JORMA International Journal of Health and Social Sciences
ISSN: 2978-0942 (Online)
Volume 3 No. 4 Issue 4 Pages 17-26

JORMA IJHSS
DOI: https://doi.org/10.64074/vn5b4a90

Bacteriological Examination of Water from Different Sources in a Tertiary Institution in Nigeria

Kenneth Oshiokhayamhe Iyevhobu[1,2,3] || Sunday Onyemaechi Okparaku[1] || Andrew Oseghale Eigbedion[4,5] || Kennedy Oberhiri Obohwemu[6] || Edmund Dongyeru[7] || Benedicta Agumeile Ken-Iyevhobu[3,8] || Rosemary Ebokhasomi Innih[2,8] || Promise Nnamudi[1,3] || Kawthar Osilama Momodu[1,3] || Success Olalekan Owolabi[1] || Christian Izokpu[1] || Funke Abolade Adumashi[6,9]

[1]*Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.*

[2]*Department of Medical Laboratory Science, Faculty of Applied Health Science, Edo State University, Iyamho, Edo State, Nigeria.*

[3]*Saint Kenny Diagnostic and Research Centre, Ujoelen, Ekpoma, Edo State, Nigeria.*

[4]*Department of Paediatrics, Faculty of Clinical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.*

[5]*Department of Paediatrics, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.*

[6]*PENKUP Research Institute, Birmingham, United Kingdom*

[7]*Northwest Community Laboratories (NWCL), United States of America.*

[8]*Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria.*

[9]*Faculty of Health, Wellbeing & Social Care, Pearson, GBS Partnership, Manchester, United Kingdom.*

Corresponding Author:
Kenneth Oshiokhayamhe Iyevhobu
ORCID: https://orcid.org/0000-0001-6577-0637
kennylamai@yahoo.com

Abstract

Water is the only naturally occurring inorganic liquid with two atoms of hydrogen and one atom of oxygen. Water is the most common indispensable, essential and useful liquid on earth. The overall aim of this study is to conduct the bacteriological examination of water from various sources at Ambrose Alli University (AAU), Ekpoma, Edo State. A total of four (4) different sources of water (Ambrose Alli University Dam, Ambrose Alli University hostel water tanks, Ambrose Alli University sachet water and Ambrose Alli University bottle water) at Ambrose Alli University, Ekpoma, Edo State were investigated. Result showed that Ambrose Alli University Dam water has the highest total bacteria count of 8.0 x 104cfu/ml. Ambrose Alli University hostel tank water has a total bacteria count of 6.4 x 104cfu/ml, Ambrose Alli University bottle water has a total bacteria count of 5.6 x 104cfu/ml while Ambrose Alli University sachet water has the least total bacteria count of 3.2 x 104cfu/ml. The most probable number of coliforms per 100ml sample ranged from 24 to 92 with Ambrose Alli University Dam having the highest value. Ciprofloxacin, Augmentin, Pefloxacin, Tarivid and Sparfloxacin showed 100% susceptibility to all isolates. Amoxicillin was intermediate to all the bacteria isolates. Staphylococcus aureus showed resistance to four antibiotics. The presence of these pathogens in water calls for public health attention. Tap water, bore-hole water, and publicly sold sachet and bottled water should be adequately treated before use and NAFDAC should ensure and enforce strict compliance with the standards as regards the production and sales of packaged sachet water. To achieve this goal the manufacturers, the consumers and government should work together to achieve this common goal for the betterment of all.

Keywords: Bacteriology || Water || Tertiary || Institution || Antibiotics.

INTRODUCTION

Water is the only naturally occurring inorganic liquid with two atoms of hydrogen and one atom of oxygen. Water is the most common indispensable, essential and useful liquid on earth. No living thing can survive for a long time without water; this is because the body requires water to conduct its metabolic activities and it is also required by plants both for growth and metabolic activities (Venkatesha, Rao & Kedare, 2020). It is an absolute essential for the continuance of life and a universal solvent, right from the onset of human civilization. Man has continued to settle close to water source, along rivers, beside lakes and near natural springs, this is because man requires water to meet his domestic needs like drinking, watering his crop, watering his livestock etc. (Joshi, Kothuri & Yadav, 2024).

Industrially, man uses water for power generation, for washing and dumping site for its industrial waste. Man has a will to continue to use water as a means of transportation, using ships, canoes, etcetera. Water as an element is very important to human in particular, its importance can be understood by the fact that it constitutes about 65% of the body weight of a normal adult and is also a medium in which biological and chemical processes take place in all organisms including man (Lin, Yang & Xu, 2022). Due to this fact, sanitary surveillance should not be overlooked, this is because water contaminated with microorganisms, especially pathogenic forms, always cause great threat to humans when consumed. This is particularly well established for diseases such as diarrhoea, cholera, infective hepatitis, amoebic and bacillary dysentery. It has been estimated that as many as 80% of all diseases in the world are associated with unsafe water (WHO, 2022). Depending on the climatic and workload, the human body needs about 1-30litre of water per day for normal functioning of the body, part of this water is derived from food (Islam et al., 2021).

