International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 12 (1), pp. 001-005, January, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of Microbial Communities in Rural Zimbabwean Drinking Water Sources

Zvidzai C.[1]*, Mukutirwa T.[2], Mundembe R.[2] and Sithole-Niang I.[1]

[1]Biochemistry Department, University of Zimbabwe, Box MP167, Mt. Pleasant, Harare, Zimbabwe
[2]Bindura University of Science and Education, P. O. Box, Bindura, Zimbabwe.

Accepted 16 October, 2023

Traditional methods employing selective, differential and non-selective media were used to isolate and identify different species of bacteria from rural drinking water reservoirs of Mount Darwin district of Zimbabwe. The colony counts from non-selective nutrient agar plates gave an indication of the overall level of bacterial activity from each water sample. Open deep wells, shallow wells and rivers were found to be the most heavily contaminated water sources. Borehole water sources had very low total microbial loads and absent in some of the water samples. The prevalent bacteria found were the Gram negative *Escherichia coli, Shigella, Salmonella, Enterobacter aerogenes* and one cocci species that was not further characterized. The presence of faecal pathogenic species in the river water and open wells poses epidemiological cases of diarrhoeal diseases in the district studied.

Key words: Drinking water, microbial analysis, faecal bacteria, bacteriophages, phenotypic

INTRODUCTION

Majority of Zimbabwean rural water sources for drinking are still the traditional ones that are dams, wells, rivers, streams and ponds which might harbour waterborne and vector-borne diseases. Among these diseases there is cholera, guinea worm, schistosomiasis, lymphatic filariasis, onchocerciasis, fungal, shigellosis, salmonellosis, yersiniosis, campylobacteriosis, parasitic and viral infections (Simango et al., 1992; Obi et al., 2002; Fenwick, 2006). However, drinking water can be carefully evaluated for microbial contamination to ensure informative updates on the quality of water and provide authorities with on-time records for national control programs before any microbe could pose any health and economic problems. This will alleviate strained meager financial resources of the developing countries channelled towards such national issues.

Above all, microbial monitoring exercise ensures safe supply of drinking water without compromising on the rural people's health. As a result of scarce protected water sources (e.g. boreholes) that are expensive to maintain in rural areas, majority of people in rural communities resort to such identified open water reservoirs. Hence there is a need for a development of a simple, reliable and sound scientific approach to monitor as well as avoid costly interventions using expensive drugs when microbial waterborne diseases happen to arise.

Several developing and developed countries have embarked on programmes to endeavour to reduce contamination of rural water sources by waterborne diseases (Ganoza et al., 2005; Roe and Cardinale, 2005; Hörman, 2005; Fenwick, 2006). Typical methods normally advised for inactivating microbes for rural water treatment at such levels are disinfection treatment (use of hypochlorite) and boiling (use of heat) that have some efficiency on microbial content reduction (LeChevallier and Au, 2002). However, other proven methods that can be recommended are retention in containers that will allow contamination inactivation through sedimentation and predation (Garcia et al., 2003, 2004; LeChevallier and Au, 2002). Generally, drinking water contamination can arise from chemical (industries and farms) and other sources (sewage material) . Microbial faecal contaminant indicators are *Escherichia coli, Clostridia, Enterococci* that could be of human and non-human origin and *Streptococci* (Binnie et al., 2002; Simpson et al., 2002; Scott et al., 2002). Overally, community drinking water sources and even municipal water systems require accurate and reliable microbial evaluation as well as identification for critical public safety and industrial economics.

This research was carried out as a simple and scientific survey approach to monitor rural drinking water contami-

*Corresponding author: E-mail: cjzvidzai@science.uz.ac.zw

Table 1. Colony count after spread plating 40 µl of neat samples from the sources and incubated at 37°C for 24 h.

