THE EFFECT OF WATER QUALITY ON THE OUTCOME OF HAND HYGIENE

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND AND MOTIVATION

Hand washing has been promoted for decades as an effective strategy to prevent the spread of infectious disease (Larson *et al*., 2003). It does not only assist in minimizing the risk of person-to-person transmission in hospital environments but also plays an important role in preventing the spread of disease via water and food and during food preparation. A number of studies have been conducted to determine the role of various factors on the efficacy of hand washing (Montville et al, 2002). Factors that have specifically been addressed in poor developing communities include the initial level of contamination, the type of rubbing agent used (soap, mud or ash), the source of the water used for rinsing and the procedure followed during the drying of the hands (Hoque *et al*, 1995). The degree to which the quality of the water used for hand washing contributes towards hand hygiene has not previously been addressed. This is a very important issue for households and health care facilities that do not have access to safe water.

OBJECTIVES OF THE PROJECT

The scope of this project was to investigate the extent to which the quality of water used for hand washing affects the outcome of the hand washing process. The specific aim of the study was:

- To determine whether water of varying quality in combination with the use of soap and drying could result in a reduction of the level of bacteria on the hands of volunteers.

RESEARCH APPROACH

The study was conducted in the laboratory to ensure the easy comparison of treatments. The hands of participants were initially spiked with *E. coli* and were thereafter subjected to different washing procedures using water of different quality. Three washing procedures were followed. The first only involved washing (rinsing) the hands and the second procedure involved washing the hands with a normal bar of body soap and rinsing it afterwards. The last procedure was identical to the second procedure but it was followed by drying of the hands on paper towel. Each experiment was repeated 10 times.

The hand washing procedures were repeated 4 times using water of different quality. For the study normal tap water, tap water spiked with *E. coli* to a level of 10^3 CFU / ml (Medium), tap water spiked with *E. coli* to a level of 10^6 CFU / ml (High), and naturally contaminated water collected from a stream in a rural area of the Limpopo Province, were used. The level of bacteria on the hands of the participants was determined using the modified glove-juice technique and the Colilert system for the enumeration of *E. coli.* An Anova one-way of analysis was performed to determine differences between treatments at the 5% level of significance.

SUMMARY OF MAJOR RESULTS AND CONCLUSIONS

None of the procedures using highly polluted water (10^6 *E. coli* / ml) for hand washing resulted in an improvement (decrease) of the bacterial load on the hands. When drying was applied, the level of bacteria was similar to that of the untreated control whereas for the other two treatments the bacterial levels even increased.

Overall there was very little difference between the outcomes of the different treatment procedures when moderately spiked water (10^3 *E. coli* / ml), natural water from a rural stream, or tap water were used. All the procedures involving the use of water with moderate to low levels of contamination showed a significant improvement when compared to the control. The use of soap did not decrease the bacterial load substantially when compared with only rinsing the hands. In all cases the procedure that included the drying of the hands showed the largest reduction in the bacterial load. This procedure resulted in at least a two log reduction in the bacterial load on the hand of participants.

This study showed that hands with a high bacterial load can be washed with water of even moderate contamination levels but that highly polluted water would not be suitable. Little difference was noted between the procedure of only rinsing the hands with water and that of washing the hands with soap. The best reduction in bacterial levels on hands was achieved when the full procedure of washing with soap followed by the physical drying of the hands was followed.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future research should address the issue of whether the suitability of water to be used for hand washing is dependant on the bacterial load of the hands to be washed.

