Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.7, 2013

Microbiological Dynamics of Potable Water under Storage Durations

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Abstract
The microbiological dynamics of potable water under different storage durations was carried out. tap and sachet water samples obtained from Elele were analyzed weekly for changes in total aerobic plate bacterial count, coliform count, *Escherichia coli* count, *Salmonella-Shigella* count, *Vibrio cholerae* count and fungal count during storage. Nutrient agar, MacConkey broth, eosin methylene blue agar, *Salmonella-Shigella* agar, thiosulphate citrate bile salt sucrose agar and Sabouraud dextrose agar were respectively used for the enumeration. The pour plate technique was employed and the statistical analyses used were analysis of variance and standard deviation. The total aerobic plate bacterial count for the borehole and satchet water samples ranged from 4.26 ± 0.4Log_{10}cfu/mL to 4.78 ± 0.1Log10cfu/mL and 0 ± 0.00Log_{10}cfu/mL to 4.53 ± 0.2Log_{10}cfu/mL respectively. The coliform count ranged from 9MPN/100mL to 240MPN/100mLand 0 ± 0.00MPN/100mL to 28MPN/100mL respectively. The *Escherichia coli* count ranged from 1.78 ± 0.5Log10cfu/mL to 3.59 ± 0.2Log_{10}cfu/mL and 0 ± 0.00Log_{10}cfu/mL respectively. The *Salmonella-Shigella* and *Vibrio cholerae* counts were 0 ± 0.00Log_{10}cfu/mL. The fungal count ranged from 0 ± 0.00Log_{10}cfu/mL to 2.90 ± 1.0Log_{10}cfu/mL and 0 ± 0.00Log_{10}cfu/mL. The bacteria isolated from the water samples were *Escherichia coli, Enterobacter* species, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* species and *Bacillus* species. Drinking of potable tap water stored for over a period of time may pose threat to the consumers if sanitary measures are not put in place during treatment of water.
Key words: Dynamics, durations, microbiological, potable, storage

1. Introduction

Drinking water or potable water is water of sufficiently high quality that can be consumed or used with low risk of immediate or long term harm. In most developed countries, the water supplied to households, commerce and industry is all of drinking water standard, although only a very small proportion is actually consumed or used in food preparation. Humans in most parts of the world have inadequate access to potable water and use sources contaminated with disease vectors, pathogens or unacceptable levels of toxins or suspended solids. Such water is not potable and drinking or using such water in food preparation leads to widespread acute and chronic illnesses and is a major cause of death and misery in many countries. Reduction of waterborne diseases is a major public health goal in developing countries. Typical water supply networks deliver potable water from the tap, whether it is to be used for drinking, washing or landscape irrigation (Jawas, Al-Ghazali, & Kharshid, 1988).

The potable water supply is important in the socio-economic life of communities. The source and potability of water supply reflects on the health conditions of the communities as contamination of such water with pathogens is the primary cause of disease outbreaks in such communities particularly in many developing countries. The transmission of disease through drinking water is, therefore, one of the primary concerns for safe water supply. The availability of potable water becomes a problem when supply is interrupted frequently and shortages become the order of the day in many developing countries. Treated water from municipal distribution system is the most popular source of water supply in most Nigerian communities, because of the belief that it has passed through an efficient water purification system. Water from this source is also believed to have met the recommended standards for potable water supply and therefore, considered to be the safest in terms of quality control and prevention of water communicable diseases (Ahmed, Kanwal, Tahir, & Raul, 2004).

Government-managed water treatment plants constitute the main source of such treated water supply to both urban and rural settlements. Due to some reasons the supply of potable water through public water from the main municipal distribution network is very unstable and unpredictable as supplies are often erratic. In other situations, the authorities saddled with responsibility of water supply are unable to meet up with the demand. Interruptions in water supply could be for a short period or even last for months during which period people opt for alternative sources of water from unconventional sources particularly streams and rivers (Ukhun, Tobi, & Okolie., 2005) to meet their immediate needs. This trend makes people resort to storing treated water from the water treatment plants. Elsewhere, situations such as earthquake, flooding and related natural disasters result in interruptions in water supply which often lead to domestic water storage (Georgia, 1999).