Agar medium type	Sample	Colony forming units (cfu/ml)	Number of colony morphology types
Nutrient medium	BH	Mean: 3.00×10^{2}; SD: 2.5×10^{1}	1
	RW	Mean: 1.01×10^{3}, SD: 2.5×10^{2}	2
	DW	Mean: 6.13×10^{3}, SD: 1.2×10^{1}	4
	SSW	Mean: 1.19×10^{4}, SD: 6.3×10^{2}	2
Blood base agar	BH	Mean: 0 SD: 0	0
	RW	Mean: 1.30×10^{4}, SD: 1.7×10^{3}	1
	DW	Mean: 4.54×10^{3}, SD: 4.2×10^{1}	3
	SSW	Mean: 1.39×10^{3}, SD: 2.2×10^{1}	2
Koser citrate	BH	Mean: 0, SD: 0	0
	RW	Mean: 0, SD: 0	0
	DW	Mean: 3.6×10^{1}, SD: 1.2×10^{1}	1
	SSW	Mean: 0, SD: 0	0
Deoxycholate citrate	BH	Mean: 0, SD: 0	0
	RW	Mean: 8.4×10^{1}, SD: 0.4×10^{1}	1
	DW	Mean: 6.78×10^{2}, SD: 0.1×10^{1}	2
	SW	Mean: 0, SD: 0	0
SS medium	BH	Mean: 0 ,SD:0	0
	RW	Mean: 1.32×10^{2}, SD: 3.2×10^{1}	2
	DW	Mean: 1.80×10^{2}, SD: 2.4×10^{1}	2
	SSW	Mean: 5.52×10^{2}, SD: 3.8×10^{1}	1
Brain heart infusion	BH	Mean: 0 ,SD:0	0
	RW	Mean: 0 ,SD:0	0
	DW	Mean: 3.35×10^{1}, SD: 0.3×10^{1}	3
	SSW	Mean: 2.4×10^{1}, SD: 0.5×10^{1}	2

nants. The work also creates a drinking water candidate list that advocates for contamination identity from faecal sources. Traditional methods employing selective, differential and non-selective media were used to isolate and identify different species of bacteria from rural drinking water of Mount Darwin district. Microbiological bioindicator parameters that were evaluated for safe water status included *E. coli*, total coliforms, thermotolerant coliforms, heterotrophic bacteria, and aerobic spore-forming bacteria.

MATERIALS AND METHODS

Water sources

The water samples for bacteriological analysis were collected from selected boreholes designated as BH1, BH2 and BH3, river sources (R1, R2 and R3), swampy area shallow wells (SSW1, SSW2 and SSW3) and deep wells (DW1, DW2, DW3 and DW4) in Mount Darwin district of Zimbabwe. Water sources were cited as central points commonly used by the community. Water samples were collected in 50 ml sterile vials that were fitted with screw caps. Sterilisation of the vials was performed by autoclaving at 121°C for 15 min prior to sampling. Samples were transported to the Microbiology and Fermentation laboratory, University of Zimbabwe, Harare, Zimbabwe and analysed for microbial presence within 8 h on the same day.

Microbiological determinations

Selective, differential and non-selective media were prepared and used to cultivate and confirm the presence of specific bacteria from the water samples. Using sterile tips, 40 µl of undiluted water was pipetted and spread plated onto MacConkey agar as a presumptive medium because it is both selective and differential for members of the enteric pathogenic bacteria, that is, *Enterobacteriaceae* (Roe and Cardinale, 2005). MacConkey medium has bile salts, which inhibit all other microbes except those fastidious ones found in the intestines. Lactose and pH indicator (bromocresol purple) are used to detect lactose-fermenting organisms like *E. coli*. Coliforms ferment lactose to produce acid, which is indicated by a yellow colouration of the broth and gas indicated by the displacement of broth in the small Durham tubes placed in each test tube. Brilliant green bile broth medium was used to confirm the presence of the coliform group because it suppresses the growth of anaerobic lactose fermenters like C*lostridium perfringens,* which may give rise to false positives in MacConkey broth. Eosin methylene blue agar was used for identifying lactose fermenting bacteria (*E. coli, Kleb-siella* and *Enterobacter*) through acidifying the medium such that the colonies would appear black with a greenish sheen. Tryptone water was used for detection of indole production for identification of *E. coli.* Koser citrate medium was used as a differential medium for *E. coli* verification from *Enterobacter aerogenes* based on citrate utilisation according to standard methods for the examination of water and wastewater.

