

Short Communication

Effectiveness of boiling in eradication of common pathogens in water

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Abstract

This Prospective analytic study was conducted at the microbiology laboratory of Dr. Ziauddin Hospital to find out minimum temperature and duration required to eradicate common bacterial pathogens from water samples. High concentration (>10¹⁴ cfu/ml) of five common diarrheal pathogens were added to 100 ml water samples and heated till 40°C, 60°C, 100°C, and two samples were further heated at 100°C for 5 and 10 minutes respectively. Another set of samples were contaminated with stool specimen positive for diarrheal pathogens and heated in the similar fashion. Samples were inoculated on culture media to find out the growth of any organisms. All samples heated to 60°C or beyond did not show any growth of bacteria on culture. So it is reliably proved that heating water up to 100°C is sufficient to eradicate common disease causing bacteria even in stool contaminated samples and further heating would not be required.

Introduction

Clean potable water has been a demand of human society and considered as one of the fundamental rights of every citizen.¹ Published local regional and global data, documents spread of water borne diseases like, diarrhoea, dysentery, amoebiasis, jaundice and typhoid in the community.²⁻³ Therefore it becomes essential for the governments to supply clean potable water to each citizen.

Different methodologies like boiling, filtering, and chemical treatment have been recommended and applied for purification of drinking water.⁴ CDC has recommended that boiling water will inactivate all major water borne bacterial and protozoal pathogens.⁵ Still the actual temperature and the time period has not been scientifically verified and hence different temperatures and time periods have been suggested by different resources.⁶

This study is trying to scientifically prove the effectiveness of boiling as the method of choice for purification of water by finding out a clear cut temperature and time period for boiling to get rid of common pathogens. Once established the effective time and temperature will not only be recommended for public use but it can also pave way for future studies based on comparison of other methods of water purification with boiling.

Material, Methods and Results

This Prospective analytic study was conducted in the department of Clinical Microbiology at Dr. Ziauddin Hospital. All tests were performed in duplicate. Hundred ml tap water samples were taken in beakers and were divided into two groups. First six samples were mixed with 1ml each of 1% McFarland solution (aprox. 10¹⁶ cfu/ml) of five common diarrhoeal pathogens, i.e. Salmonella typhi, Salmonella paratyphi B, Shigella, Vibrio cholerae, and E-coli (ATCC strains) giving them a final strength of 10¹⁴ cfu/ml after dilution.

Other six water samples were mixed with stool samples positive for faecal coliforms. A large scoop of stool was added to water samples, representing the faecally contaminated samples.

First beaker of each set was labeled as growth control, and 10µl samples were inoculated onto Sheep blood agar (SBA), Macconkey's agar (MKA) and Chocolate agar (CA), and incubated at 37°C for overnight incubation. Second set of beakers were heated till 40°C, 3rd set were heated at till 60°C, 4th set till 100°C, 5th set heated at 100°C for 5 minutes and the last set of beakers were heated at 100°C for 10 minutes.

All samples were cooled down to room temperature, and 10µl samples from each were quantitatively inoculated on SBA, MKA and CA, and incubated at 37°C. Plates were viewed at 24 hours initially; all negative plates were reincubated for another 24 hours. Colonies were quantified and organisms identified by using biochemical methods following standard microbiological protocols.⁷

After 24 hours incubation, heavy growth (>10⁸ cfu/ml) was observed on growth control plates, yielding all five organisms initially mixed in water samples. Inoculation of samples from beaker that was heated till 40°C showed = 10⁸ colonies of two organisms that were identified as Salmonella typhi and E-coli. Sample heated to 40°C did not show any growth of Salmonella paratyphi B, Vibrio cholerae and Shigella species.

Other four samples heated to 60°C, 100°C, 100°C and 100°C for 5 and 10 minutes respectively yielded no growth on any plates even after 48 hours incubation. (Table1)

The stool contaminated sample showed positive

Table 1. Results of water samples with bacterial pathogens.

Samples	Water temperature (°C)	Surviving Holding time (minute)	Total colony count Per ml
Control	Room temperature (34°C)	None	Too numerous to count cfu of all 5 organisms
Sample 1	40°C	40°C	>109 cfu of (E-Coli & Salmonella typhi
Sample 2	60°C	60°C	< 1 colonies
Sample 3	100°C	100°C	< 1 colonies
Sample 4	100°C	100°C	< 1 colonies
Sample 5	100°C	100°C	< 1 colonies

Table 1. Results of water samples with bacterial pathogens.

Samples	Water temperature (°C)	Surviving Holding time (minute)	Total colony count Per ml
Control	Room temperature (34°C)		>103 cfu of E-coli & Vibrio cholera
Sample 1	40°C	0 minutes	>10 cfu of E-coli
Sample 2	60°C	0 minute	< 1 colonies
Sample 3	100°C	0 minutes	< 1 colonies
Sample 4	100°C	5 minutes	< 1 colonies
Sample 5	100°C	10 minutes	< 1 colonies

growth of E-coli and Vibrio cholerae in control samples, bacterial growth was also observed in the sample heated to 40°C, but no growth on any plates were seen from samples that were heated till 60°C and over that.(Table 2).

Our results have confirmed that 60°C is the critical temperature for killing of bacterial pathogens, even when the water is contaminated with stool specimen. Technically 60°C is the temperature at which proteins get denatured so there is always a likelihood of microorganisms getting disrupted after loss of the cell internal structure.

This study did not include the other parasitic or viral diarrhoeal pathogens, or other important non diarrhoeal pathogens transmitted by water. The reasons for non inclusion, was firstly, the difficulty in standardizing the quantity of parasitic cysts and viruses, as well as the confirmation of their eradication. Secondly if we look at the published data from the region and the country, bacterial pathogens are the main causes for diarrhoeal illnesses.⁸⁻¹⁰

Published literature has shown that boiling water upto 100°C for one minute will also inactivate all other

diarrhoeal pathogens other than bacteria like viruses and parasites.⁸⁻¹⁰ Though the information regarding other non diarrheal viruses is incomplete, but evidence supports that Hepatitis A considered a rather heat resistant virus is also rendered non infectious by boiling it for one minute.³

Conclusion

On the basis of the results we can safely conclude that 60°C is the critical temperature for eradication of bacterial pathogens. Therefore heating the water upto 100°C for 5-10 minutes is sufficient to eradicate common disease causing bacteria even in stool contaminated samples and any further heating will not be required.

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