# Drinking Water Stored in Copper Vessel - Reveals Antibacterial Activity

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## ABSTRACT

**Introduction and Aim:** Recently About 2.2 million diarrheal deaths per year especially in developing countries, in which 1.4 million deaths among children under age five. Safe drinking water, especially in developing countries, is still a major problem. The present study was designed to explore the antibacterial effect of copper vessel stored with drinking water inoculated with diarrhea-causing bacterial strains enterotoxigenic Escherichia coli (ETEC), Vibrio cholera and Shigella flexneri.

**Materials and Methods:** The bacterial strains isolated from fecal sample, identified by colony morphology and biochemical test. About 500 Colony Forming Unit (CFU/mL) of the bacterial strains of E. coli, V. cholera and S. flexneri were inoculated separately in drinking water stored in the copper vessel and non- copper vessel for 12 hrs. After incubation 100 µL of samples was taken from each copper vessel and non-copper vessel container and spread on nutrient agar for the enumeration of bacteria colonies. After 24hrs of incubation at 37°C, dishes were observed for visible bacterial colonies using colony counter.

**Results:** The bacterial strains E. coli (ETEC) (532 CFU), V. cholera (502 CFU) and S. flexneri (512 CFU) was inoculated in copper vessel observed no growth on the selective media and when the bacterial strains inoculated in the non-copper vessel observed growth upon inoculated in selective media E. coli (ETEC) (624 CFU), V. cholera (328 CFU) and S. flexneri (483 CFU).

**Conclusion:** The antibacterial activity may be due to the oligodynamic effect of copper which destroy the cell wall and cell membrane results in membrane damage. The present study recommends the use of cost-effective copper vessels to store drinking water especially in rural areas for protection from water-borne infection.

**Key Words:** Diarrheal, Copper vessel, Drinking water

## INTRODUCTION

The 70% of the World is covered by water of which only 2.5% is of fresh water, which 1% of the freshwater is accessible easily, remaining trapped in glaciers and snowfields and from the 1% of fresh water only 0.3-0.5% is available for drinking purpose. As the human population enhances, day by day and daily consumption of fresh water also increased several folds.

Water plays an essential dual role - one is vital for life, as more than 60% of human body is composed of water and sustains the natural environment, contributes to the development of economic, health, social, recreational and cultural activities. On the other hand, it plays a vital role in the transmission of water-borne infectious diseases. It is estimated to cause about 2.2 million diarrhea deaths per year especially in developing countries in which 1.4 million deaths among children under age five (1). Worldwide, an economic burden for a water-borne disease is about 12 billion US dollars per year (2). Waterborne infections are caused by ingestion, airborne or contact with contaminated water by a variety of infectious agents which includes bacteria, viruses, protozoa and helminths (3). In addition, flood accelerates the risks of outbreaks of waterborne diseases. About 1415 species of microorganisms have been reported to be pathogenic, among which approximately 348 are water-borne, causing 115 infectious diseases (4).

From 1991 to 2008, about 1,428 water-borne outbreaks were reported (5).

The major bacterial agents that account for millions of diarrhoeal deaths particularly in developing countries, are enterotoxigenic Escherichia coli (ETEC), Vibrio cholerae, and species of Shigella, which spread through contaminated water, food or from person to person. Shigella flexneri known as acute bacillary dysentery causes approximately 10% of all diarrhoeal episodes among children aged less than five years (6). Infection with ETEC is associated with traveler's diarrhea, and the rate of infection is higher in India compared to other developing countries (7).

Currently, about 780 million people do not have access to a purified drinking water, and 2.5 billion people lack access to improve sanitation worldwide especially in the developing countries (8). It is estimated that about 3.2% of deaths globally are attributable to consume unsafe water caused by poor sanitation and hygiene (9). The WHO recommends that improving water quality may reduce the global diseases burden by approximately 4%. Thus, there is an urgent need to move all possible efforts to minimize the water-borne infection. Though detection methods play an essential role in monitoring water quality, surveillance, and quantitative microbial risk assessment but providing safe drinking water globally especially in developing countries like India is still a challenging one.

