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BACTERIOLOGICAL PROFILE OF DRINKING WATER SOURCES IN ILORIN METROPOLIS, NIGERIA

Isaac D. Olorunshola[1*], Oladapo O. Oludairo[1], Julius O. Aiyedun[1], John O. Bale[1], Opeoluwa O. Akintola[1], Samuel Omorigie[2], Faith F. Folahan[3] and Jeremiah O. Ogah[4]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, [3]Dept. of Medical Microbiology and Parasitology, College of Health Sciences and [4]Infectious Diseases and Environmental Health Research Group, University of Ilorin, Ilorin, Kwara State, Nigeria and [2]Infectious Diseases and Environmental Health Research Group, University of Benin, Benin City, Edo State, Nigeria.

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ABSTRACT

This study was undertaken to determine the bacteriological quality of three selected sources of drinking water sold in Ilorin metropolis. Representative water samples were collected from 87 sources consisting of 60 boreholes, 20 packaged sachet water and 7 river water samples from seven different locations across three Local Government Areas in Ilorin metropolis. The samples of water were collected in sterile McCartney bottles, placed in ice-packed flasks and immediately transferred to the laboratory for bacteria isolation, determination of bacterial load (total bacterial and coliform counts) and biochemical identification respectively. Results from this study showed that the most common bacteria isolates obtained were Staphylococcus aureus (33 isolates), Proteus species (28 isolates) and Salmonella species (26 isolates). Other bacteria isolates obtained included Klebsiella species, Pasteurella species, Bacillus species and Escherichia coli. Furthermore, results showed that river water samples had the highest bacterial count with an average of 8.56 x 10^2colony forming units (CFU)/100 ml, while the packaged sachet water samples had the least colony count with an average of 2.32 x 10^2CFU/100 ml. The total coliform counts of the borehole and packaged sachet water were within the WHO limit for portable drinking water although the total bacterial count was higher. The distribution patterns of the isolates based on sampling sites revealed that most of these organisms were encountered at Ilorin West. The disparity of the bacteriological quality of the different water sources could be related to the varying level of exposure to contaminants within and across the sampling locations. The results of this study emphasize the need for periodic assessment of water quality for safety purposes.

Keywords: *Water Quality, Bacterial Pathogens, Ground and Surface Water, Ilorin, Nigeria*

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Correspondence: E-mail: idolorunshola@yahoo.com; Tel: +2347061156376

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INTRODUCTION

Access to potable water still remains a major problem in many parts of Nigeria. Currently, over 100 million Nigerians do not have access to basic sanitation facilities, with over 63 million Nigerians not having access to potable water [1].

Water makes up about 75 percent of the human body making it impossible for human beings to live without water. In the domestic environment, water is used for drinking, cooking, bathing and cleaning, however, access to safe drinking water and sanitation is critical in terms of health. According to the United Nations, access to clean potable water is an essential human need. Unsafe drinking water has contributed to numerous infectious diseases such as cholera, hepatitis, typhoid, and diarrhoea [2]. Although water may appear to be clear and pure and has no specific taste or odor, it may contain elements that can have undesirable effects on health.

Nigeria, as a developing nation, has seen unparalleled forms of water pollution from various sources of water available to the populace due to a high rate of urbanization characterized by high population concentration, increasing industrial and agricultural activities coupled with environmental pollution/degradation, and indiscriminate disposal of all kinds of wastes especially in urban areas [3]. In Nigeria, the lack of an adequate supply of potable water is a critical challenge that has been attributed to several factors amongst which are weak government policies and interventions on the infrastructure necessary for ensuring proper hygiene. This gap in policy enactment and implementation has led to incidences of water crisis, scarcity, and contamination and has accounted for the rise in waterborne illnesses, morbidity and mortality rate amongst people in rural and urban centers across the country [4,5,6]. Public or municipal water supply is inaccessible to a large proportion of urban dwellers and where available the supply is highly inadequate, unreliable, and irregular. Consequently, there is a high dependency on untreated water.

