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Original Research Article

A MODEL TO STUDY THE EFFECT OF BOILING ON WATER BORNE BACTERIAL DIARRHEAL AGENTS

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ABSTRACT

This simple and easy study was conducted to trace out the actual temperature and time period to get rid of pathogenic bacteria from the water sample simply by boiling method. There is no perfect scientific basis yet exists from the search of literature regarding effect of boiling on water borne pathogens. Four common water borne pathogens were selected (*Salmonella typhi, Shigella. dysenteriae*, *Vibrio cholerae*, and *Escherichia. coli*), artificially contaminated the samples and were subjected to 40^{o}C, 60^{o}C, 80^{o}C and 100^{o}C various temperatures. The result findings indicate that all four pathogens survived till 10 minutes at 40^{o}C. However; two bacterial cultures i.e *S. typhi* and *V. cholerare* (105cfu/ml) survived at 60^{o}C in 5 minutes but not in 10 minutes. None of the growth of four pathogens were observed in rest of the exposure of temperature at 80^{o}C and 100^{o}C. It concludes that absolute eradication can be achieved at 80^{o}C and therefore, boiling of water is quite safe for human consumption.

Key Words: *Salmonella typhi, Shigella. dysenteriae*, Diarrheal agents

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INTRODUCTION:

Water is the most essential component of life. The global burden of diseases from water, sanitation and hygiene is magnanimous, about 4% of all deaths and 5.7% of all the diseases [1]. Infectious diarrhea probably is the largest contributor of this burden [1]. According to WHO, unsafe water and sanitation account for 2 million diarrheal deaths per annum including majority of children 5 years or less [2]. A diverse variety of infectious agents is associated with diarrhea, including several bacteria, viruses and protozoa. However, bacteria are clinically most important as well as are relatively easier to handle and diagnose. Globally, several waterborne bacteria are involved in the development of diarrhea, including *Campylobacter jejuni*, *Escherichia coli, Salmonella, Shigella* and *Vibrio*. Stutman in his

study reported Salmonella, Shigella and Campylobacter as the most common causes of bacterial diarrhea in United States [3]. A study from Bangkok, Thailand reported shigellosis and entero-invasive E. coli infection to be significantly associated with bloody diarrhea [4]. In neighbouring countries, like in a major city of Bangladesh, Dhaka, water analysis results indicated high bacteriological contamination containing *Klebsiella spp, Enterobacter spp., Pseudomonas spp. and Proteus spp. Staphylococcus spp. and Salmonella spp.* (5).Similarly, outbreak of potential diarrheal agents have also been reported globally as *Vibrio cholerae* reportedly caused a severe outbreak of diarrhea in West Bengal, India in April 2006, with 298 reported cases [6]. Though many temperatures and time periods have been suggested [7] yet the time period and the actual temperature has not been scientifically documented [8]. The rationale of our study is to develop a model and evaluate and compare the effects of boiling on different water-borne bacterial agents causing diarrhea.

MATERIALS AND METHODS:

This simple study was designed and executed in the Department of Microbiology, Federal Urdu University of Arts, Sceince and Technology, Karachi, Pakistan. In this study, four groups were made.100 ml of tap water was taken into four beakers labelled with the respective name of pathogens used in this study (*Salmonella typhi, Sh.* dysenteriae, *Vibrio cholerae*, and *E. coli*). In each water sample, 1ml of 1% MacFarland index solution cultural suspension was mixed. One additional beaker containing 100 ml of tap water was used as a control. First group containing four beakers were heated up to 40^{o}C, in the second group containing four beakers were heated four beakers up to 60^{o}C,in the third group containing four beakers were heated up to 80^{o}C and forth group containing four beakers were heated up to 100^{o}C. All groups were heated for two different time periods i.e 5 minutes and 10 minutes. Later all the water samples were cooled down and around 10 ul sample from each were inoculated on Blood agar and MacConkey's agar and incubated at 37^{o}C for micobiological investigation. All plates were observed after 24 hrs. All colonies that were obtained were isolated properly, gram stained and counted. For the sake of identification of isolates, biochemical tests were also performed [15].