Water is obtained through the precipitation of rain as it falls on the earth, drains through the soil, to enter the streams and rivers through these to the lakes, ocean, and seas from where it evaporates and returns to the atmosphere. It eventually falls as precipitate completing a cycle called hydrologic cycle (Wen et al., 2020). The provision of adequate portable water of high bacteriological quality has been a necessity due to various waterborne diseases associated with unsafe water. Majority of the populace in Ekpoma and environs depends on packaged or sachet water as the most reliable source of portable water (Troeger et al., 2020).

Coliform organisms, if present in packaged or sachet water, there is also the probability of pathogenic organisms like *Escherichia coli, Salmonella spp, Shigella spp, Vibrio cholerae, Enterococcus faecalis* and *Clostridium perfringens* to be present, hence not safe for consumption (Ochei & Kolhatkar, 2000). Thus, there is a need to continuously assess the bacteriological quality of packaged or sachet water to ensure proper treatment process for this water in this region. This study is therefore conducted to assess the bacteriological quality of water from various sources in Ambrose Alli University, Ekpoma, Edo State by using Multiple Tube Method and to add to the existing knowledge base within the study area.

MATERIALS AND METHODS

Study Area

This study was conducted in Ekpoma. The administrative headquarters of Esan West Local Government Area of Edo State which lies between latitude 6.45`N to 6.75`N of the Equator and longitude 6.08`E to 6.13`E of the Greenwich Meridian with altitude of about 332m above sea level. It is made up of quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukpenu, Idua, Ukhur, Egoro, Emehi, Igor and Idumebo (Aziegbe, 2006). Ekpoma has a population of 89,628 in 1991 and 127,718 in 2006 (NPCN, 2012), majority of which are civil servants, traders, businesspeople, transporters, farmers, teachers/lecturers, and students by occupation. The Ambrose Alli University, Ekpoma is situated in this town (Iyevhobu et al., 2024). The main sources of water in the locality are rainfall and wells. It has two (2) distinct seasons, wet and dry seasons. The wet season occurs between April and October with peak in August, average rainfall ranging from 150cm to 250cm. The dry season occurs between November and

March with cold harmattan between December and January, average temperature of about 25^0C (Edo State of Nigeria, 1992).

Materials Used

Glassware such as bottles of different sizes, universal bottles, beakers, bijou bottles, Durham tubes, test tubes, pipette, Syringes, conical flask were used. Double strength MacConkey broth medium, single strength MacConkey broth medium were made and MacConkey agar plate.

Media Preparation and Sterilization of Materials

The media, Nutrient agar and MacConkey agar were prepared in accordance with the manufacturer specification and after which the MacConkey broth was prepared. The entire work bench was disinfected with 0.5 hypochlorite solution, and all the glassware was washed with detergent and sterilized along with the MacConkeys broth on the bottles in the hot air oven at 160^0C for 1 hour.

Sample Collection

This study was conducted between July and August 2018. A total of four (4) different sources of water (Ambrose Alli University Dam, Ambrose Alli University hostel water tanks, Ambrose Alli University sachet water and Ambrose Alli University bottle water) in Ambrose Alli University, Ekpoma, Edo State. After collection, it was analysed for microbial contents.

Sample Analysis

Enumeration of aerobic and anaerobic mesophillic bacterial counts, coliforms bacteria were conducted.

Presumptive Test: Double strength MacConkey's broth medium was used to which bromocreso purple indicator is added. For each sample different sizes of bottle were obtained which contain inverted Durham's tubes. Then 50ml of the double strength MacConkey broth was added to a 200ml capacity bottle and equal volume of the water sample (i.e., 50ml water sample) was added to the bottle. 10ml of double strength MacConkey broth was added to five different universal bottles and equal volume of water sample (i.e., 10ml of water sample) were also added to all the universal bottles. The sample and the broth were properly mix and the Durham's tube placed upside down was filled with the samples, the whole bottles were incubated at 37ºC for 48hrs. The tubes in which there is a characteristic colour change and gas production was regarded as positive. The colour change indicates acid production while the gas is indicated by bubbles at the top of the Durham's tube. The tubes which had no gas production will be regarded as negative (Ochei & Kolhatkar, 2000; Bell et al., 2021).

Multiple Tube Technique: The method is used for the detection of coliforms and involves the presumptive test.