The quality of water is of vital importance whether it is for industrial or domestic purposes. The quality of water influences the health status of any individual hence, analysis of water for the bacteriological property is very important from the public health perspective. Water constitutes a large percentage of body mass although it is not a source of nutrients. However, untreated water can be a source of serious environmental and health problems [2]. However, the objectives in providing portable water include freedom from harmful micro-organisms and freedom from undesirable or harmful chemicals. Therefore, both the physicochemical and bacteriological assessment of portable water is of paramount importance and monitoring must be given the highest priority. Cases of water-associated diseases and deaths have been reported globally especially in developing countries [7]. Periodic assessment of water quality is highly imperative and also recommended to ensure that man consumes safe water that will guarantee his safe health conditions. It is a fact that both surface and subsurface sources of water are available for man's varied uses. The fact that these sources are open to pollutants which often distort the integrity of such water has made it important that their quality is assessed over time.

Water in sachets and in other forms is readily available and affordable but there are concerns about their purity. The integrity of the hygienic environment and the conditions where the majority of the water in sachets are produced has also been questioned [8]. There are claims of past outbreaks of water-borne illnesses that ensued from consumption of polluted water in sachets [9]. An understanding of their microbiological quality and safety are therefore imperative and should be a cause of concern to consumers, water suppliers, regulators and public health authorities [10].

Occurrence of diseases and deaths associated with consumption of water of poor quality have been reported [11,12]. Furthermore, varying levels of bacterial counts have been reported across Nigeria and these include high bacteria counts in river water [13], high bacteria count in ground water [14] and high coliform count [15,16] where species of *Enterobacter, Proteus, Escherichia, Salmonella* and *Shigella* were mostly isolated. Fausat and Kolawole, [17] reported a 14% presence of *Klebsiella sp., Escherichia coli, Staphylococcus aureus,* and *Streptococcus* species in a quality assessment study of bacteria content on a segment of Asa River

in Ilorin. Furthermore, in a bacteriological assessment of selected hand-pumped borehole water sources in Malete environs, Kwara State, Nigeria, *Klebsiella species, Staphylococcus aureus, Escherichia coli,* and *Streptococcus* species were identified [18]. Oyedeji *et al.* [19] confirmed the concentrations of *coliforms and E. coli* in sachet water [19]. The objective of this study was therefore to evaluate the bacteriological quality of water from three selected sources (boreholes, sachet and river) of drinking water in the Ilorin metropolis.

MATERIALS AND METHODS

The study area

Ilorin metropolis is the capital of Kwara State in North-central Nigeria. It consists of three Local Government Areas (LGAs) namely Ilorin East, Ilorin South and Ilorin West covering approximately 50.2km^{2}of Guinea savannah land (Fig. 1). According to Federal Republic of Nigeria Official Gazzette, 2007, Ilorin metropolis has about 2,371,089 inhabitants. The metropolis lies between Longitude 8°05′ and 10°15′ N; and Latitude 2°73′ and 6°13′ E. The major sources of employment are agriculture and cottage industries, which engage almost 80% of the workforce.

Water Samples

Three different types of water sources of drinking water in Ilorin were sampled. These included borehole water, packaged sachet water, and river water. Borehole water described in this study are underground water sources brought to the surface with the aid of a submersible pumping machine and stored in storage tanks where they are accessed for drinking and other domestic uses. Packaged sachet water is a popular source of drinking water in Nigeria that is processed, sealed in polythene bags of 500 ml and often receive Government regulatory approval for drinking before they are sold. On the other hand, river water sources used in this study refer to water from free-flowing streams and rivers often used as a major source of drinking water in rural Nigeria.

The purposive sampling method was used to collect water samples. A grand total of 87water samples in triplicates were collected from the different drinking water sources across the three LGAs of Ilorin metropolis. Triplicates of water samples were collected from 60 different boreholes, 20 packaged sachet water samples from different manufacturers, and water samples from7 different rivers. The water samples were collected in sterile bottles of 50 ml each placed in ice-packed flasks and immediately transferred to the laboratory for analysis. Table 1 shows the type and number of water samples collected in each LGA.