Water supply from the mains that is presumed safe is often stored in plastic tanks and other plastic containers for several months without considerations for the possible implications of storage on the quality of stored water. Studies have shown that water may become contaminated at any point between collection, storage and usage (Tambekar, Hirulkar, Bhokre, Gulhane, & Bhanginwar, 2006a; 2006b). Also, storing water in containers and handling procedures of water at home, hotels or restaurants cause water quality deterioration to such extent that the water becomes potential risk of infection to consumers (Jagals, Bokaka, & Grabow, 1999).

The aim of this study is to investigate the effect of storage duration on the microbiological quality of potable water supplied to a Nigerian community.

2. Materials and Methods

2.1 Collection of samples

Samples of borehole water and sachet water were collected from Elele, Rivers State, Nigeria. The Samples were stored at room temperature in sterile screw capped plastic containers and in the rubber sachets. This was to simulate storage of water as it is practised in many homes. At weekly interval, for up to 10 weeks, samples were taken from the stored water for microbiological analyses. Duplicates of each water samples were examined.

2.2 Chemical Reagents

Chemical reagents used in the study were of analytical grade and were products of Hach Company, Colorado, USA; BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories England. They were nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification and for stock culture; Sabouraud dextrose agar used for the isolation of fungi, *Salmonella-Shigella* agar for the isolation of *Salmonella* and *Shigella*, thiosulphate citrate bile sucrose agar for the isolation of *Vibrio cholerae,* eosin methylene blue agar for the isolation of *Escherichia coli* and MacConkey broth for coliform counts.

2.3 Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the borehole and sachet water were serially diluted in ten folds. Total viable heterotrophic aerobic bacterial counts were determined using pour plate technique. Then the molten nutrient agar, Sabouraud dextrose agar, *Salmonella-Shigella* agar and thiosulphate citrate bile sucrose agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi, *Salmonella-Shigella, Vibrio cholerae* respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30^oC for 48h and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

2.4 Enumeration of Coliforms

The coliforms were estimated using the Most Probable Number techniques (multiple tube fermentation technique) as described by Cheesbrough, 2005.

2.5 Characterization and Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized and identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative/fermentation (O/F), utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges-Proskaur reaction and urease production. The tests were performed according to the methods of Cheesbrough, 2005; Adeoye, 2007; Agwung-Fobellah & Kemajou, 2007; Ochei & Kolhatkar, 2008. Microbial identification was performed using the keys provided in the Bergey's Manual of Determinative Bacteriology, 1994.

Fungal isolates were examined macroscopically and microscopically using the needle mouth technique. Their identification was performed according to the scheme of Barnett & Hunter, 1972 and Larone, 1986.

3. Results

The results of the microbiological examination of potable (tap and sachet) water are recorded in Tables 1– 3.

Table 1 shows the mean count of microorganisms isolated from stored tap water. Total aerobic plate counts ranged from 4.26 ± 0.4Log_{10}cfu/mL to 4.78 ± Log_{10}cfu/mL. The week10 had the highest of 4.78 ± Log_{10}cfu/mL and the week0 had the least count of 4.26 ± 0.4Log_{10}cfu/mL. The *Escherichia coli* count ranged from 1.78 ± 0.5 Log_{10}cfu/mL to 3.59 ± 0.2 Log_{10}cfu/mL. The week4 had the highest count of 3.59 ± 0.2Log_{10}cfu/mL while week0 had the least count of 1.78 ± 0.5Log_{10}cfu/mL. The coliform count ranged from 9 MPN/100mL to 240 MPN/100mL. The week5 recorded the highest cont of 240MPN/100mL and week10 recorded the least count of 9MPN/100mL. The fungal count ranged from 0 ± 0.00Log_{10}cfu/mL to 2.90 ± 0.6Log_{10}cfu/mL. The highest count of 2.90 ± 0.6Log_{10}cfu/mL was recorded in week10 while weeks 0, 1, 2 and 3 recorded the least count of 0 ± 0.00Log_{10}cfu/mL. The ANOVA, $P < 0.05$ shows that there was a significant difference in the mean counts of total aerobic plate bacterial count, *Escherichia coli* count, coliform count and fungal count in stored tap water at different storage durations. The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean

counts of *Vibrio cholerae* count and *Salmonella-Shigella* count in the stored tap water at different storage durations.