Deoxycholate citrate agar (DCA) was opted as a selective and differential medium for the isolation of *Shigella* and *Salmonella. Salmonella Shigella* agar which is a selective differential medium for the isolation of enteric pathogens was used for that purpose. Five

Table 2. Bacteriological assessment using MacConkey broth and brilliant green bile broth.

Sample	Working quantities	Coliform Presumption colony test (MacConkey)	Coliform Confirmatory colony test (BGBB)	Most Probable number (MPN) (cfu/100mml)
BH	5 X 10 ml	5	5	Mean: 23
	5 X 1.0 ml	1	1	SD: 12
	5 X 0.1 ml	1	1	
RW	5 X 10 ml	5	5	Mean: 171
	5 X 1.0 ml	5	5	SD: 25
	5 X 0.1 ml	5	5	
DW	5 X 10 ml	5	5	Mean: 114
	5 X 1.0 ml	5	5	SD: 32
	5 X 0.1 ml	5	5	
SSW	5 X 10 ml	5	5	Mean: 469
	5 X 1.0 ml	5	5	SD: 102
	5 X 0.1 ml	5	5	

plates of each medium type was inoculated with the 40 µl of undiluted water and spread plated for colonies to developed and then enumerated on a colony counter. Colony morphology and colour developments were noted. The cultures were Gram stained and other routine traditional and classical biochemical tests were performed as confirmatory tests (Gerhardt et al., 1994).

Determination of thermotolerant halophiles

Salt tolerance test was performed with a halophile medium at 7.5 and 15% sodium chloride concentration on agar plate.

Assaying for bacteriophages

To achieve an enriched phage water sample, 45 ml of each water sample was first cultured by adding 5 ml of Deca Strength Phage Broth (DSPB) and incubating the mixture at 37°C overnight in an INNOVA shaker incubator at 200 rpm. Nutrient agar plates were prepared and allowed to set meanwhile the enriched phage water samples were processed by first dispensing 12.5 ml of each culture water sample into Falcon tubes of 15 ml working volume. The tubes were then centrifuged using a bench centrifuge at 3 700 rpm for 10 min and supernatant collected. The centrifugation was repeated at 4°C and the supernatant dispensed into 50 ml Falcon tubes. The resultant supernatant was further filtered using a 0.2 µm cellulose acetate sterilizing membrane filter to separate the bacteriophages from total prokaryotic cells. A volume of 5 ml soft agar was mixed with 1 ml of an overnight prepared *E. coli* culture and 0.4 ml of the phage filtrate. The resultant mixture was poured onto the nutrient agar plates then incubated at 37°C. A control was used and plaques were observed after an overnight incubation (Gerhardt et al., 1994).

RESULTS

Microbial diversity studies

From table 1, it was noted that the water samples had four different types of colonies grew on nutrient agar. The microbial counts obtained were quite high ranging from 3.00 x 10^2 to 6.13 x 10^3 cfu/ml. Distinct colonies could be identified using colony morphology appearance, Gram reaction, catalase reaction and wet-mount microscopy observation. Cocci, bacilli and motile cells were observed on wet-mounts. One rod type of colony isolated on Deoxycholate citrate agar was Gram positive and another was Gram negative. Borehole water samples did not have any colony developments on all the medium agar tests except on nutrient agar. Shigella and Salmonella agar medium had high faecal (human and non-human) coli-forms that developed except from all borehole water sources. Since DCA is a selective and differential medium for the isolation and identification of *Salmonella* and *Shigella*, the colony appearances clearly distinguished between the two. Two colony forms were obtained on DCA medium from the river and deep wells water sources. Notably, one colony type was observed from swampy shallow water sources.

Coliform tests

Spread plating of water samples on MacConkey agar, Brilliant green bile agar and Eosin methylene blue agar broth media showed development of colonies that are of *Enterobacteriaceae* species, however, all borehole water samples had very low such microbial growth developments. Borehole water sample designated BH2 had no coliform growth. The protected water sources had low levels of 23 cfu/ml MPN as compared to unprotected ones that produced high levels of coliforms by several magnitudes ranging from 23 to 469 cfu/ml^{-1} (Table 2). *E. coli* from m-Endo plates were plated on MacConkey / sorbitol medium to confirm the presence of potential pathogenic strains. Results indicated that there is a high probability of pathogenic *E. coli* on blood base agar

Inoculation of the water samples into lactose-fermentation broth cultures showed production of gas in Durham tubes except in BH2 samples further confirming the absence of coliforms. All the lactose-fermentation broths turned yellowish except in the same sample, BH2.

