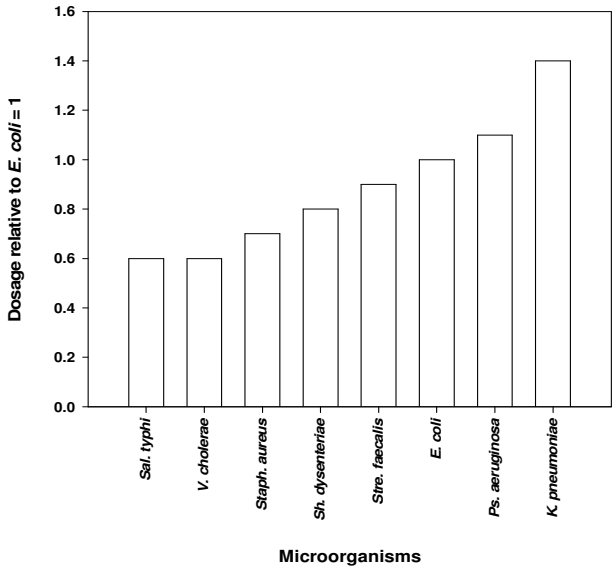

Fig. 3: Relative hypochlorite doses required for 99.9% ($C \cdot T_{99.9\%}$) inactivation of various pathogenic bacteria to that for *E. coli*

This effective lethal dose ($C \cdot T_{99.9\%}$) of 12 mgl^{-1}–min indicated that the branded hypochlorite solution will effectively eliminate bacteria in household drinking water at the standard recommended dose of 0.5 mgl^{-1} for 30 minutes. The treatment of drinking water with point-of-use chemical disinfectants in households will significantly reduce the incidence of water-borne infections particularly in the rural communities where central treatment of water is mostly unavailable. Epidemiological studies have implicated the failure to properly disinfect drinking water to be a major contributing factor to the propagation of cholera, diarrhoea and many other waterborne diseases (WHO, 2010a; 2010b). These have resulted in enormous loss in terms of reduced economic activity, decreased productivity, unemployment and job loss, and even death especially maternal and infant mortality (WHO, 2010b). Numerous studies have clearly shown that provision of microbiologically safe drinking-water can significantly reduce, directly or indirectly, the morbidity and mortality of water-borne diseases (WHO, 1997). Hence, efficient disinfection of drinking water must never be compromised.

*Conclusion***:** Overall, the results obtained in this study imply that the branded hypochlorite solution should effectively inactivate almost all vegetative bacteria in household drinking water at the manufacturer's recommended dosage of 0.5 mgl^{-1} after at least 30 minutes contact time. The study also suggests that non-detection of *E. coli* in hypochlorite-treated water indicates that there are no enteric bacteria but may not adequately guarantee that the drinking water is devoid of other microorganisms. During emergencies and for small households particularly in developing countries, portable chlorine-based disinfection products should be made available and affordable as point-of-use drinking water treatment alternative. However if necessary, it is advised that, water should first be filtered before chlorination to increase the efficiency of the hypochlorite solution and reduce its chances of reacting with natural organic compounds to form potentially harmful chemical by-products.

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