The household boiling water for disinfection is in danger of leaching harmful chemicals from the plastic bottles and container, also prone to recontamination during handling and storage. At present three main water treatment methods are available includes distillation, reverse Osmosis and solid block activated carbon, apart UV, Ozone, activated alumina, sediment filters, ion exchange, granular activated carbon, and boiling are also used but combined with other methods for effective outcomes. Many of the currently available water purification systems are more expensive, required regular maintenance, electricity and beyond the reach of the rural population in countries such as India is questionable. Even inexpensive Candle filters (with diatomaceous earth) require regular cleaning and replacement are usually ignored by users.

In most of the rural places at India, people usually collect drinking water from the lake, ponds, wells, running streams, municipal pipes, from stored water tanks and water, may become contaminated at any point between collection, carrying and storage before use. The use of copper by human civilizations dates behind to between the 5th and 6th millennia B.C. It was the 1st metal used, presumably because it could be found in a native, metallic form which did not demand to smelt. The Indian Ayurveda describes storing water in a copper vessel overnight and drinking it in the morning had many health benefits. The present study was designed to explore the antibacterial effect of copper vessel container by storing the drinking water inoculated with diarrhea causing bacterial strains enterotoxigenic E. coli (ETEC), V. cholera and S. flexneri.

## MATERIALS AND METHODS

### Isolation and Identification of Bacterial Strains

Bacterial strains enterotoxigenic E. coli (ETEC), V. cholera and S. flexneri were isolated in the faecal sample. Bacterial strains enterotoxigenic E. coli (ETEC), V.cholera and S. flexneri were isolated in the stool samples by culturing on Mac conkey agar, thiosulphate-citrate-bile salt sucrose agar (TCBS) and Salmonella shigella agar respectively. Initially colony morphological identification was Mac conkey plates showed pure lactose fermenting, translucent, glossy, smooth colonies and were identified as E. coli. On TCBS agar V. cholera produce large, flattened yellow colour colonies with opaque centers due to fermentation of sucrose in the medium. S. flexneri is non lactose fermenter appear as a transparent colorless colonies. Biochemical test showed Indole and Methyl Red (MR) showed Positive and negative for Voges-Proskaure (VP) and citrate for E. coli. For identification of V. cholera Catalase and oxidase test showed positive, urease test showed negative, indole, citrate and MR test showed positive and Negative for VP test. For identification of S. flexneri showed Catalase, MR and nitrate reduction showed positive and negative for Citrate, Oxidase, and VP test. After morphological and biochemical identification single colonies of E. coli (ETEC), V. cholera and S. flexneri were isolated, inoculated in sterile Luria Bertani broth as a starter culture and incubated at 370Cfor 12- 16 hrs, followed by serial dilution with normal physiological saline.

### Serial Dilution Method

A pure culture may be obtained by serially diluting (tenfold-1 in 10 dilutions-1:10, 1:100 or 1/10, 1/100, 1/1000 or 10-1, 10-2, 10-3) the sample with sterile

water or saline to the point of extinction in numbers of cells and transferred on an agar plate. In serial dilution technique sample has been diluted serially. It is assumed that each viable bacterium in the original sample can produce one discrete colony (colony forming unit or CFU) and thus, the number of colonies represents the number of bacteria that can grow under the conditions. Petridish with between 30 and 300 colonies are ideal for completing a standard plate count.

**Spread Plate Method**

An aliquot of the serially diluted sample (0.1ml) is placed onto the agar surface in a petri dish and is spread uniformly with a sterile, bent 'L' shaped glass rod until the surface becomes dry and the spreader begins to stick to the surface. Petri dishes are turned upside down to avoid condense on the agar and incubated at 37oC for 24 hours and after 24 hours of incubation the Petri dishes are examined for visible the colony formation. Above said procedure repeated in triplicate and average mean of the three was calculated.