Table 3. *E. coli* confirmatory test for indole growth in tryptone water.

Sample	Kovac's colour change	*E. coli* (present /absent)
BH1	Red	+
BH2	Yellow	-
BH3	Red	+
R1	Red	+
R2	Red	+
R3	Red	+
DW1	Red	+
DW2	Red	+
DW3	Red	+
DW4	Red	+
SSW1	Red	+
SSW2	Red	+

Brilliant green blue broth produced colour changes in all water samples except in BH2 as a result of aerobic coliforms but not of anaerobic lactose fermenters (Table 3).

All colonies were able to grow in the NA media with 7.5 % (w/v) and 15 % (w/v) NaCl concentrations showing that they could sustain the thermotolerant halophilic conditions.

The assaying for the determination of bacteriophages had no plaque developments on it from all the six water samples indicating that the water is not contaminated with lytic viruses. It further indicates that there were no cells infected as none were lysed.

DISCUSSION

The results of the bacteriological analysis of drinking water from Mt Darwin showed that most drinking water sources are contaminated with coliforms and pathogenic bacteria. However, there are no lytic bacteriophages.

The bacterial species identified were members of the *Enterobacteriaceae* family. The microbes were mainly Gram negative, non-spore forming bacilli. Pathogens like *Shigella* and *Salmonella* were identified using the selective and differential medium DCA (Hynes) agar. On DCA agar, *Shigella* were non-lactose fermenting pale coloured colonies while *Salmonella* produce non-lactose fermenting pale coloured colonies with black centres (Cheesbrough, 2000; Ashbolt et al., 2001; Ashbolt, 2004). This description fits very well with the appearance of colonies that were observed. The *Shigella* pathogen was isolated from deep well water and river water sources. Other coliforms were obviously present. It is important to note that coliform bacteria are widely found in nature and do not necessarily indicate faecal pollution (Binnie, 2002; Griffith et al., 2003).

Use of Koser citrate medium helped to differentiate *Enterobacter aerogenes* from *E. coli* that were identified using MacConkey and Brilliant green blue broth as total coliform units in the samples. These two media types support growth of coliforms. *E. aerogenes* is a gram negative rod, which is capable of using citrate as carbon source for metabolic energy in the absence of fermentable glucose or lactose (Hörman, 2005; Cappuccino and Sherman, 1996; Ashbolt, 2004). Koser citrate medium does not have glucose or lactose therefore *E. coli*, which is citrate negative, does not grow on the medium. *E. aerogenes* have citrate permease, which facilitates transport of citrate in the cell hence could grow but there are very low levels of the bacterium that grew (36 cfu/ml). The *E. coli* was therefore shown to be present in all samples but using KCA analysis then deep well water sources were the only ones with *E. aerogenes* . Tryptophan degradation test proved that all samples had *E. coli* except BH2 (Table 3).

Enteric pathogens cannot normally multiply in water hence water is not its mode of transmission to humans (WHO, 1996). However, the infective dose in people whose local or general natural defence mechanisms are impaired would be significantly low. The people likely to be at risk would be the very old or the very young as well as patients undergoing immunosuppressive therapy. Other immuno- compromised individuals suffering from AIDS would also be at risk. Also, water polluted by bacteria when permitted to contaminate food would lead to the multiplication of the pathogens to very large doses.

Since the sample sites were remote from laboratory facilities, the physicochemical changes were inevitable before analysis. Water from deep boreholes is normally free of pathogenic microbiological contaminants. This was observed from the results on plate counts on media inoculated with borehole water where in most cases no bacteria were observed to grow. Ground water in boreholes is generally of good quality due to the relatively slow subsurface movement of water compared to surface water sources like rivers. Shallow and deep wells, which are open, were found to be heavily polluted.