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THE EFFECT OF WATER QUALITY ON THE OUTCOME OF HAND HYGIENE

1.1 BACKGROUND

Since the initial work by Holmes and Semmelweis during the second half of the nineteenth century hand washing has been promoted as an effective strategy to prevent the spread of infectious disease (Wendt, 2001; Larson et al., 2003). It does not only assist in minimizing the risk of person to person transmission in hospital environments but also plays an important role in preventing the spread of disease via water and food especially during food preparation. A number of studies have been conducted to determine the role of various factors on the efficacy of hand washing. Most of the studies have focused on health care settings and food processing plants (Wendt, 2001; Montville et al, 2002; Banfield and Kerr, 2005) but the domestic environment has also received some attention (Curtis and Cairncross, 2003; Larson et al.,2003). Factors that have specifically been addressed in poor developing communities include the initial level of contamination, the type of rubbing agent used (soap, mud or ash) the source of the water used for rinsing and the procedure followed during the drying of the hands (Hoque et al, 1995). The importance and promotion of hand washing in homes, particularly in developing countries have, however, received limited attention (Curtis and Cairncross, 2003)

Access to safe water remains a serious problem worldwide, especially in developing countries. Rapid population growth, inadequate infrastructure and limited water resources (scarcity) are some of the main factors that have contributed to this problem. Contamination of water sources by human and animal wastes is common in these countries and has serious health and economic consequences for communities who rely on such sources for domestic use. The degree to which the quality of the water used for hand washing contributes towards hand hygiene has not previously been addressed in detail. This is an important issue for households and health care facilities that do not have access to safe water.

1.2 OBJECTIVES OF THE PROJECT

The scope of this project was to investigate the extent to which the quality of water used for hand washing affects the outcome of the hand washing process. The specific aim of the study was:

- To determine whether water of varying quality in combination with the use of soap and drying could result in a reduction in the level of bacteria on the hands of volunteers.

1.3 MATERIALS AND METHODS

1.3.1 Volunteers

The study was conducted under controlled laboratory conditions using 40 volunteers. The volunteers were all staff members or students from the Department of Microbiology and Plant Pathology at the University of Pretoria. The volunteers were limited to this group as they all had knowledge of microbiology and were able to understand the procedures and the potential risk involved. Both male and female volunteers representing the various racial groups were included. None of the participants were subjected to more than 4 treatments.

1.3.2 Cultures and contamination levels

To ensure that all treatments started with comparable levels of contamination, the hands of the volunteers were spiked with a non-pathogenic laboratory strain of *E coli*. Each participant received 2.5 ml of a suspension containing approximately 10^6 *E. coli* /ml. The participant was asked to spread the bacterial suspension to the whole surface area of both hands by rubbing the hands together. The *E. coli* was grown overnight for 18 hours at 37^oC in Nutrient broth (Biolab, Merck).

1.3.3 Water quality

The hand washing procedures were repeated 4 times using water of different quality. For the study normal tap water, tap water spiked with *E. coli* to a level of 10^3 CFU / ml (Medium), tap water spiked with *E. coli* to a level of 10^6 CFU / ml (High), and naturally contaminated water collected from a stream in a rural area of the Limpopo Province, were used.

1.3.4 Washing procedures

For each of the different types of water three washing procedures were followed. The first only involved washing (rinsing) the hands with 100 ml of water. The second procedure involved washing the hands with a normal bar of body soap and rinsing it afterwards. For this procedure each participant was supplied with 100 ml of water. The last procedure was identical to the second procedure but it was followed by drying of the hands on paper towel. Each procedure was repeated 10 times.

The list of washing procedures that were used is as follows:

- Washing (rinsing) with water spiked with 10^6 *E.coli* / ml
- Washing (rinsing) with water spiked with 10^3 *E.coli* / ml
- Washing (rinsing) with naturally contaminated river water
- Washing (rinsing) with clean water
- Washing with soap and water spiked with 10^6 *E.coli* / ml
- Washing with soap and water spiked with 10^3 *E.coli* / ml
- Washing with soap and naturally contaminated river water
- Washing with soap and clean water
- Washing with soap and water spiked with 10^6 *E.coli* / ml followed by drying with a paper towel
- Washing with soap and water spiked with 10^3 *E.coli* / ml followed by drying with a paper towel
- Washing with soap and naturally contaminated river water followed by drying with a paper towel
- Washing with soap and clean water followed by drying with a paper towel

1.3.5 Bacterial counts on hands

The level of bacteria on both hands of each volunteer was determined by using the modified glove-juice technique as was described by Larson et al. (2003). The subject first inserted one hand into a sterile polyethylene bag containing 100 ml of the sampling buffer. After massaging the entire hand through the wall of the bag for one minute, the other hand was inserted and the procedure was repeated.

