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An Observational Study on the Effectiveness of Point-Of-Use Chlorination

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Abstract

Although the efficacy of chlorine disinfection under controlled laboratory conditions is well known, the effectiveness of chlorine under field point-of-use (POU) conditions is still not clearly understood and may be impacted by a variety of factors. This study evaluated the effectiveness of POU chlorine disinfection in rural Ecuador under typical use conditions and compared this effectiveness with the efficacy in controlled laboratory conditions. While reductions of indicator organisms were slightly higher in households that used chlorination, no significant differences were seen between households employing POU chlorination and the households with no chlorination (1–1.5 $\log_{10}$ median reductions for chlorinating households and 0.31–0.55 $\log_{10}$ for nonchlorinating households, depending on the indicator organism). In contrast, significant reduction of all test organisms was found when simulating POU conditions in the laboratory. This study demonstrates that POU chlorination can be considerably less effective under actual field conditions than would be predicted based on its laboratory efficacy (3–5 $\log_{10}$ median reductions for chlorinated and 0–0.3 $\log_{10}$ for non-chlorinated samples). Human factors (including improper storage and chlorine dosing) and uncontrolled water quality effects are hypothesized to impact significantly the effectiveness of chlorine disinfection.

Introduction

Consumption of fecally contaminated water is a leading cause of death in rural regions of less-developed countries (Briscoe, 1986). According to the World Health Organization (WHO, 2004), 1.8 million people die each year from diarrheal diseases, 90% of which are under the age of five. Most of these deaths are in less-developed countries, especially in rural areas where there is limited access to safe water and adequate sanitation. In fact, WHO attributes 88% of diarrheal disease to consumption of unsafe water, lack of adequate sanitation, and poor hygiene. WHO further suggests that simple improvements in drinking water quality using point-of-use (POU) treatment, including chlorine, can lead to a reduction in diarrheal episodes by between 25% and 40% (WHO, 2004).

POU chlorination currently plays a major role in providing safe drinking water in many rural areas without piped distribution systems (Rangel, Lopez, Mejia, Mendoza, & Luby, 2003; Reller et al., 2003). While it is widely understood that chlorine can achieve a significant inactivation of most waterborne pathogens in controlled laboratory and controlled POU settings (Blaser, Smith, Wang, & Hoff, 1986; Whan, Grant, Ball, Scott, & Rowe, 2001),

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many variables may still influence the effectiveness of POU chlorination in the field, such as uncontrolled water quality parameters, behavioral factors associated with rural water treatment, and so on (Fernandez Gomez et al., 1993; Patel & Isaacson, 1989). These variables might contribute to reduced effectiveness of POU chlorination in practice, as compared to more controlled laboratory and intervention field settings, and are not typically included in rural drinking water studies.

Studies on POU drinking water treatment interventions are challenged by many aspects such as the complex transmission pathways of diarrheal disease, lack of consistency in field methods, and controversy over the best indicator to represent diarrheal pathogens (Gleeson & Gray, 1997; Young, Clesceri, & Kamhawy, 2005). Additionally, human factors and uncontrolled water quality parameters are not always included in POU studies.

Luby and co-authors conducted a study in Karachi, Pakistan, that compared drinking water quality in control households to intervention households that received a safe container and a diluted hypochlorite solution. They found a significant difference (2 $\log_{10}$) in thermotolerant coliform (TTC) concentration between these two groups of households (Luby et al., 2001). Sobsey and co-authors conducted a similar study in Bangladesh and Bolivia that also combined safe storage with chlorine use. Their study showed some reduction of indicator organisms as well as incidence of diarrhea when chlorine was used effectively (Sobsey, Handzel, & Venczel, 2003). These intervention studies, however, exclude real-world chlorine-use variables such as chlorine storage time, concentration, and proper dosing, because participants are more likely to use chlorine properly after initial training or with assistance from the investigators than they are in actual nonresearch study situations. In nonresearch villages, chlorine training and assistance are not common components of household chlorine use.

In addition, human behavior is likely to change over time, making study length an important variable. In fact, Arnold and Colford (2007) found attenuation in the reduction of childhood diarrhea as study length increased. Most interventions are relatively short, while real-world chlorine use should be indefinite and consistent in order to be effective. Longer studies and observational studies are likely to be more accurate descriptions of real-world chlorine use.