However, highly specific molecular techniques that in-

clude immunological use of kits, fluorescence, use of DNA probes and polymerase chain reactions (PCR) specific for *Shigella flexneri*, *Leptospira* sp., *Vibrio cholerae* and other host of microbes have been developed which are expensive for developing countries (Nascimento et al., 2004; Wegley et al., 2006; Ganoza et al., 2006). These new techniques provide the necessary specificity and sensitivity for biomonitoring the bacteriological quality of water but are expensive. The use of conventional methods is still relevant and valid to isolate and identify bacteria in drinking water particularly in developing countries riling under economic hardships.

REFERENCES

Ashbolt NJ (2004). Risk analysis of drinking water microbial contamination versus disinfection by-products. Toxicology. 198: 255-262.

Ashbolt NJ, Grabow WO, Snozzi M (2001). Indicators of microbial water quality. In Fewtrell L, Bartram J (editors.) Water Quality: Guidelines, Standards and Health.World Health Organization and IWA Publishing, London, UK. pp. 289-316.

Binnie C, Kimber M Smethurst G (2002). Basic Water Treatment. Royal Society of Chemistry, Cambridge, UK.

Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Cambridge University Press, Cambridge, UK.

Cappuccino JG, Sherman N (1996). Microbiology: A Laboratory Manual. The Benjamin Cummings Publishing Company Inc., California, USA.

Fenwick A, (2006). Waterborne Infectious Diseases-Could they be consigned to History? Science. 313: 1077-1081.

Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, Segura ER, Gilman RH, Gotuzzo E, Vinetz JM (2006). Determining Risk for Severe Leptospirosis by Molecular Analysis of Environmental Surface Waters for Pathogenic Leptospira. PLoS Med. 3: 1329-1340.

García J, Vivar J, Aromir M, Mujeriego R (2004). Initial contaminant removal performance factors in horizontal flow reed beds used for treating urban wastewater. Water Res. 38: 1669-1678.

García J, Vivar J, Aromir M, Mujeriego R (2003). Role of hydraulic retention time and granular medium in microbial removal in tertiary treatment reed beds. Water Res. 37: 2645-2653.

Gerhardt P, Murray RGE, Wood WA, Krieg NR (1994). Manual methods of general bacteriology. 1st edition John Wiley and Sons Inc, Washington DC.

Griffith JF, Weisberg BS, McGee DC (2003). Evaluation of microbial source tracking methods using mixed faecal sources in aqueous test samples. J. Water Health. 1:141-151.

Hörman A (2005). Assessment of the microbial safety of drinking water produced from surface water under field conditions. PhD Thesis, Helsinki, Finland.

LeChevallier MW, Au KK (2002). Water treatment for microbial control: A review document. World Health Organization.

Nascimento ALTO, Verjovski-Almeida S, Van Sluys MA, Monteiro-Vitorello CB, Camargo L, EA, Digiampietri LA, Harstkeerl RA, Ho PL, Marques MV, Oliveira MC, Setuba JC, Haake DA, Martins EAL (2004). Genome features of Leptospira interrogans serovar. Braz. J. Med. Bio. Res. 37: 459-478.

Roe D, Cardinale JA (2005). Evaluation of microbial a structures and persistence in the Alfred Wastewater Treatment Plant Reed Bed Sludge Treatment System.

Obi CL, Potgieter N, Bessong PO, Matsaung G (2002). Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. W. South Afr. 28:287-292.

Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J (2002). Microbial source tracking: Current methodology and future directions. Appl. Environ. Microbiol. 68: 5796-5803.

Simango C, Dindiwe J, Rukure G (1992). Bacterial contamination of food and household stored drinking water in a farm working community in Zimbabwe. Centr. Afr. J. Med. 38: 143-148.

Simpson JM, Santo Domingo JW, Reasoner DJ (2002). Microbial source tracking: state of the science. Environ. Sci. Technol. 36: 527-5288.

Wegley L, Mosier-Boss P, Lieberman S, Andrews J, Graff-Baker A, Rohwer F (2006). Rapid estimation of microbial numbers in water using bulk fluorescence. Environ. Microbiol. 10: 1775-1782.

World Health Organization (1996). Guidelines for Drinking Water Quality (Vol. 2): Health Criteria and Other Supporting Information, Geneva, Switzerland.