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Bacteriological Quality Assessment of Harvested Rain Water Stored in Excavated Tanks in Rural Communities of Ogbadibo Area, North Central Nigeria

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Abstract
Bacteriological quality of harvested rain surface water stored in randomly selected 49 cased and 77 uncased excavated tanks in Ogbadibo area was assessed during the dry season of January to March 2011. Multiple-tube fermentation technique and biochemical tests for identification of bacteria were used. Forty five (91.8%) of cased and 75 (97.4%) of the uncased reservoir water were positive for coliform and non-coliform bacteria, giving a total of 120 (95.2%) out of 126 sampled. Contamination of the reservoirs by bacteria was high throughout the communities, ranging from 94.1% in Orokam to 96.9% in Owukpa. The water quality was bad with most probable number (MPN) index ranging from 28 to above 278. The bacterial species identified and the number of affected tanks were *Enterobacter* species in 104 (82.5%), *Klebsiella* species 83 (65.9%), Proteus species 72 (57.1%), *Citrobacter* species 66 (52.4%), *Escherichia coli* 29 (23.6%), *Salmonella* species 15 (11.9%) and *Shigella* species 11 (8.9%). Every reservoir was contaminated with at least 3 species of the identified bacteria. There is urgent need for public health education, reservoir design for cased elevation and encasement, and stored water treatment to prevent outbreak of water borne diseases.
Keywords: Coliform bacteria, tube-fermentation technique, contamination, biochemical tests

1. Introduction

Availability of safe portable water is a serious problem in the rural communities of North Central Nigeria. Majority of the rural dwellers therefore usually drink untreated and polluted water from wells, ponds, streams and harvested rain water (Adesiyun *et al.* 1983). Harvested and stored rain and runoff water in excavated ground tanks during the dry season period of scarcity has almost become the trend in Ogbadibo area of Benue State, North Central Nigeria. This is because wells and boreholes usually yield little quantity of water from the deepest aquifers, and streams dry up during dry season, due to the porous nature of the soil and the basement rocks. These stored waters are reported to have severe health hazards (Enujiugha *et al.* 1994; Gracey 1979). This is because stored waters in tanks are recipients of contamination of all sorts, full of silt, colloidal matter, and pathogens, especially immediately after the rain (Park 2007). Many workers have reported presence of enteric pathogens like *Escherichia coli*, *Salmonella* species, *Klebsiella* species, *Shigella* species and *Enterobacter* species in water drawn from these types of tanks (Morse *et al.* 1978; Gracey 1979; Peters & Odeyemi 1990; Inyang & Aderemi 2003).

Unfortunately, these stored waters are usually used without any sort of treatment for drinking and other domestic purposes during the period of scarcity by the rural folks (Ofukwu *et al.* 2012). The direct use of the untreated water has been reported to cause incalculable number of water borne bacterial diseases in poor rural communities (WHO 1985). The effect of these diseases include loss in man-hour in agriculture, leading to low harvest, socioeconomic problems and some death due to disease outbreaks. Despite these deleterious effects, no documented data exists for the quality of water stored in this form in the community. This study assessed the bacteriological quality of these harvested water stored in excavated tanks in Ogbadibo area of North Central Nigeria.

2. Materials and Methods

Study Area: Ogbadibo area, a composite autonomous rural community in south eastern part of Benue State sharing boundaries with Enugu and Kogi state in North Central Nigeria. It has a population of about 212, 400, 90% of who are rural farmers, living in villages. The area lies between latitude 6°45' and 7°15'N and longitude 7°30' and 8°00'E. The area has average annual temperature of 28°C and altitude of about 300 meters above sea level within the Guinea Savannah Zone with a mean annual rainfall of 1,450 mm. It has a tropical climate with distinct rainy season (March to November) and dry season period of water scarcity (December to February). The area basically sits on cretaceous sedimentary basin and basement complex with lateritic clay on the surface that yields water for wells and boreholes (Agbede & Adegoye 2003).

Collection of Samples: Water samples were randomly collected from 49 cased and 77 non-cased excavated tanks over a period of five months, November 2010 to March 2011, from the three traditional headquarters of the autonomous communities using the multistage method as described by Steel and Cowan (1985).

Analysis of Samples: Cased tanks refer to those with concrete basement and sides while uncased refer to those plastered with clay. One hundred milliliters (100 ml) of water sample from each reservoir were collected into sterile Erlenmeyer flask and taken to the laboratory within 2 to 3 hours. Multiple-tube fermentation technique, as described by Park (2007) with minor modification, was used to determine the grade of quality of water and most probable number (MPN) index. Bacteriological assessment was carried out using standard procedures described by Inyang & Aderemi (2003). Multiple-tube fermentation technique consists of presumptive, confirmed and completed tests. For the presumptive test, 3 sets of 5 tubes in racks, each containing 10 ml of Lauryl sulphate tryptose broth (oxoid) and inverted Durham vials were inoculated with 10 ml, 1 ml and 0.1 ml of the water sample respectively. These were incubated at 35°C for 48 hours. Tubes that were cloudy and produced gas were considered positive presumptive test for coliform bacteria and were subjected to confirmed phase.

For the confirmed test, detection of total coliforms and faecal coliforms was done by taking 1 ml of broth from positive tubes with the smallest inoculums, using sterile Pasteur pipette, into 10 ml of 2% brilliant green lactose bile (Oxoid) in duplicates. These were incubated at 35°C and 44°C respectively for 48 hours and observed for cloudiness and gas production. Tubes that were cloudy with gas production at both temperatures were considered confirmed positives for faecal coliforms while those that showed growth with gas production were non-faecal coliforms. The numbers of confirmed positive tube combinations from the sets of brilliant green lactose bile tubes were used to determine the most probable numbers (MPN) of coliforms/100ml of the water using the MPN index statistical table.