Table 2 shows the mean count of microorganisms isolated from stored sachet water. Total aerobic plate counts ranged from 0 ± 0.00Log_{10}cfu/mL to 4.53 ± 0.2Log_{10}cfu/mL. The week10 had the highest count of 4.53 ± 0.2Log_{10}cfu/mL while the weeks0, 1 had the least count of 0 ± 0.00Log_{10}cfu/mL. The coliform count ranged from 0MPN/100mL to 28MPN/100mL. The highest count of 28MPN/100mL was recorded in the week10 while the least count of 0MPN/100mL was recorded in weeks0-5. The ANOVA, $P > 0.05$ shows that there was significant difference in the mean count of total aerobic plate bacterial count and coliform count in stored sachet water at different storage durations. There *Escherichia coli* count, *Salmonella-Shigella* count and *Vibrio cholerae* count had counts of 0 ± 0.00Log_{10}cfu/mL respectively. The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean counts of *Escherichia coli* count, *Salmonella-Shigella* count and *Vibrio cholerae* count.

Table 3 shows the microorganisms isolated and their percentage occurrence. The organisms isolated were *Escherichia coli, Enterobacter* species*, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* species and *Bacillus* species. The *Escherichia coli* had the highest occurrence of 37.04% while *Enterobacter* species and *Klebsiella* species had the least occurrence of 7.41% in the stored tap water. The *Staphylococcus aureus* had highest occurrence of 66.67% and *Escherichia coli, Enterobacter* species, *Pseudomonas aeruginosa* and *Klebsiella* species had the least occurrence of 0% in the stored sachet water.

4. Discussion

The bacterial species isolated from the water samples include *Staphylococcus aureus, Bacillus* species*, Escherichia coli, Enterobacter* species, *Pseudomonas aeruginosa* and *Klebsiella* species. These bacteria have been isolated from most community drinking water supplies in Nigeria and have been implicated in the transmission of water-borne diseases in various parts of the country (Antai & Anozie, 1987; Onuh & Gonchi, 1998, Inyang & Aderemi, 2001; Edema, Omemu, & Fapetu, 2001). The population of these microorganisms in stored water increased progressively with storage until after the 7th week when their population began to decline. *E. coli*, *Staphylococcus aureus* and *Enterobacter* species were bacteria that were frequently encountered particularly within the first seven weeks of storage. *E. coli* was constantly encountered at all stages of storage.

The occurrence of *Escherichia coli* and *Enterobacter* species in freshly collected water samples is indicative of faecal contamination which may be as a result of seepage into the broken pipes and also an indication of inadequate treatment of post-chlorination system. This implies that water entering the distribution system was not entirely devoid of coliforms. The United States Environmental Protection Agency (USEPA, 2005) recommends zero contamination level of coliforms in drinking water. The occurrence of coliforms in freshly treated water may be due to unhygienic conditions. Clark *et al.,* 1982 reported that coliforms could be found in both chlorinated and unchlorinated water as the complete elimination of coliforms in water requires knowledge of their population in such water to determine the quantity of chlorine that would be required for complete elimination. The observed count on freshly treated water samples, therefore, put to question the effectiveness of the water treatment procedures for water supplied in the area under study. Many treatment plants designed and installed in many third world countries have inherent operational problems (Ogedengbe, 1982). Some are very old and are never frequently maintained. The implication of this is that treated water obtained from taps and distribution points within the treatment plant premises are not potable as they are not entirely devoid of coliforms.
Staphylococcus aureus, which is a normal flora of the body, indicates contamination from handlers especially those fetching water from the taps. This organism when ingested can cause diarrhea and vomiting due to enterotoxin produced by it (Singleton, 1995; Frazier & Westhoff, 2004; Eze, Okoye, Agwung, & Nnabueke, 2008). The higher load of this organism in the tap water than in the sachet water samples may be as a result of handling of the taps and washing of hands or other parts of the body on the taps by individuals who may carry it on their bodies as normal flora thereby leaving the organism on the surfaces of the taps.

Bacillus species are Gram positive aerobic spore-formers and most members of the genus are saprophytic prevalent in the soil, water and air and on vegetation (Books, Butel, & Morse, 2004).