Table 1. Distribution of water samples in Ilorin metropolis Local Government Areas

Source of Water	Ilorin South	Ilorin East	Ilorin West	Total
Borehole	20	15	25	60
Packaged Sachet Water	6	8	6	20
River	3	1	3	7
Total	29	24	34	87

Media preparation

The Nutrient agar and Eosin Methylene blue were prepared according to manufacturer's instructions and sterilized using the autoclave at 121^{o}C for 15 minutes.

Total Bacteria Count

The total bacteria in the water samples were estimated and each water sample was serially diluted in the ratio of 1:100 and the aliquot was inoculated into nutrient agar. This was properly mixed and poured into a sterile petri dish [20]. Streaking method was used to obtained pure bacterial isolates by sub-culturing a previously incubated plate onto a freshly prepared sterile plate.

Isolation of Enterobacteriaceae
For the isolation of Enterobacteriaceae, the membrane filtration method was used [13]. In this method, phenol red indicator was combined with lauryl sulphate broth (LSB) after it was purified. Relative to the LSB containing the impure dye or its major contaminant, the purified phenol red provided clear visualization of discrete yellow colonies observed against a white background. The colonies remained stable for at least 24 h at 25ºC under standard laboratory lighting conditions [13].

Non-selective and selective enrichment
For the non-selective media, the water samples (50 ml) were filtered through a sterile membrane filter with pores of 0.45 µm. After the water samples were passed through the filter, the filter was transferred aseptically into 90 ml of buffered peptone water and gently mixed then incubated overnight at 37°C. For the selective media, 1 ml of the pre-enrichment agar was transferred with a pipette into 10 ml Rappaport-Vassiliadis Soy Peptone (RVS) broth was incubated at 37°C.Serial dilution of 10^{-6} was done using normal saline and a loopful of culture was streaked on selective agar Salmonella-shigella agar (SSA) and incubated at 37°C overnight. Colonies on the *Salmonella-shigella* agar were then counted and subjected to biochemical test.

Isolation of Coliforms
For the isolation of coliforms, the multiple tube fermentation method is also referred to as the most probable number (MPN). In this technique, presumptive, confirmed, and completed tests were carried out to enumerate for coliforms. The most probable number (MPN) of total coliform counts was calculated using the Hoskins.

Biochemical Test
Colonies from various media plates were further subjected to biochemical/physiologic tests namely; Gram staining, motility, catalase, oxidase, methyl-red, Voges-Proskauer, coagulase, indole, citrate utilization; gelatin hydrolysis, acid from carbohydrates (KlieglerIron Agar), and sugar utilization as described by APHA [21].

Statistical analysis
All data generated was analyzed statistically by calculating the mean of triplicate value. Data were presented in percentage and also represented in tables and figures respectively.

RESULTS
Results from this study show that the most common bacterial isolate obtained was *S. aureus* with 33 isolates, *Proteus sp* with 28 isolates *Salmonella sp* with 26 isolates. Other bacterial isolates obtained include *Klebsiella* sp., *Pasteurella*sp, *Bacillus*sp, *E. coli* (Figure 2). Table 2 shows that most of the bacterial isolates were obtained from water samples collected from the Ilorin West metropolis. Furthermore, while the borehole had more bacterial isolates than other water groups, *E. coli* was mostly isolated from the river water samples (Table 3). Furthermore, results from Table 4 showed that river water samples had the highest bacterial count with an average bacterial count of 8.56 x 10^2CFU/100 ml, with the borehole water samples having the least colony count with an average of 2.32 x 10^2CFU/100 ml.

DISCUSSION
The results of this study indicated contamination of the water samples with heterotrophic bacteria found naturally in water, soil, or vegetation which showed a problem with the overall quality of drinking water in Ilorin, Nigeria. Furthermore, the presence of bacterial contamination in the water samples is suggestive of problems with the processing of water which could be due to ineffective treatment, or no treatment at all, as some producers just bag and seal well or pipe-borne water without any form of treatment. Also, intrusion of contaminated water into the potable water supply or re-growth problems could also be contributory [22]. Improper storage conditions on un-cemented floors with direct contact with soil could lead to contamination [23].