The sampling buffer was a 75 mMol /l phosphate buffer (pH 7.9) that contained 0.1% polysorbate 80; 0.1% sodium thiosulphate and 0.03% lecithin. These compounds were added to neutralize any residual antiseptics or chlorine in the sample or to disperse bacterial clumps into single cells for quantification purposes.

The level of *E. coli* present in each sample was determined using the Colilert system (IDEXX). Serial dilutions were made of the samples and the final dilution was made in 90 ml of ¼ Ringers solution. This was done to ensure that 100ml of sample was available for analysis. The content of one Colilert -18 snap pack was added to 100 ml of the sample. After the content was fully dissolved the sample reagent mixture was poured into a Quanti-Tray/2000 and sealed. The trays were incubated at 35°C for 18 hours after which the results were scored and the level of *E. coli* in the sample determined by using the corresponding MPN table. The results were expressed as CFUs/ set of hands.

1.3.6 Control values

The initial level of contamination (untreated control levels) was determined by measuring the E. coli levels of twenty participants directly after spiking of the hands. These experiments were conducted on two occasions to record any possible variations that might be present during the study which was conducted over a period of 4 days.

1.3.7 Statistical analysis

The student t test was performed on the two sets of control values to determine whether there was any variation between the results obtained on the two separate occasions. The Anova one-way analysis of variance test was performed on the data set to determine if there were significant differences between the various treatments at a 5% significance level.

1.4 RESULTS

The control *E. coli* levels as well as the level of *E. coli* after each individual treatment are provided in Appendix 1. The values are expressed as CFU/ set of hands. The data sets were summarized by determining the logarithmic value of the mean of each set as well as the standard deviation of the set. This information is summarized in Table 1.

The student t test showed that there was no significant difference between the two sets of control values collected on different occasions. For the rest of the statistical analyses these two sets were combined and treated as one data set.

Table 1. Summary of the basic statistical analyses of the data sets for the different treatments

Treatment	Number of repeats	Logarithmic value of the mean	Standard deviation
No washing	20	8.234609	0.81042
Washing with spiked water with 10^6 E. coli/ml	10	8.930232	0.080631
Washing with spiked water with 10^3 E. coli/ml	10	6.848767	0.592695
Washing with naturally contaminated river water	10	7.610858	1.005221
Washing with clean water	10	7.347228	0.317466
Washing with soap and spiked water with 10^6 E. coli/ml	10	9.009539	0.644627
Washing with soap and spiked water with 10^3 E. coli/ml	10	6.772885	0.222584
Washing with soap and naturally contaminated river water	10	7.016804	0.622452
Washing with soap and clean water	10	7.329512	0.488367
Washing with soap and spiked water with 10^6 E. coli/ml followed by drying with paper towel	10	8.074431	0.224825
Washing with soap and spiked water with 10^3 E. coli/ml followed by drying with paper towel	10	6.262006	0.39398
Washing with soap and naturally contaminated river water followed by drying with paper towel	10	6.164592	0.23177
Washing with soap and clean water followed by drying with paper towel	10	5.824303	0.433036

The significance values as calculated for the whole data set using the one-way Anova analysis are given in Appendix 2. All of this data have been summarized graphically in Figure 1.