For the completed test, organisms from positive confirmed tubes were isolated in pure culture on both Eosin Methylene Blue (EMB) and MacConkey (MCA) agar (Difco). Loopful from selected positive 2% brilliant green lactose bile broth tubes were streaked on EMB agar plates and incubated at 35°C for 48 hours. One or more typical colonies or colonies considered most likely to consist of coliform bacteria were picked and then tested for growth and gas production in Lauryl sulphate tryptose broth incubated at 35°C for 48 hours; and a negative reaction in the Gram stain. Also from each plate, one or more colonies of non-lactose fermenting bacteria typical of members of *Enterobacteriaceae* were picked for identification. Standard biochemical tests were done to identify the isolated Gram-negative bacilli as described by Barrow and Felthan (1993).

3. Results

One hundred and twenty (95.2%) of the total number of 126 reservoirs sampled were positive for coliform bacteria and non-lactose fermenting members of *Enterobacteriaceae* (non-coliform bacteria) (Table 1). Forty five (91.8%) cased and 75 (97.4%) uncased reservoirs were positive coliform and non-coliform bacteria across the communities. There were no significant differences (P>0.05) between the number of contaminated reservoirs located in the three different traditional headquarters of Orokam (94.1%), Otukpa (95.3%) and Owukpa (96.7%). Table 2 shows the Most Probable Number of coliforms per 100 ml of water (MPN index). The MPN index for the reservoirs ranges from minimum of 28 to maximum of >278.

The number of reservoirs and species of bacteria identified through various biochemical tests are shown in Table 3. Every reservoir was contaminated with more than one bacterium. *Enterobacter*, *Kblebsiella*, *Citrobacter* species and *Escherichia coli* were the coliform bacteria identified. The non-coliform members of *Enterobacteriaceae* identified in this study included *Proteus*, *Salmonella* and Shigella species (Table 3).

Table 1. Distribution of coliform and non-coliform positive excavated reservoirs in Ogbadibo area of North Central, Nigeria.

	Cased reservoir Number		Uncased reservoir Number		Total Number	
Location	**Sample**	**Positive (%)**	**Sample**	**Positive (%)**	**Sample**	**Positive (%)**
Orokam	19	17 (89.5)	32	31 (96.9)	51	48 (94.1)
Otukpa	20	18 (90.0)	23	23 (100.0)	43	41 (95.3)
Owukpa	10	10 (100.0)	22	21 (95.5)	32	31 (96.9)
Total	**49**	**45 (91.8)**	**77**	**75 (97.4)**	**126**	**120 (95.2)**

Table 2. Most Probable Number (MPN) index of 126 sampled excavated reservoir water in Ogbadibo area, North Central Nigeria

No. Coliform Positive Reservoir (%)	MPN index	Rating
18 (14.3)	28 – 46	
7 (5.6)	47 – 130	
53 (42.1)	131 – 172	
37 (29.4)	173 – 278	
5 (4.0)	>278	

*6 of the reservoirs were coliform negative

Table 3. Biochemical characterization of coliform bacteria isolates from excavated tanks in Ogbadibo area, North Central Nigeria

Bacteria	No. positive tanks (%)	Gram reaction	Motility	Lactose	Citrate	Indole	Oxidase	Urease	Voges Proskaver	Methyl red	H_2S production
Coliforms											
Enterobacter sp.	(82.5)	– Rod	+	+	+	–	–	–	+	–	–
Klebsiella sp.	83 (65.9)	– Rod	–	+	+	–	–	+	+	–	–
Citrobacter sp.	66 (52.4)	– Rod	+	–	+	–	–	+	–	+	–
Escherichia coli	29 (23.0)	– Rod	+	+	–	+	–	–	–	+	–
Non-coliforms											
Proteus sp.	72 (57.1)	– Rod	+	–	–/+	+	–	+	–	+	+
Salmonella sp.	15 (11.9)	– Rod	+	–	+	–	–	–	–	+	+
Shigella sp.	11 (8.7)	– Rod	–	–	–	–	–	–	–	+	–

4. Discussion

The rate (95.2%) and degree (all >2.2 cfu/100ml) of contamination of the sampled reservoir water confirmed the reports of Park (2007), Peters & Odeyemi (1990) and Gracey (1979) who stated that surface water held in excavated reservoirs were highly contaminated with enteric bacteria organisms and will be unsafe for human consumption without treatment. *Enterobacter* and *Klebsiella* species were most predominant amongst coliforms and non-coliforms isolated in this study. The frequency of isolation of these coliforms agrees with the findings of Alabi & Adesiyun (1986) who reported high rates of isolation of coliforms from filtered water in households of a university community in Nigeria.

The occurrence of some enteric pathogenic organisms like *E. coli*, *Salmonella* sp and *Shigella* sp may be due to human and animal waste or faeces contamination of the surface water (Adesiyun *et al.* 1983; Ogedengbe 1982) during the rainy season because the isolates in this study grew at 44°C. Some of these pathogens, *E. coli*, *Salmonella* sp and *Shigella* sp could cause serious gastroenteritis, diarrhea, weight loss, fever, and debilitation in humans who drink the water without boiling or treatment (Rao 1988).

Since this reservoir storage system is the main source of accessing drinking water during the dry season, there is the need to boil, filter and treat the water with chlorine before drinking. The edges of the cased tanks should be raised above the ground and properly covered to avoid human and animal waste contamination. Weeds should be periodically removed and tanks properly cleaned before the beginning of rainy season.

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