**Inoculation of bacterial culture in sterilized water stored in copper and non-copper vessel**

Drinking water (2 liters) was sterilization at 120°C for 15-20 minutes and transferred to sterile copper vessel and non-copper vessel. Then sterilized water was inoculated with 500 CFU/mL of test bacterial strain. The CFU was carried by serially dilution and spread plate method (for each bacterial culture separate copper / non-copper containers were used). Before and after inoculation bacterial population was enumerated by spread plate method. Then the copper vessel and non-copper vessel's with bacterial cultures were incubate at room temperature (28±2 °C) for 12hours. After incubation 100 µL of samples was taken from each copper vessel and non-copper vessel container and spread on nutrient agar for the enumeration of bacteria colonies. After 24hrs of incubation at 37°C, dishes were observed for bacterial colonies using colony counter.

## RESULTS

The bacterial strains E. coli (ETEC) (532 CFU), V. cholera (502 CFU) and S. flexneri (512 CFU) was inoculated in copper vessel showed no growth on the selective media and when the bacterial strains inoculated in the non-copper vessel showed growth upon inoculated in selective media E. coli (ETEC) (624 CFU), V. cholera (328 CFU) and S. flexneri (483 CFU) shown in Table No. 1.

**Table 1: Effect of copper vessel container on water sample with diarrhoeagnic bacteria stored for 12 hrs**

| Sl. No | Bacterial sample | Before incubation number of CFU* | After incubation number of CFU for sample taken copper vessels container | After incubation number of CFU for sample taken non-copper vessels container |
|---|---|---|---|---|
| 1 | Escherichia coli (ETEC), | 532±24 | 0 | 624±35 |
| 2 | Vibrio cholera | 502±12 | 0 | 328±24 |
| 3 | Shigella flexneri | 512±11 | 0 | 483±56 |

* Number of colony forming unit (CFU)

## DISCUSSION

Water is essential for maintaining life on Earth, but can also serve as a media for many pathogenic organisms. The present study was designed to provide safe water to people especially at the rural places where the water purifier cannot reach since more expensive, required regular maintenance which is beyond for the rural population. The use of copper by human civilizations dates back to between the 5th and 6th millennia B.C. Copper vessel is merely passive storage of water, and it will be a one-time investment with no recurring costs for further maintenance. It is suitable for developing countries like India where there is a frequent intermittent supply of drinking water, necessitating storage of drinking water for days. In such conditions, copper vessels can be introduced during storage of drinking water. In the present study, the result showed, no growth was observed after 12hrs of incubation of bacterial strains stored in a copper vessel and when the water stored in non-copper vessel showed the presence of colony forming unit indicates its viability (Table No. 1).

When the water is stored in a copper vessel overnight a detectable amount of copper ions gets dissolved into the water called Oligodynamic effect. The dissolved copper result in structural and membrane damage, by observing to inactivate bacteria by destroying their cell wall and cell membrane (10,11). Damage to the membrane leads to leakage of potassium or glutamate through the outer membrane of

bacteria results in osmotic shock. In addition, copper ions binding to proteins that cause oxidative stress by generating hydrogen peroxide and damaging DNA may result in genotoxicity (11).

As copper vessel are not very common today since the costly and easy availability of plastic and stainless steel containers with less cost. The present study recommends to use a copper vessel to store drinking water since Copper vessel is easy to use, requires no electricity/battery and does not need any maintenance and replacements like other commercial water purifiers, making it ideal for situation prevailing in developing countries like India especially in rural areas. This study also recommends that canned drinking water supply companies can use copper coated containers for processing the water and also to use copper coated pipelines.

## CONCLUSION

The antibacterial properties of water stored in copper vessel inoculated with bacterial strains were firmly established. The result showed no bacterial colony counts were enumerated which implies that storage of water in the copper vessel is a promising additional tool alongside other hygienic measures to curb the number and severity of waterborne diarrhoeal infections. At this point, additional studies would be recommended to help in determining the most cost-effective way to give maximal protection for other water-borne diseases.

**Conflicts of Interest**

Author's declared that no Conflict of Interest

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