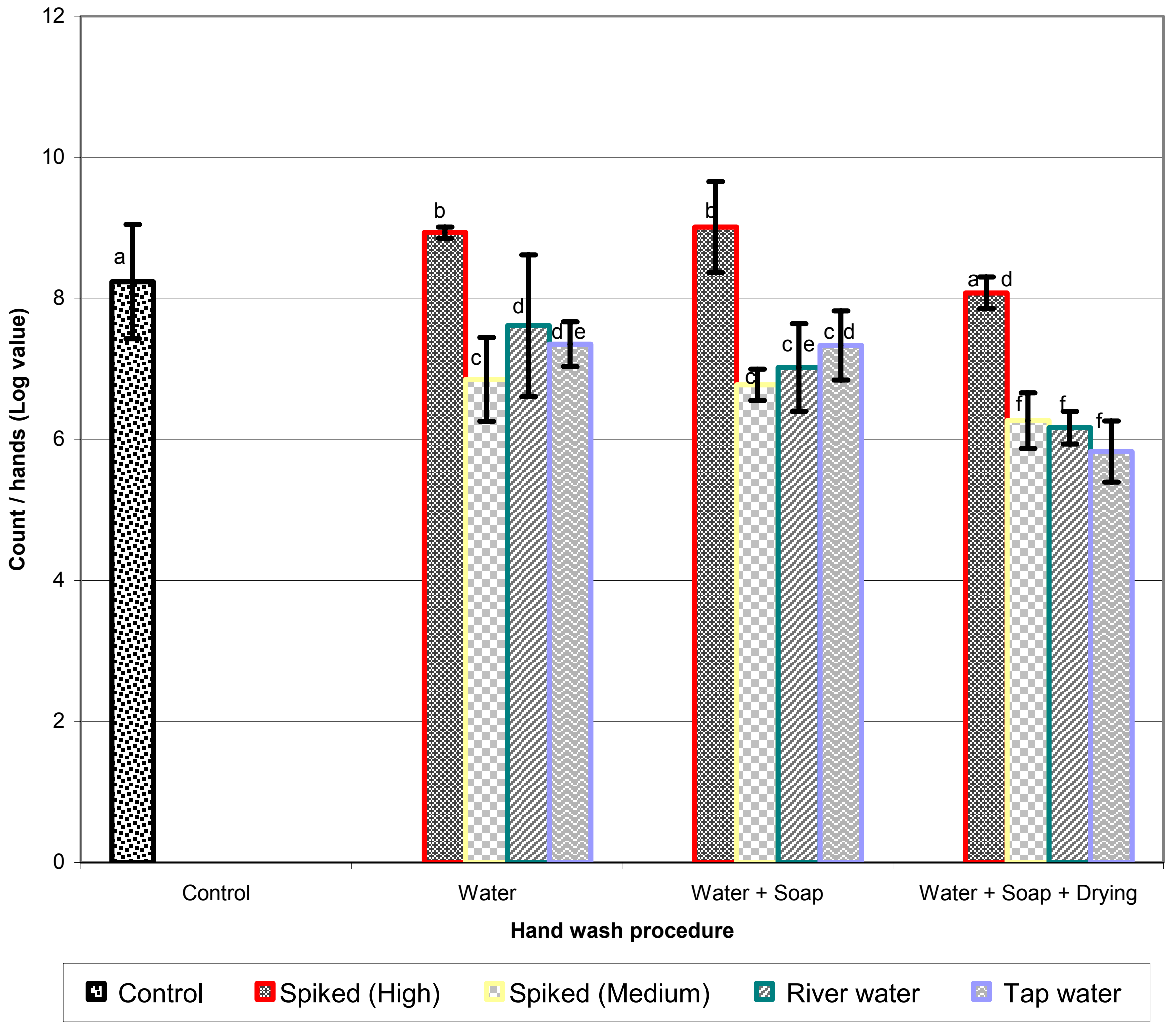

Figure 1. Graphic representation of the data sets for the different hand wash procedures using water of varying quality. Data sets where no significance (<5%) existed between the different treatments are labelled with the same letter.

From Figure 1 it can be seen that none of the procedures using highly polluted water (10^6 *E. coli* / ml) resulted in an improvement of the bacterial load on the hands. When drying was applied the level of bacteria was similar to that of the untreated control whereas for the other two treatments the bacterial levels even increased.