Efficacy describes the potential of an intervention under *ideal* conditions, e.g., $\log_{10}$ reduction of microorganisms with a proper chlorine dose and contact time. Effectiveness is a more general term that includes efficacy. Effectiveness is a measure of benefit resulting from POU chlorination under real-world conditions of implementation. As such, it considers the efficacy of an intervention as well as its application and its acceptance by those who use it. In addition to human and use variables, effectiveness includes various water quality factors that affect chlorination. Both physical (pH, temperature, etc.) and chemical (inorganic compounds and ions, dissolved organic compounds, particulates, etc.) parameters greatly influence disinfection *efficacy* (Sobsey, 1989). Additionally, chlorine efficacy is much lower in water with high oxidant demand, a factor of which chlorine users may not be aware (Crump et al., 2004). *Effectiveness* of POU chlorination includes all variables that affect the $\log_{10}$ reduction of microbial contamination by chlorine from the time of water collection until consumption (efficacy *plus* human factors).

For the current study, an observational design was chosen to minimize bias toward higher microbial reductions in treatment households, which is inherent in intervention studies. This observational study design captures variables such as compliance and difficulty-of-use. It tests the real-world effectiveness of POU chlorination. This study is valuable because it examines the effectiveness of chlorine in a village where it was available, but no education, marketing, or additional steps (safe containers or chemical flocculation) had been recently

provided. This project was designed based on the current chlorine-use practices in the Borbón region in northern coastal Ecuador. Participants had previous experience with chlorine disinfection but were given no training on the most effective way to use chlorine beyond initially reading the instructions on the chlorine bottle to participants as a safety precaution. Participants were encouraged to continue using chlorine in the same way that they had used it in the past.

Methods

Field Study

Site—This study was carried out in a rural village in the Borbón region of northern coastal Ecuador (Esmeraldas Province), approximately 50 km south of the Colombian border. It is one of the largest villages in the Borbón region (approximate population 700), with intermittent electricity and no centralized water distribution system. Most residents of this village collect their household water from a local stream, although some residents rely on shallow wells or rainwater. A map of the village is shown in Figure 1. Water storage containers are typically filled once per day or less often for use inside the home. This study was conducted as part of a larger five-year National Institute of Health (NIH)–funded diarrheal disease transmission project in approximately 20 villages in the Borbón region.

Household Selection—Using data from the parent project, 30 households that potentially used chlorine for water treatment were identified. These households answered a more detailed chlorine-use survey to identify households that were likely to use chlorine for water treatment during the three-week sampling period of this study. A total of 10 households gave consent to participate and answered *yes* to these more detailed chlorine-use survey questions. These 10 households were enrolled in the study. It is important to note that these households were asked about their *current* chlorine use patterns. They were not asked to change their chlorine use behavior for this study. While households that currently used chlorine were selected, researchers made an effort not to influence their chlorine-use practices, maintaining an observational study design. The chlorinated POU samples represent real-world households who choose to use and have access to chlorine as a drinking water treatment option. One household dropped out of the study due to illness in the family, leaving nine households for the final analysis. These nine households were spread out throughout the study village and collected water from four different places on the source stream. Contact with human subjects was reviewed by the IRB committees at University of Washington; University of California, Berkeley; and Universidad San Francisco de Quito.

Water Storage Containers—All households in this study used common water storage containers (buckets, jerry cans, or gallon milk jugs) made of low-density polyethylene (LDPE). Drinking water was always collected from the source stream.

Field Sampling—Field sampling was conducted over three weeks during August 2005. Households were sampled between two and nine times each depending on their availability. Each time, two types of water samples were collected a source sample and a POU sample. All samples were collected in sterile 500 ml Whirlpak® bags, stored on ice, and analyzed within 24 hours. The source samples were taken at the same time and place that the family filled their storage containers with water. The POU samples were taken from household storage containers approximately 24 hours after filling the containers. Drinking water had been stored or used during this 24-hour period. In this study, household water storage containers were not sterilized. Rather, they were used in the same way during this study as they were used during other times of the year. Chlorine residual was measured for each POU sample. A POU survey was also administered at this time to determine if the household had

used chlorine for this particular water collection event. Field and sample duplicates were each taken in approximately 10% of the samples for each organism (U.S. Environmental Protection Agency [U.S.EPA], 1986).

