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Killing of enteric bacteria in drinking water by a copper device for use in the home: laboratory evidence

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Summary Water inoculated with 500–1000 colony forming units/ml of *Escherichia coli*, *Salmonella* Typhi and *Vibrio cholerae* was stored overnight at room temperature in copper pots or in glass bottles containing a copper coil devised by us. The organisms were no longer recoverable when cultured on conventional media, by contrast with water stored in control glass bottles under similar conditions. The amount of copper leached into the water after overnight storage in a copper pot or a glass bottle with a copper device was less than 475 parts per billion, which is well within the safety limits prescribed by the WHO. The device is inexpensive, reusable, easy to maintain, durable, does not need energy to run and appears to be safe. It has the potential to be used as a household water purification method for removing enteric bacteria, especially in developing countries.

1. Introduction

Around 2.2 million people die of basic hygiene- and water-related diseases, such as diarrhoea, every year, the majority being children in developing countries.[1] In developing countries the public water distribution systems are often poorly maintained. Furthermore, treated water is also often observed to be re-contaminated because of unsafe storage and handling practices within households.[2] Thus, it is important to explore effective strategies and approaches that are affordable, can be implemented at home and are acceptable to the population.

The ancient texts of Ayurveda recommend the use of metals such as gold, silver and copper for water purification[3] and, traditionally, Indian homes stored drinking water in copper and silver pots. In recent years this practice has be-

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replaced by the use of steel and plastic containers, as copper and silver have become expensive.

The antimicrobial effect of copper and copper alloys on pathogens such as *Escherichia coli*,[4,5] *Mycobacterium tuberculosis*,[6] methicillin-resistant *Staphylococcus aureus* (MRSA),[7] and influenza A virus[8] has been reported. Here, we report the effect of storing water inoculated to contain 500–1000 colony forming units (CFU)/ml of *E. coli*, *Salmonella* Typhi and *Vibrio cholerae* overnight in copper pots or in glass bottles containing an inexpensive copper coil device that we have developed.

2. Materials and methods

2.1. Test and control

Copper pots of 2 l capacity with a surface area of approximately 750 cm^2 (Supplementary Figure 1) were purchased from a kitchenware shop in Bangalore, India and were thoroughly cleaned each time before use with tamarind and salt as is traditional in Indian homes. Glass bottles (Schott Duran, Mainz, Germany) of 1 l capacity with a surface area of approximately 518 cm^2 were used as controls. The test and control containers were sterilised by autoclaving before use.

2.2. Bacterial strains

Escherichia coli NCIM 2065 was obtained from the National Chemical Laboratory, Pune, India. A S. Typhi isolate was obtained from a patient (ST/40/05) and its identity confirmed at the Central Research Institute, Kasauli, Himachal Pradesh, India. The isolate was sensitive to chloramphenicol, ampicillin, co-trimoxazole, cefuroxime, ceftriaxone, cefepime and ofloxacin, but moderately sensitive to norfloxacin and resistant to nalidixic acid.

A *V. cholerae* isolate obtained from a patient (ST/60/06) was confirmed to be biotype El Tor, serotype Inaba; Basu Mukherjee phage type 2; new phage typing T 27 at the National Institute of Cholera and Enteric Diseases, Kolkata, India. The isolate was sensitive to tetracycline, ampicillin, chloramphenicol, norfloxacin and gentamicin and resistant to nalidixic acid, co-trimoxazole and polymyxin B.

Experiments using *E. coli* were performed in the Microbiology Laboratory of the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore, India and experiments using S. Typhi and *V. cholerae* were performed in the Department of Microbiology, Sri Devaraj Urs Medical College (SDUMC), Tamaka, India between August and December 2007.

2.3. Inoculum preparation

Escherichia coli was inoculated into 3 ml of sterile nutrient broth and incubated overnight at 37 ± 2 °C. *Salmonella* Typhi and *V. cholerae* were inoculated into 3 ml of sterile peptone water and alkaline peptone water, respectively, and incubated overnight at 37 ± 2 °C. The overnight cultures were serially diluted with saline and plated on appropriate solid media to quantify the bacterial content.[9]

Six litres of sterile distilled water were taken in a large, sterile flask. To this, appropriately diluted inocula from the above cultures were added so as to obtain 500–1000 CFU/ml. The bacterial content of the inoculated water was ascertained by plating on solid media.