Overall there was very little difference between the outcomes of the different treatment procedures when moderately spiked water (10^3 *E. coli* / ml), natural water from a rural stream, or tap water were used. Rinsing the hands with moderately contaminated water had a slightly better outcome compare to when the other two types of water were used. The data sets obtained for washing with soap and drying with a towel showed no significant differences. All the procedures involving the use of water with moderate to low levels of contamination showed a significant improvement when compared to the control. The use of soap did not decrease the bacterial load substantially compared with only rinsing the hands. In all cases the procedure that included the drying of the hands showed the largest reduction in the bacterial load and should be the procedure of choice. This procedure resulted in at least a two log reduction in the bacterial load.

1.5 DISCUSSION

In order to be able to evaluate the effect of water quality on hand washing practices it was decided to spike the hands of all the participants with the same level of bacteria. Having similar initial levels of bacteria allowed for the direct comparison of different treatment procedure using water of varying quality. *E.coli* was selected as the bacterium to spike the hand with for a number of reasons. *E. coli* is commonly found in faecal material, is used as an indicator of faecal contamination and its behaviour is, in many respects, similar to that of many of the water-borne enteric bacterial pathogens. A non-pathogenic strain of *E. coli* and a rapid method for its detection were also available in the laboratory.

A community based study by Larson et al. (2003) showed that the bacterial load on the hand of primary care takers in households of a Latino neighbourhood in New York, USA, had a mean value of about 1×10^6 / set of hands. However, values as high as 3×10^7 / set of hands were measured and in 15% of the cases higher values were indicated. The lack of both water supply and sanitation systems in rural areas often restrict hygiene behaviour. Higher bacterial levels on hands would therefore typically be expected for rural areas than for urban areas. During the present study it was decided to use slightly higher values than what was recorded in the study of Larson et al. (2003). The mean bacterial level on the hands of the participants in this study was 1.7×10^8 / hands after spiking with *E. coli*.

Water of various levels of contamination was used in this study to represent the quality of water that might be encountered in rural areas. These areas often lack basic water supply and sanitation services and the surface water may be highly polluted. In a study performed by Venter et al., (1996) in an underdeveloped peri-urban area of South Africa it was determined that the mean level of bacteria in streams was in the order of 5×10^4 /ml. Values as high as 1×10^7 were, however, recorded. In the present study neutralized tap water was spiked to a high level of 10^6 *E. coli* / ml and to a moderate level of 10^3 *E. coli* / ml. Un-spiked tap water with no *E. coli* present and water from a rural stream with an *E. coli* level of about 2000 CFU / 100ml were also used.

The study only focused on three basic procedures typically associated with hand washing in developing urban and rural communities (Hoque et al., 1995). The rinsing of hands with water, washing with soap without drying the hands (only air drying allowed) and washing with soap followed by the drying of hands with a towel. In the study the best reduction in bacterial levels on hands was achieved when the full procedure of washing with soap followed by the physical drying of the hands, was followed. In the worst case it resulted in a 2 log reduction of the levels. A number of other studies also confirm this result (Hoque et al., 1995; Montville et al., 2002)

Little difference was noted between the procedure of only rinsing the hands and that of washing the hands with soap without drying. Results on the benefit of soap in reducing microbial densities on hand are varied. Larson et al. (2003) did not notice significant differences in the bacterial density after washing with soap whereas other groups reported positive outcomes (Hoque et al., 1995; Curtis and Cairncross, 2003).

The effect of water quality on hand washing was varied. None of the procedures involving highly polluted water (10^6 *E. coli* / ml) resulted in a reduction in the bacterial load on the hands. When drying with a paper towel was applied, levels similar to that of the control were noticed (Figure 1). Rinsing or washing the hands with soap using the highly contaminated water even lead to a significant increase in the bacterial load on the hands. Surprisingly, no significant differences in the bacterial loads were notice when clean or moderately contaminated water were used. The water collected from a stream in a rural area provided similar results. These results clearly indicate that hands with a high bacterial load can be washed with water of even moderate contamination levels but that highly polluted water would not be suitable.