Table 2: Frequency distribution of bacteria isolates from the three LGA of Ilorin metropolis

	No. (%) of positive samples			
	Total (N=87)	**Ilorin West (n=34)**	**Ilorin East (n=24)**	**Ilorin South (n=29)**
Staphylococcus aureus	33 (37.9**)	26 (83.9*)	3 (14.3*)	4 (11.4*)
Proteus sp	28	19 (67.9 %)	4 (14.3%)	5 (17.9%)
Salmonella sp	26	17 (65.4%)	6 (23.1%)	3 (11.5%)
Pseudomonas sp	12	7 (58.3%)	2 (16.7%)	3 (25.0%)
Klebsiella sp	10	4 (40.0%)	0 (0.0%)	6 (60.0%)
Pasteurella sp	8	5 (62.5%)	2 (25.0%)	1 (12.5%)
Escherichia coli	7	6 (85.7%)	1 (14.3%)	0 (0.0%)
Shigella sp	7	5 (71.4 %)	0 (0.0%)	2 (28.6%)
Enterobacter sp	5	3 (60.0%)	1 (20.0%)	1 (20.0%)
Bacillus sp	4	1 (25.0%)	3 (75.0%)	0 (0.0%)
Moraxella sp	3	3 (100.0%)	0 (0.0%)	0 (0.0%)
Streptococcus sp	3	1 (33.3%)	2 (66.7 %)	0 (0.0%)
*Morganella*sp	3	2 (66.7%)	1 (33.3%)	0 (0.0%)
Acinetobacter sp	2	2 (100.0%)	0 (0.0%)	0 (0.0%)
Corynebacterium sp	2	1 (50.0%)	1 (50.0%)	0 (0.0%)
*Plesiomonas*sp	1	1 (100%)	0 (0.0%)	0 (0.0%)
Listeria sp	1	1 (100.0%)	0 (0.0%)	0 (0.0%)

N=total number of samples analyzed; n=number of samples from each of the three LGA
**=% of total number of samples analysed; *=% of number of samples from each location

A major highlight from this study is the presence of coliforms such as *E. coli* in some of the water samples (Table 2) which is indicative of faecal contamination of water. *Escherichia coli* is a member of the Total coliform group that is found in the faeces of warm-blooded animals and humans and not in the environment [24]. Faeces can also be a source of pathogenic viruses, protozoa and helminths [24,25]. The presence of these coliforms in these water samples, shows that the water purification process of the packaged sachet water was poor.

In spite of this, *E.coli* was absent in some of the water samples. Although *E.coli* serves as a useful indicator of contaminated water, it has its limitations. Its absence may however be insufficient to justify the purity of the analyzed packaged water from contamination with pathogenic viruses, protozoa and helminthes [10] which are now increasingly transmitted through drinking water. *E. coli* are naturally present in the environment but are generally not harmful to individuals with intact immune systems but may cause disease in people with impaired local or general immune defense mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy or those with acquired immunodeficiency syndrome [26]. If water used by such persons for drinking or bathing contains sufficient numbers of these organisms, they can produce various infections of the skin and the mucous membranes of the eye, ear, nose and throat [26].

Another important finding from this study is the presence of *Klebsiella* and *Enterobacter* species in the different water samples. While these organisms are natural inhabitants of many water environments, and may

multiply to high numbers in waters rich in nutrients, such as pulp mill wastes, textile finishing plants and sugarcane processing operations, they have been documented to colonize washers in taps and can grow in water distribution systems [27]. They are also excreted in the faeces of many healthy humans and animals, and are readily detected in sewage-polluted waters. Their presence in drinking water could have serious health implications. Some of the isolates found are important human pathogens associated with a variety of infectious diseases, such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infection etc [28,29]. Major *et al*. [30] reported the presence of similar organisms from borehole water samples in Ilorin, Nigeria.