2.4. Evaluation of bacterial growth

The inoculated distilled water was distributed between copper pots (2 l) and glass bottles (1 l) and incubated overnight for 16 h at room temperature (27 ± 2 C). Duplicates were maintained each time. The experiment was conducted three times.

Aliquots of 100 μl were withdrawn after overnight incubation and the bacterial count determined by plating on nutrient agar for *E. coli* or MacConkey agar for S. Typhi and *V. cholerae* (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Serial dilutions were carried out for accurate enumeration of bacteria, when necessary.

2.5. Estimation of pH and copper content

The pH and copper content of the inoculated water were measured before and after incubation using a pH meter (DI 707; Digisun Electronics, Hyderabad, India) and Spectroquant (Merck, Darmstadt, Germany), a commercially available, ready-to-use kit, respectively. The kit was used as per the supplier's instructions. The colorimetric quantification of copper using the kit relies on the royal blue colour complex $[Cu\ (cuprizone)_2\ (NH_3)_2]^{2+}$ that is produced on the addition of the reagents provided in the kit. The upper and lower limits of detection of copper by this kit are 6.0 and 0.02 mg/l respectively. Distilled water acted as the negative control and positive controls were copper solutions obtained using copper turnings as per the procedures of the Bureau of Indian Standards.[10]

2.6. Fabrication and evaluation of the copper device

We also contrived an inexpensive copper coil device (Supplementary Figure 2) using copper cables purchased from a hardware shop in Bangalore, India (4 mm diameter, > 98% purity) and tested at a commercial laboratory (Essen & Co., Bangalore, India, a UKAS and ISO 9001:2000 certified laboratory). The minimum surface area required to exhibit antimicrobial activity was arrived at after testing copper cables and sheets of different surface areas, ranging from 7 to 45 cm^2/l of water, against *E. coli*. The effective surface area of copper to volume of water against the organisms tested was determined to be 15.2 cm^2/l.

Four litres of sterilised distilled water were inoculated with logarithmically growing cultures of bacteria as described earlier, and distributed equally between four glass bottles with (test) or without (control) the copper device with a surface area of 15.2 cm^2 (Supplementary Figure 2). After overnight incubation at room temperature (27 ± 2 C), samples of water were withdrawn to enumerate bacteria as described earlier. The pH and copper content of the water samples were also measured as described before.

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Table 1 Effect of overnight storage of water inoculated with enteric bacteria in copper pots and control glass bottles

Bacteria inoculated	Copper pots[a]		Glass bottles[a]	
	Before incubation	After incubation	Before incubation	After incubation
Escherichia coli NCIM 2065	984 ± 4	No growth	984 ± 4	32916 ± 2036
Salmonella Typhi ST/40/05	687 ± 14	No growth	687 ± 14	3150 ± 156
Vibrio cholerae ST/60/06	502 ± 6	No growth	502 ± 6	2520 ± 115

[a] Mean of six observations; bacterial counts given as colony forming units/ml.

Table 2 Effect of overnight storage of water inoculated with bacteria in glass bottles with a copper device and glass bottles without the copper device

Bacteria inoculated	Copper device[a]		No device[a]	
	Before incubation	After incubation	Before incubation	After incubation
Escherichia coli NCIM 2065	935 ± 2	No growth	935 ± 2	26458 ± 832
Salmonella Typhi ST/40/05	688 ± 12	No growth	688 ± 12	3366 ± 401
Vibrio cholerae ST/60/06	502 ± 6	No growth	502 ± 6	2267 ± 15

[a] Mean of six observations; bacterial counts given as colony forming units/ml.

3. Results

The effect of overnight storage of water inoculated with enteric bacteria in copper pots and in control glass bottles is shown in Table 1. Water containing *E. coli*, S. Typhi and *V. cholerae* stored in copper pots did not yield any growth after 16 h storage. The inoculated water stored in control bottles gave evidence for the presence and growth of bacteria: more than 30-fold growth was observed for *E. coli* and more than four-fold growth was observed for S. Typhi and *V. cholerae*.