Laboratory Study

To reflect observed field conditions, four LDPE sample bottles were filled with autoclaved lake water from Portage Bay, Seattle, Washington. Three of the bottles were seeded with field-isolated strains of *Enterococcus*, *E. coli*, and a laboratory strain of somatic coliphage (ΦX174). The last sample bottle (control) was not seeded with the indicator organisms. After seeding with indicator organisms, one sample bottle was dosed with a free chlorine concentration of 2.9 mg/l, equivalent to one drop per liter (corresponding to the instructions on the chlorine bottle in the field). The other two seeded sample bottles were not dosed with free chlorine and were used to determine the effect of temperature. One bottle was stored at 26.5°C (along with the control and chlorine-dosed sample bottle), and the other at 4°C. The average temperature in the study village during the time of the field experiment was 26.5°C. Samples were taken from the sample bottles at the beginning of the experiment (time zero), immediately following free chlorine addition, and 24 hours after free chlorine addition (time one). Laboratory experiments were conducted four times and samples were analyzed in duplicate.

Microbiological Assays

Bacteria—In the field study, *E. coli* was analyzed on 3M® Petrifilms using 1 ml of each undiluted sample. Blue colonies were counted as *E. coli* and red (coliform) colonies were not quantified or used in this study. *Enterococcus* was analyzed using a modified sterile membrane filtration field technique based on U.S. EPA Method 1600. Ten ml sample volumes were used, regardless of the sample type (chlorinated, nonchlorinated, source, POU, or control). Colonies were grown on mEI agar and incubated at 41°C for 24 hours. For the laboratory study, *E. coli* and *Enterococcus* were isolated and assayed by membrane filtration on MacConkey agar and mEnterococcus agar, respectively. Preliminary investigation demonstrated that results from the two types of *E. coli* assays (membrane filtration on selective media and Petrifilms) were within 10% of each other (data not shown).

Coliphages—Somatic coliphage concentrations were analyzed using a modified version of the double agar layer (DAL) (Adams, 1959). The top agar layer was pre-poured into tubes, capped, sealed, and solidified prior to sampling. At the field site, these tubes were boiled and individually remelted. *E. coli* CN13 (American Type Culture Collection #700609) was added to 2 ml water samples mixed with the top agar layer and poured over the bottom agar layer of trypticase soy agar containing naladixic acid. Plates were then incubated at ambient temperatures (approximately 26.5°C) overnight. The laboratory phage protocol was modeled after the field double agar layer protocol, except the top agar was not solidified and remelted as it was in the field.

Data Analysis

In both the laboratory and the field samples, log reductions were calculated based on the difference in indicator organism concentrations prior to adding chlorine and approximately 24 hours following chlorine addition. Log_{10} reductions were compared for chlorine and nonchlorine POU field samples and for treated and untreated laboratory samples. In cases when no indicator organisms were detected following chlorination, the log reduction was reported as “greater than” the maximum detectable reduction, and a concentration of one half the organism’s detection limit was used to calculate a surrogate reduction for the

purposes of statistical analysis. All field samples with initial (time zero) organism concentrations below the detection limit were excluded from the analysis.

All data were analyzed using Stata 9 (College Station, TX). Medians and median average deviations (MADs) of log reductions were compared between chlorinated and nonchlorinated samples in both field and laboratory experiments. The Wilcoxon rank sum hypothesis test was used to determine statistical significance between $\log_{10}$ reductions in chlorinated and nonchlorinated samples.

Results

Field Study

A total of 46 paired samples (source and POU) taken from nine households were analyzed. Twenty-four samples were reported as chlorinated and twenty-two samples were reported as nonchlorinated. Samples were grouped as chlorinated or nonchlorinated, independent of household (Figure 2). Two households always reported chlorinating their water, and one household never reported chlorinating their water. The remaining households reported water chlorination in a portion of samples taken over the course of the study.