Table 3. Frequency of bacteria isolates from different water sources in Ilorin metropolis

	No (%) of water source positive for identified bacteria			
Bacteria Isolate	**Borehole (n=60)**	**Sachets (n=20)**	**River (n=7)**	**Total**
Staphylococcus aureus	23	37	33	
Proteus sp.	19	3	6	28
Salmonella sp.	8	0	18	26
Pseudomonas sp.	5	2	5	12
Klebsiella sp	3	2	5	10
Pasteurella sp.	5	1	2	8
Escherichia coli	2	0	5	7
Shigella sp.	2	0	5	7
Enterobacter sp.	2	1	2	5
Bacillus sp.	1	0	3	4
Streptococcus sp.	2	0	1	3
Moraxella sp.	1	0	2	3
*Morganella*sp.	1	0	2	3
Acinetobacter sp.	1	0	1	2
*Corynebacteria*sp.	2	0	0	2
Plesiomonas sp.	1	0	0	1
Listeria sp.	1	0	0	1

n= Number of Samples

Table 4. Bacterialogical analysis of the water samples

Water Source (Sample)	Total Bacterial Count (100/ml)	Total Coliform Counts (mpn/ml)
Ilorin South (29)		
Borehole (20)	118	5
Packaged sachet water (6)	256	0
River (3)	820	11
Ilorin East (24)		
Borehole (15)	738	7
Packaged sachet water (8)	293	0
River (1)	800	9
Ilorin West (34)		
Borehole (25)	214	3
Packaged sachet water (6)	120	0
River (3)	950	13
WHO Standard	100	10

Results from the study also showed the presence of *Pseudomonas* species in all the water samples. *Pseudomonas* sp. are common environmental organism that can be found in faeces, soil, water and sewage. They can multiply in water environments and also on the surface of suitable organic materials in contact with water. The presence of high numbers of *P. aeruginosa* in potable water, notably in packaged water, can be associated with complaints about taste, odour and turbidity.

Proteus species especially *Proteus mirabilis* are widely distributed as free-living organisms in soil and water in the natural environment. In humans, *Proteus* is found as part of the normal flora of the gut. Its main pathological role is in infections of the urinary tract, but it can also cause wound infections and septicaemia. [31].

Coliform bacteria are commonly associated with water quality. The World Health Organization/U.S Environmental Protection Agency (EPA) standard for acceptable drinking water is a total *Coliform* count of zero [32,33,34]. Accordingly, *Aeromonas, Salmonella* and *Shigella* species are potential bacterial pathogens in packaged drinking water [35,36].

Other studies conducted on the microbiological quality of water from different sources in Nigeria have shown various levels of contamination [17, 19]. Enforcing compliance by the regulator body NAFDAC to ensure good quality assurance and the maintenance of internationally defined drinking water standards has also remained a challenge as many are not registered and even those that are registered do not always meet the standards required [37].

The quality of drinking water is getting severely affected because of the wide spread pollution of surface water. Besides, discharge of untreated waste water through bores and from unscientific disposal of solid waste also contaminates water sources, thereby reducing the quality of fresh water resources. The occurrences of bacteria in water is however common, treatable, and in most cases preventable. The quality of drinking water is a powerful environmental determinant of health. Water is vital to sustaining life and it needs to be treated that way. A satisfactory (adequate, safe and accessible) supply must be available to all [38]. Diseases related to contamination of drinking water constitute a major burden on human health, interventions to improve the quality of drinking water provides significant health benefits.

CONCLUSIONS

In the past, there was a city-wide distribution of pipe borne water across the Ilorin metropolis. This overall helped to improve the quality of drinking water in Ilorin. However, the breakdown of this basic amenity has led to the reliance on other sources of drinking water as espoused in this study. Future studies will focus on evaluating the chemical residues in drinking water sources in Ilorin metropolis.

RECOMMENDATIONS

Microbiological assessment of drinking water quality should be done periodically with the regulatory body, the National agency for food and drug administration and control (NAFDAC) ensuring good quality assurance and maintenance of internationally defined drinking water standards. Efforts are needed to increase public awareness of the hazards of drinking contaminated water and of ways to prevent such contamination. There is also need to ensure that government authorities enforce water hygiene rules in water factories in order to ensure public health.

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