The effect of overnight storage of water inoculated with enteric bacteria in glass bottles with and without the copper device is shown in Table 2. The findings were similar to those observed with copper pots. There was no growth of bacteria after overnight incubation with the copper device, whereas control bottles without the device showed more than a 30-fold increase in *E. coli* counts and more than a four-fold increase in S. Typhi and *V. cholerae*.

The pH and levels of copper in the test containers were well within the permissible limits set by the WHO (<8.5 and 2000 parts per billion, respectively)[11] (Table 3).

4. Discussion

In the experiments described here we were unable to recover bacteria from water inoculated with *E. coli*, S. Typhi and *V. cholerae* after overnight storage in copper pots. By contrast, water stored similarly in glass bottles showed clear evidence for the persistence and growth of bacteria, more than three- to four-fold in S. Typhi and *V. cholerae* and more than 30-fold in *E. coli*. We attribute the multiplication of bacteria in control bottles to the nutrients present in the nutrient broth or peptone water used for inoculum preparation. The observations were reproducible in multiple experiments conducted in three different batches and showed complete killing of the enteric pathogens in water when stored in copper pots. Our experiments with a copper device developed by us gave similar results. The copper leached into the water during overnight storage was within the limits stipulated by WHO for human consumption.

Our study substantiates the ancient claim by Ayurvedic texts that water stored in copper vessels can promote health. This is the first time that an antimicrobial effect of copper pots and a copper device on *V. cholerae* and S. Typhi has been reported. The inexpensive copper device developed by us has immense potential as a point-of-use intervention at household level for improving the quality of drinking water by removing enteric pathogens. The cost of our copper device is one-tenth the cost of a copper pot and can be used in a regular plastic pot.

Many of the currently available water purification systems are expensive and beyond the reach of the rural

Table 3 Mean copper content and pH of water stored overnight in copper pots or glass bottles with a copper device ($n = 18$)

Container	pH	Copper content (ppb)
Copper pot	7.16 ± 0.03	426.83 ± 33.64
Glass bottle (control)	6.80 ± 0.05	Undetectable
Copper device	7.17 ± 0.03	417 ± 12
Glass bottle (control)	6.83 ± 0.06	Undetectable

ppb: parts per billion.

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population in countries such as India. The available, inexpensive methods for household purification of water have shortcomings. Candle filters (with diatomaceous earth) require regular cleaning and replacement, which are tasks usually ignored by users. The use of regular, household, solar heaters for disinfection[12] is an innovative idea, but the water is in danger of re-contamination during handling; moreover the functioning of the heaters on a cloudy day is not dependable. The device described by us seems to circumvent these shortcomings; it is durable, does not require electricity or fuel and can be reused. Our studies have indicated that water removed after storage in a copper pot for 16 h and then inoculated with bacteria (500 CFU/ml) retains the antibacterial activity (FRLHT preliminary findings, unpublished). The surface area of the copper device can be easily scaled up to handle different volumes of water (Supplementary Figure 3) and the device could, therefore, be adopted by different sized households in rural communities of developing countries.

Our study does not address parameters such as turbidity and chemical purification, which are required to meet WHO criteria for safe drinking water. However, it has been estimated that reduction of water contamination by faecal coliforms alone by two orders of magnitude can lead to a 40% reduction in diarrhoea.[13] Household use of the copper device described here could, thus, go a long way to providing safe drinking water. As this study was conducted using distilled water the effect of copper on bacteria in natural water, possibly containing chelating agents, still needs to be tested. The surface area:volume ratio of the copper device may also need to be standardised for other conditions of water and pathogens (viruses and protozoa).

Field trials using the device need to be carried out in diarrhoea-prone areas where water and sanitation are a problem in order to demonstrate its benefits and adaptability. Nevertheless, owing to the low cost and simplicity of the device, we think there is a role for it in the decontamination of water and the subsequent reduction of mortality and morbidity due to enteric pathogens, particularly *V. cholerae*, in rural areas and the urban slums of developing countries.

Authors' contributions: PV conceived the methodology, supervised all aspects of the study and finalized the manuscript; SRP guided the experiments conducted using *V. cholerae* and S. Typhi; VBPS and KOS performed the experiments; PV, SRP and VBPS analysed the results; VPBS drafted the manuscript; SRP edited the manuscript. All authors read and approved the final manuscript. PV is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2009.01.019.

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