The concentration of indicator organisms in source water ranged from 1.5 to 3.6 log Colony-Forming Units (log CFUs)/100 ml, 0 to 4.2 log CFUs/100 ml, and 0 to 4.1 log Plaque-Forming Units (log PFUs)/100 ml for *Enterococci*, *E. coli*, and coliphage, respectively. The median and MADs of $\log_{10}$ reductions for each organism in chlorinated and nonchlorinated samples are shown in Figure 3. The differences between $\log_{10}$ reductions in chlorinated and nonchlorinated samples ranged from 0.40 to 0.94 depending on the indicator. Reductions, however, were not significantly different between chlorinated and nonchlorinated field samples ($p > .05$ for all tests). The median log reductions for chlorine and nonchlorine households were 1.0 and 0.53, 1.50 and 0.55, and 1.0 and 0.37, for *E. coli*, *Enterococci*, and somatic coliphage, respectively (Figure 3). Differences in $\log_{10}$ reductions between chlorinated and nonchlorinated samples were not significant for any indicator organism ($p = .08$ for *E. coli*, $p = .10$ for *Enteroccoci*, and $p = .16$ for somatic coliphage). Chlorinated POU samples had detectible concentrations in 50%, 58%, and 21% of the *E. coli*, *Enterococci*, and phage samples, respectively. Likewise, nonchlorinated POU samples had detectible concentrations 86%, 82%, and 32% of the time for *E. coli*, *Enterococci*, and phage, respectively.

Chlorine residual was also measured in each POU sample (data not shown). The total chlorine residuals observed in the households that responded yes when asked if they had chlorinated their water were slightly higher than for households that answered no; however, the results were not statistically different.

Laboratory Study

E. coli and *Enterococcus* were initially seeded at a level of 10^6 CFUs/ml and coliphage was seeded at 5×10^3 PFU/ml. The median and median absolute deviations of $\log_{10}$ reductions for each organism under laboratory conditions are shown in Figure 4. None of the test organisms was significantly reduced in nonchlorinated samples; in fact, *E. coli* was in slightly higher in concentration after 24 hours. Median $\log_{10}$ reductions of −0.56, 0.28, and 0.03 were observed in nonchlorinated samples for *E. coli*, *Enterococci*, and coliphage X174, respectively. *E. coli* was the most sensitive test organism and coliphage ΦX174 was the most resistant organism to chlorination at the tested dose. Median $\log_{10}$ reductions for chlorine-treated samples were observed to be 5.23, 3.82, and 3.20 for *E. coli*, *Enterococci*, and coliphage ΦX174, respectively. Differences in $\log_{10}$ reductions between chlorinated and

nonchlorinated samples ranged from 3.2 to 5.9 depending on the indicator. All differences were observed to be significant ($p<.05$).

Discussion

In strict epidemiological language, efficacy would refer to the impact of chlorine treatment in an experimental trial. This differs from effectiveness, which refers to the impact of chlorine treatment under real-world conditions. The current study is an observational study measuring the effectiveness of water chlorination under typical use (real-world) practices, not the efficacy under ideal use practices that are typically evaluated in intervention studies.

This observational study had two main findings. First, chlorine water treatment was not used every day in this village, even among households who claimed to use it. Only two households reported always chlorinating their water during the study. Other households reported chlorinating their water 14%–77% of the time (median 50%), and one household reported never chlorinating their water during the course of the study. Second, despite well-demonstrated laboratory efficacy for chlorination at the doses and contact times used in this field study, no significant reductions of indicator organisms in chlorinated household samples were observed as compared to nonchlorinated samples. This suggests that POU chlorination, as implemented in the Borbón region of Ecuador, is ineffective and should not be expected to control waterborne disease in that region.

In contrast, the results of some previous *intervention* field studies suggest that POU chlorination is an effective method of control when used in combination with a safe container (Sobsey, Handzel, & Venczel, 2003), additional treatment to remove turbidity (Crump et al., 2004), or an education campaign (Quick et al., 2002). These additional steps are not always introduced with POU chlorination in real-world households, unfortunately. Intervention studies are also likely to have a higher compliance rate than actual household water treatment situations, due to direct oversight of investigators. As a result, it is more likely that chlorine is used properly when applied in conjunction with an intervention research project. Outside of research projects, however, most POU water treatment has little or no oversight from an outside group. Chlorine is often donated or purchased with no follow-up training or compliance work. Even if additional steps are introduced with POU chlorination, these practices must be suitably reinforced to prevent the attenuation in compliance observed by Arnold and Colford (2007). The observations of the current study also support a need for reinforcement of appropriate POU chlorination procedures. This study did not intend to comprehensively identify all unsafe water samples; rather, it evaluated the effectiveness of household chlorination by comparing sample concentrations of indicator organisms before and after chlorination. The Petrifilm method used in this study was not sensitive enough to ensure the WHO *E. coli* standard for drinking water of 0 CFUs/100 ml due to the small sampling volume (1 ml) (WHO, 2006). Still, it is important to note that several water samples were found to have elevated levels of *E. coli* using the Petrifilms (>100 CFUs/100 ml), even after chlorination.