It has been observed that sterile water devoid of microorganisms rarely exists except in the laboratory (Ogbulie & Akujiobi, 2006). The population of coliforms decreased with storage time, while the total bacterial count in the stored water samples increased. This may be as a result of accumulation of debris from long storage which may encourage microbial growth.

This study has shown that there is a need for an improvement in water treatment procedures. There is also the need for awareness programmes to be put in place to educate communities on the possible health implications of drinking water which has been stored for a long time.

Conclusion

It has shown that storing potable water for a long period increases the microbial load. Therefore consumers of potable water stored over a period of time should take precautions either by boiling, filtration or other methods of treatment of water to avoid being contaminated by microorganisms that may be pathogenic to humans resulting from such storage durations.

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Table 1: The Mean counts of microorganisms isolated from stored tap water.

	Log_{10}cfu/mL					MPN/100mL
Storage (weeks)	**TAPC**	EC	VC	**SSC**	FC	CC
0	4.26 ± 0.4	1.78 ± 0.5	0 ± 0.0(	0 ± 0.00	0 ± 0.00	20
1	4.30 ± 0.4	3.04 ± 1.0	0 ± 0.0(	0 ± 0.00	0 ± 0.00	75
2	4.41 ± 0.3	3.38 ± 0.5	0 ± 0.0(	0 ± 0.00	0 ± 0.00	120
3	4.51 ± 0.1	3.57 ± 0.2	0 ± 0.0(	0 ± 0.00	0 ± 0.00	150
4	4.57 ± 0.4	3.59 ± 0.1	0 ± 0.0(	0 ± 0.00	2.00 ± 0.5	210
5	4.62 ± 0.4	3.40± 0.2	0 ± 0.0(	0 ± 0.00	2.30 ± 0.5	240
6	4.69 ± 0.1	3.40 ± 0.4	0 ± 0.0(	0 ± 0.00	2.60 ± 0.9	93
7	4.72 ± 0.4	3.30 ± 0.2	0 ± 0.0(	0 ± 0.00	2.60 ± 0.5	39
8	4.72 ± 0.3	1.95 ± 0.2	0 ± 0.0(	0 ± 0.00	2.70 ± 0.9	23
9	4.77 ± 0.2	1.85 ± 0.3	0 ± 0.0(	0 ± 0.00	2.78 ± 1.0	15
10	4.78 ± 0.1	1.90 ± 0.4	0 ± 0.0(	0 ± 0.00	2.90 ± 0.6	9

Legend: TAPC = total aerobic plate count; EC = *Escherichia coli* count; VC = *Vibrio* count; SSC = *Salmonella-Shigella* count; CC = Coliform count; FC = Fungal count

Table 2: The Mean counts of microorganisms isolated from stored sachet water.

	Log_{10}cfu/mL					MPN/100mL
Storage (weeks)	**TAPC**	EC	VC	**SSC**	FC	CC
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
2	2.48 ± 0.3	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
3	2.70 ± 0.1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
4	2.95 ± 0.4	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
5	4.23 ± 0.4	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
6	4.34 ± 0.5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0
7	4.36 ± 0.4	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	9
8	4.42 ± 0.1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	15
9	4.51 ± 0.5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	21
10	4.53 ± 0.2	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	28

Legend: TAPC = total aerobic plate count; EC = *Escherichia coli* count; VC = *Vibrio* count; SSC = *Salmonella-Shigella* count; CC = Coliform count; FC = Fungal count

Table 3: Microorganisms isolated and their Percentage Occurrence

Bacteria	Tap water (%)	Sachet water (%)	Total (%) occurrence
Escherichia coli	10(37.04)	0(0.00)	13(33.33)
Staphylococcus aureus	5(18.52)	2(66.67)	7(23.33)
Bacillus species	5(18.52)	1(33.33)	6(20.00)
Klebsiella species	2(7.41)	0(0.00)	2(6.67)
Pseudomonas aeruginosa	3(11.11)	0(0.00)	3(10.00)
Enterobacter species	2(7.41)	0(0.00)	2(6.67)
Fungi			
Aspergillus species	1(14.29)	0(0.00)	1(14.29)
Rhizopus species	4(57.14)	0(0.00)	4(57.14)
Saccharomyces species	2(28.57)	0(0.00)	2(28.57)