RESULTS AND DISCUSSION:

With the passage of time, the availability of safe drinking-water is becoming a major concern of human health. In Pakistan, contamination of water is a severe concern and has been reported by multiple studies. A study conducted at Khairpur showed 80% water samples collected from different areas of the city to be contaminated with E. coli [9]. Another study by Rasheed et al reported isolation of a large variety of pathogenic bacteria including *V. cholerae, E .coli and Shigella* from water samples of different regions of Pakistan [10]. In an investigation, in Karachi, the cosmopolitan city of Pakistan, an outbreak of typhoid fever in the community was found owing to the consumption of water loaded with the pathogenic Salmonella enterica serovar Typhi in about 100% well water, 65% household water samples [11]. Akhlaque et al showed that all (100%) of the 32 samples taken from different areas of Karakoram highway were contaminated and henceforth, unfit for human consumption [12]. Similarly, 95.8% of the domestic pumps of Punjab were found to be contaminated using H2S strip technique [13]. Furthermore, 81% of the water samples from Peshawar contained bacteriological impurities [14]. Thus, reports from almost every region of Pakistan have proved this as a matter of high concern that requires immediate actions to prevent any further mortalities and outbreaks of diseases due to waterborne pathogens. This study is quite simple

but provides a scientific basis to trace out the actual temperature during boiling for getting rid of potential human bacterial pathogens. According to the research findings of study, very dense growth (of 4 pathogens were obtained both time periods 5 mins and 10 mins up to heating of 40^{o}C.

Table 1: Exposure of water samples with bacterial pathogens at various temperatures for 5 minute time interval:

Groups of water samples	Water Temperature (^{o}C)	Colony count (cfu/ml)	Pathogens recovered
GP1	40	Countable colonies >106	*S.typhi, Sh. dysenteriae, V. cholerae and E.coli*
GP2	60	> 10 2	*S.typhi and V. cholerae*
GP 3	80	No significant colonies	--------
GP4	100	No significant colonies	----------
Control	35 (Rom temperature)	Uncountable colonies	*S.typhi, Sh. dysenteriae, V. cholerae and E.coli*

Table 2: Exposure of water samples with bacterial pathogens at various temperatures for 10 minute time interval.

Groups of water samples	Water Temperature	Colony count (cfu/ml)	Pathogens recovered
GP1	40	Countable colonies >104	*S.typhi, Sh. dysenteriae, V. cholerae and E.coli*
GP2	60	No significant colonies	----------
GP 3	80	No significant colonies	--------
GP4	100	No significant colonies	----------
Control	35 (Room temperature)	Uncountable colonies	*S.typhi, Sh. dysenteriae, V. cholerae and E.coli*

However, moderate nature of growth of *S. typhi* and *V. cholerare* (10^{2}cfu/ml) at 60^{o}C in 5 minutes but not in 10 minutes was observed. In rest of the exposures of temperature, none of the growth was observed. It reflects the growth of pathogens stopped propagating just after 60^{0} C. A similar study conducted in Pakistan on a slightly different wayy but supported the idea that heating water up to 100°C is adequate to eradicate common disease causing pathogens [15].United States Centers for Disease Control and Prevention (CDC) recommends induction of simple and economical household methods for treatment and storage of water [16]. Various methods have been implicated in the treatment of water throughout the world, including chlorination and boiling. However, a study from Lahore, Pakistan reported only 27% water samples to be positive for chlorine [17]. Boiling of the drinking water is a more renowned treatment method for executing the microorganisms. It is achieved by thermal inactivation of the pathogenic bacterial components [18]. However, different species of the bacteria must have varying ranges of temperatures required for proper and complete inactivation. Apparently, hot surfaces, may not necessarily have killed the enteropathogens, however, heating following appropriate time over 650C have completely killed all enteropathogens [19]. In another study conducted in which effect of boiling was studied, 169

boiled samples from Community was collected and only 48 were reported to have coliforms after microbiological analysis [20]. Campylobacter jejuni has been considered as very important causes of diarrhea.. However, a study from Rawalpindi and Islamabad, Pakistan reported C. jejuni to account for 18% diarrheal cases of children up to 12 years of age [21]. Heat inactivation of microbes is exponential and implicates first-order kinetics. Thus, thermal death is achieved in lesser duration at high temperatures, while lower temperatures are useful with a prolonged duration [22]. Recently, a study from Lahore, Pakistan showed that 37% of the water samples collected from different areas were contaminated with bacteria. It also showed that maximum contamination was found in areas with low socio-economic conditions [23].

CONCLUSION:

The results of our study help us to confidently suggest our community members regarding boiling of water, as largely population prefer boiling at home, as it costs nothing rather installing filters or adopt other means to make water germs free. Moreover, people just boil water however; boiling does not merely enough yet temperature and time is also equally important. A couple of studies in this respect has been conducted in past with a bit variable conclusion, but most of them have almost the same consensus. We found out that complete killing of all the four selected pathogenic diahorreal bugs was achieved at 80^{o}C for 5 minutes heating. We may conclude broadly that if boiling is done at 100 for at least about 5 minutes.