A potential limitation of this study is the dependence on household survey responses to distinguish between chlorinated and nonchlorinated samples, which may have resulted in potential misclassification of households. Due to the short-term nature of the recall, it is likely that any misclassification was due to untruthfulness of study participants who may have wanted interviewers to believe they had chlorinated their water when they actually had not. To offset this concern, interviewers encouraged honest answers by reacting in the same way regardless of household responses to chlorine questions.

In this study, chlorine residual concentration test results were inconclusive. Even though results were higher for samples reported as chlorinated, they were not significantly different from samples reported as not chlorinated. These results could reflect a high chlorine demand of the source water that consumed free chlorine, a failure to adhere to proper chlorination practices by the user, or the use of a degraded chlorine product.

A number of potential explanations are available as to why significant increases in $\log_{10}$ reductions were not observed in chlorinated samples as compared to nonchlorinated samples in this study. First, no additional treatment occurred to remove turbidity. As mentioned earlier, various water quality factors—both physical and chemical—greatly influence disinfection efficacy (Sobsey 1989). Based on the overall appearance of the water, it is likely that most added chlorine was consumed by inorganic and organic compounds in the water, leaving too little free chlorine to achieve significant inactivation of microorganisms. Second, household water storage containers were not sterilized or required to meet safety standards. Rather, they were used in the same way during this study as they were used by the households in other times of the year. Therefore, it is possible that drinking water was contaminated by household sources such as contaminated hands. Third, villagers might add chlorine inconsistently or incorrectly to their drinking water. The poor economic situation in the region may cause villagers to use less chlorine than directed on the bottle in order to make a bottle last longer, a behavior that would render the chlorine less effective. All of these potential explanations, regardless of how difficult they are to study, are important in choosing an acceptable water treatment method and using it properly in households.

Conclusion

Intervention trials typically measure the efficacy of POU chlorination and not necessarily the effectiveness. This distinction must be considered when interpreting data from the literature on POU chlorine treatment. Arguably, effectiveness is a more accurate description of how chlorine use will affect health, suggesting that variables such as ease-of-use and uncontrolled water quality parameters may prove to be important factors in assessing POU drinking water treatment. The results of this study also suggest a need for supplements to chlorine dissemination programs in order to effectively improve water quality and thereby reduce incidence of diarrheal disease. Further research is necessary to quantify the field effects of chlorine demand, safe containers, household contamination, and other behavior aspects on the effectiveness of POU chlorination. Finally, a large-scale observational study is needed to verify the results and determine their impact on diarrheal disease in the study area.

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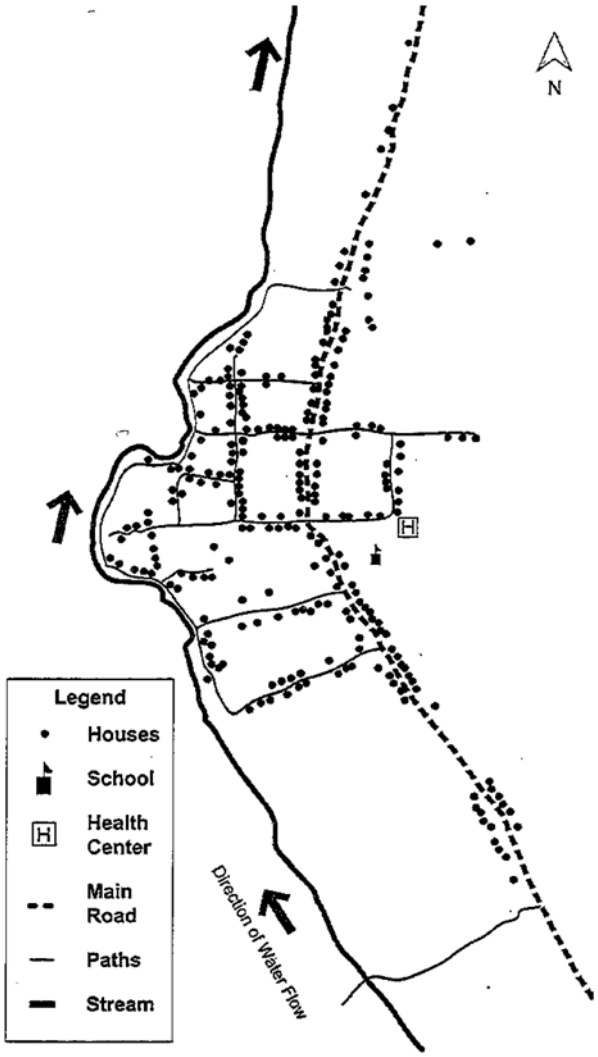