In the light of the current findings an important question emerges. Would a significant reduction in the bacterial load of hands with a lower contamination load ($10^5 – 10^6$ CFU / set of hands) be noticed when moderately contaminated water is used for washing? It may well be that the suitability of water to be used for hand washing may be dependant on the bacterial load of the hands to be washed.

1.6 CONCLUSIONS

This study showed that hands with a high bacterial load can be washed with water of even moderate contamination levels but that highly polluted water would not be suitable. It also showed that little difference was noted between the procedure of only rinsing the hands with water and that of washing the hands with soap but without using a towel for drying. The best reduction in bacterial levels on hands was achieved when the full procedure of washing with soap followed by the physical drying of the hands was followed.

1.7 RECOMMENDATIONS FOR FUTURE RESEARCH

Future research should address the issue of whether the suitability of water to be used for hand washing is dependant on the bacterial load of the hands to be washed.

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Appendix A
Raw data

	MPN count		MPN count
Control values			
1A	4.79E+08	11A	2.06E+08
2A	9.61E+08	12A	4.36E+08
3A	6.59E+08	13A	2.28E+08
4A	9.14E+08	14A	5.75E+08
5A	6.02E+08	15A	2.49E+08
6A	7.50E+06	16A	2.22E+08
7A	7.50E+06	17A	3.13E+08
8A	1.00E+06	18A	4.36E+08
9A	5.25E+08	19A	2.22E+08
10A	2.03E+07	20A	2.49E+08
Washing with clean water		Washing with spiked water with 10^3 E. coli/ml	
1B	1.72E+07	1C	1.00E+06
2B	3.44E+07	2C	2.00E+06
3B	3.28E+07	3C	3.10E+06
4B	3.61E+07	4C	1.04E+07
5B	2.04E+07	5C	2.50E+07
6B	5.04E+06	6C	2.72E+07
7B	4.36E+07	7C	2.99E+07
8B	2.31E+07	8C	1.00E+06
9B	4.79E+07	9C	1.62E+07
10B	8.55E+06	10C	1.46E+07
Washing with spiked water with 10^6 E. coli/ml		Washing with naturally contaminated river water	
1D	9.61E+08	11E	5.25E+08
2D	1.21E+09	12E	9.14E+07
3D	9.61E+08	13E	8.70E+07
4D	6.29E+08	14E	8.30E+08
5D	9.14E+08	15E	8.30E+06
6D	8.30E+08	16E	6.59E+06
7D	7.56E+08	17E	6.89E+06
8D	6.89E+08	18E	3.28E+06
9D	8.30E+08	19E	9.14E+08
10D	8.70E+08	20E	3.28E+06
Washing with soap and clean water		Washing with soap and spiked water with 10^3 E. coli/ml	
11F	2.29E+07	11G	7.22E+06
12F	9.61E+07	12G	2.99E+06
13F	4.10E+06	13G	6.02E+06
14F	1.09E+07	14G	8.30E+06
15F	7.92E+07	15G	6.02E+06
16F	1.10E+07	16G	9.61E+06
17F	1.79E+07	17G	1.90E+06
18F	9.61E+07	18G	6.89E+06
19F	2.13E+07	19G	8.30E+06
20F	6.30E+06	20G	7.92E+06

Washing with soap and spiked water with 10^6 E. coli/ml	
21H	8.30E+09
22H	6.02E+09
23H	9.14E+09
24H	9.61E+08
25H	1.86E+08
26H	5.75E+08
27H	4.79E+08
28H	6.59E+08
29H	1.94E+08
30H	4.36E+08

Washing with soap and naturally contaminated river water	
21I	5.75E+07
22I	8.30E+07
23I	7.92E+07
24I	2.28E+06
25I	1.79E+06
26I	4.57E+06
27I	8.70E+06
28I	4.79E+06
29I	9.14E+06
30I	5.49E+06