REFERENCES:

1. http://www.who.int/quantifying_ehimpacts/global/en/ArticleEHP052002.pdf
2. http://www.who.int/household_water/en/
3. Stutman HR. Salmonella, Shigella, and Campylobacter: common bacterial causes of infectious diarrhea Pediatric Annals [1994, 23(10):538-43]
4. Taylor DN, Bodhidatta L, Echeverria P. Epidemiologic aspects of shigellosis and other causes of dysentery in Thailand.Reviews of Infectious Diseases. 1991, 13 Suppl 4:S226-30]
5. Islam S, Begum HA, Nili NY. Bacteriological Safety Assessment of Municipal Tap Water and Quality of Bottle Water in Dhaka City: Health Hazard Analysis. Bangladesh J Med Microbiol. 2011;4(1).
6. Bhunia R, Ramakrishnan R, Hutin Y, Gupte MD. Cholera outbreak secondary to contaminated pipe water in an urban area, West Bengal, India, 2006. Indian J Gastroenterol. 2009:28(2):62–6.
7. Koneman EW, Allen SD, Janda WM, et al, eds. Color atlas and text book of diagnostic microbiology, 5th ed. Philadelphia: Lippincott, 1997; pp 180-88.
8. Groh CD, MucPherson DW, Groves DJ. Effect of Heat on the Sterilization of Artificially Contaminated Water J Travel Med 1996; 3: 11-13
9. Shar AH, Kazi YF, Soomro IH. Antibiotic susceptibility of Thermo-Tolerant Escherichia coli 2 isolated from drinking water of Khairpur City, Sindh, Pakistan. Pak J Biol Sci. 2009:12(8):648-52.
10. Rasheed F, Khan A, Kazmi SU. Bacteriological analysis, antimicrobial susceptibility and detection of 16S r RNA gene of Helicobacter pylori by PCR in drinking water samples of earthquake affected areas and other parts of Pakistan. Malay J Microbiol. 2009;5(2):123-127.

11. Farooqui A, Khan A, Kazmi SU. Investigation of a community outbreak of typhoid fever associated with drinking water. BMC Public Health. 2009;9:476.
12. Akhlaque M, Chaudhry NA, Khan FA. Bacteriological analysis of drinking water along the Karakorum Highway (KKH). Ann King Edward Med Uni. 2003;9(4):295-8.
13. Anwar M, Chaudhry N, Tayyab M. Bacteriological quality of drinking water in Punjab: evaluation of H2S strip test. J Pak Med Assoc. 1999;49(10):237-41.
14. Sarwar G, Khan J, Iqbal R, Afridi AK, Khan A, Sarwar R. Bacteriological analysis of drinking water from urban and peri-urban areas of Peshawar. J Postgrad Med Inst. 2004;18(1):64-9.
15. Sabir. N and Farooqi B.J (2008). Effectiveness of boiling in eradication of common pathogens in water . J Pak Med Assoc , 58 (3): ,140-141.
16. http://www.cdc.gov/healthywater/global/household.html
17. Anwar MS, Chaudhry NA, Tayyib M. Qualitative assessment of bacteriological quality and chlorination status of drinking water in Lahore. J Coll Physicians Surg Pak. 2004;14(3):157-60.
18. Spinks AT, Dunstan RH, Harrison T, Coombes P, Kuczera G. Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures. Water Res. 2006;40(6):1326-32.
19. Juan C. Bandres, MD; John J. Mathewson, PhD; Herbert L. Du Pont, MD. Heat Susceptibility of Bacterial Enteropathogens *Arch Intern Med.* 1988; 148(10):2261-2263.
20. Stephen E. Luby, Amber H. Syed , Naureen Atiullah[†], Mohammad K. Faizan MBBS[†], Susan Fisher-Hoch MD. Limited effectiveness of home drinking water purification efforts in Karachi, Pakistan International Journal of Infectious Diseases Volume 4, Issue 1, 2000,3-7
21. Ali AM, Qureshi AH, Rafi S, Roshan E, Khan I, Malik AM, et al. Frequency of Campylobacter jejuni in diarrhoea/dysentery in children in Rawalpindi and Islamabad. J Pak Med Assoc. 2003 Nov; 53(11):517-20.
22. Moats WA. Kinetics of thermal death of bacteria. J Bacteriol. 1971;105(1):165-171.
23. Anwar MS, Lateef S, Siddiqi GM. Bacteriological quality of drinking water in Lahore. Biomedica. 2010;26:66-69.