FIGURE 1.
Map of Study Village

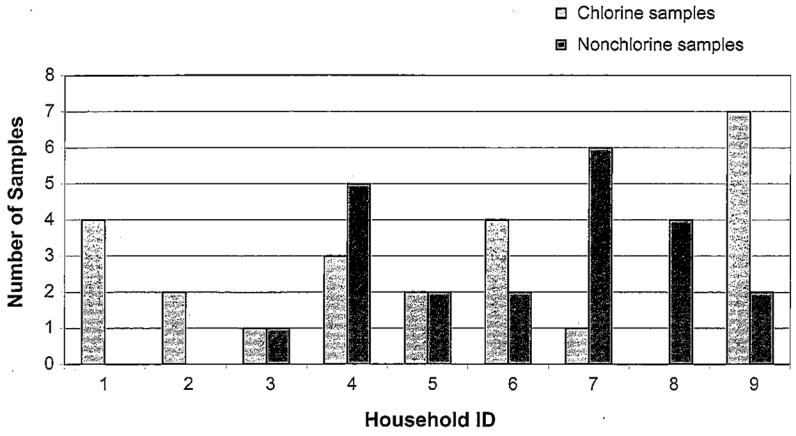

FIGURE 2.
Number of Chlorinated and Nonchlorinated Samples from Each Household

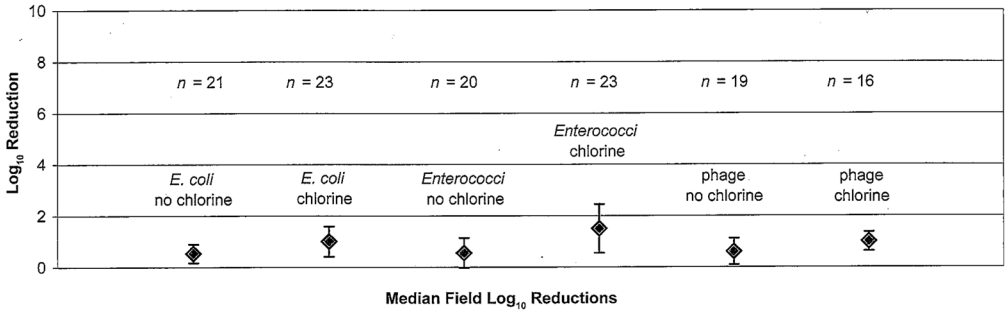

FIGURE 3.
Median and MAD Log Reductions in Field Samples

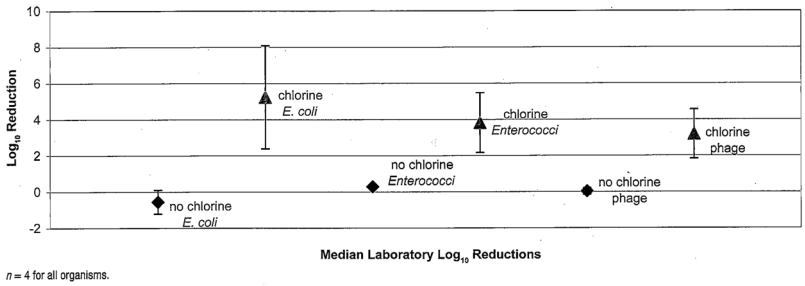

FIGURE 4.
Median and MAD Log Reductions in Laboratory Samples