Washing with soap and clean water followed by drying with paper towel	
21J	4.95E+06
22J	9.61E+05
23J	2.00E+05
24J	9.14E+05
25J	8.30E+05
26J	2.00E+05
27J	8.70E+05
28J	9.61E+05
29J	7.50E+05
30J	1.94E+05

Washing with soap and spiked water with 10^3 E. coli/ml followed by drying with paper towel	
31K	1.07E+06
32K	6.02E+06
33K	8.36E+05
34K	8.30E+06
35K	9.33E+05
36K	5.21E+05
37K	2.19E+06
38K	2.19E+06
39K	3.61E+06
40K	1.11E+06

Washing with soap and spiked water with 10^6 E. coli/ml followed by drying with paper towel	
31L	7.85E+07
32L	1.29E+08
33L	1.85E+08
34L	2.38E+08
35L	1.66E+08
36L	1.62E+08
37L	7.71E+07
38L	9.32E+07
39L	1.50E+08
40L	4.28E+07

Washing with soap and naturally contaminated river water followed by drying with paper towel	
31M	1.02E+06
32M	1.05E+06
33M	1.57E+06
34M	3.78E+06
35M	1.08E+06
36M	9.87E+05
37M	1.04E+06
38M	1.18E+06
39M	1.35E+06
40M	3.97E+06

Appendix B
Significance values of treatments

	1	2	3	4	5	6	7	8	9	10	11	12	13
1		<.0001	<.0001	0.0016	0.0046	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	0.4599	<.0001
2	<.0001		0.0479	<.0001	0.2928	0.9435	0.0230	<.0001	0.1878	<.0001	<.0001	0.0042	<.0001
3	<.0001	0.0479		<.0001	0.0028	0.0563	0.7616	<.0001	0.5019	<.0001	0.0202	<.0001	0.0070
4	0.0016	<.0001	<.0001		<.0001	<.0001	<.0001	0.7512	<.0001	<.0001	<.0001	0.0008	<.0001
5	0.0046	0.2928	0.0028	<.0001		0.2617	0.0010	<.0001	0.0188	<.0001	<.0001	0.0655	<.0001
6	<.0001	0.9435	0.0563	<.0001	0.2617		0.0275	<.0001	0.2125	<.0001	<.0001	0.0034	<.0001
7	<.0001	0.0230	0.7616	<.0001	0.0010	0.0275		<.0001	0.3302	0.0002	0.0427	<.0001	0.0162
8	0.0005	<.0001	<.0001	0.7512	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0003	<.0001
9	<.0001	0.1878	0.5019	<.0001	0.0188	0.2125	0.3302	<.0001		<.0001	0.0030	<.0001	0.0009
10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001		0.0818	<.0001	0.1751
11	<.0001	<.0001	0.0202	<.0001	<.0001	<.0001	0.0427	<.0001	0.0030	0.0818		<.0001	0.6969
12	0.4599	0.0042	<.0001	0.0008	0.0655	0.0034	<.0001	0.0003	<.0001	<.0001	<.0001		<.0001
13	<.0001	<.0001	0.0070	<.0001	<.0001	<.0001	0.0162	<.0001	0.0009	0.1751	0.6969	<.0001	

1 = Control

2 = Washing with clean water

3 = Washing with spiked water with 10^3 E. coli/ml

4 = Washing with spiked water with 10^6 E. coli/ml

5 = Washing with naturally contaminated river water

6 = Washing with soap and clean water

7 = Washing with soap and spiked water with 10^3 E. coli/ml

8 = Washing with soap and spiked water with 10^6 E. coli/ml

9 = Washing with soap and naturally contaminated river water

10 = Washing with soap and clean water followed by drying with paper towel

11 = Washing with soap and spiked water with 10^3 E. coli/ml followed by drying with paper towel

12 = Washing with soap and spiked water with 10^6 E. coli/ml followed by drying with paper towel

13 = Washing with soap and naturally contaminated river water followed by drying with paper towel