Principle: Measured volumes of neat water are added to a series of tubes containing a liquid indicator growth medium. A characteristic colour change in any tube indicates the presence of indicator bacteria in the sample. The most probable number (MPN) of indicator organisms in the sample depends on the number and distribution of positive and negative reactions.

Procedure: For each sample, different sizes of bottle were obtained which contain inverted Durhams tube. 50ml of the double strength MacConkey broth was dispense into 200ml capacity bottle and equal volume of the water sample was also added to the bottle. 10ml of double strength MacConkey broth was dispensed to five (5) different universal bottles and equal volume of water sample was also added to all the bottles. The whole bottles were incubated at 37^0C overnight/24hours using water bath. Gas production and colour change in the bottles were known as positive. The bottles which have no gas production were known as negative. The most probable number (MPN) of organisms in the sample was read using Maccradys table.

Enumeration of Total heterotrophic Bacteria: Samples of the swimming pool water samples were serially diluted in ten folds. Total viable aerobic heterotrophic plate counts were determined using pour plate technique. Then the molten nutrient agar, Sabouraud dextrose agar, Salmonella-Shigella agar, eosin methylene blue agar and MacConkey agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria, Salmonella-Shigella, Vibrio cholerae, *Escherichia coli* and coliform respectively. They were swirled to mix, and colony counts were taken after incubating the plates at 30ºC for 48hrs and preserved by subculturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

Characterization and Identification of Bacterial: Bacterial isolates were characterized and identified after studying Gram reaction as well as cell micro morphology. The other tests carried out 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 carried out according to the methods of Chesbrough (2006). Microbial identification was performed using the keys provided by Ochei and Kolhatkar (2000).

Antibiotics Susceptibility Test

In vitro susceptibility tests of the bacterial isolates to antibiotics were done using disc diffusion technique. 0.1 ml of each bacterial isolate prepared directly from an overnight broth culture and adjusted to 0.5 McFarland Standard (Iyevhobu, 2021; Iyevhobu, 2022) was inoculated using sterile pipette onto each of the nutrient agar media. The commercially available discs containing the following antibiotics; Penicillin (Pen, 10ug), Ceftazidime, (Caz, 30ug), Streptomycin (Stp, 30ug) Ciprofloxacin (Cpf, 5ug), Gentamycin (Gen, 10ug), Ofloxacin (Ofl, 5ug) Ceftriaxone (Cef, 30ug) and Cotrimoxazole (Cot, 30ug) of oxoid products were aseptically placed on the surfaces of the sensitivity agar plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37ºC for 24 hours and the zones of inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimetres (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data.

RESULTS

The results obtained from this study showed that water from Ambrose Alli University (AAU) Dam water, Ambrose Alli University (AAU) hostel tank, Ambrose Alli University (AAU) bottled water and Ambrose Alli University (AAU) sachet water. The most probable number (MPN) of coliform organism, total bacterial count on nutrient agar and MacConkey present in the water sample was used to determine the bacteriological quality of the water sample. The organisms isolated in the study are *Escherichia coli, Klebsiella* spp, *Staphylococcus aureus* and *Proteus* spp.

Table 1 shows the presumptive coliform test result: Number of Tubes Inoculated, Number of Tubes Positive and MPN per 100ml of Sampled Water. Ambrose Alli University (AAU) Dam water was the most contaminated water with a most probable number (MPN) of 92, Ambrose Alli University (AAU) hostel tank has a most probable number (MPN) of 54, Ambrose Alli University (AAU) bottled water has a most probable number (MPN) of 43and Ambrose Alli University (AAU) sachet water was the least contaminated water with a most probable number (MPN) of 24. Three (3) samples each were examined in each of the locations. All the samples were examined were contaminated except one of the samples from Ambrose Alli University (AAU) sachet water (table 2).

Bacteria isolated and total bacteria count (cfu/ml) isolated in the study are shown in table 3. In Ambrose Alli University (AAU) Dam water *Escherichia coli* and *Staphylococcus aureus* was isolated with a total bacteria count of 8.0 x 10^4/ml, in Ambrose Alli University (AAU) hostel tank, *Klebsiella* spp was isolated with a total bacteria count of 6.4 x 10^4/ml, *Proteus* spp was isolated in both Ambrose Alli University (AAU) bottle water and Ambrose Alli University (AAU) sachet water with a total bacteria count of 5.6 x 10^4/ml and 3.2 x 10^4/ml respectively.

Table 4 shows the total microbial counts of the water samples from the water samples from different sources of water in Ambrose Alli University (AAU) Ekpoma. Ambrose Alli University (AAU) Dam water has a total bacteria count of 80 cfu/ml x 10^2 which has the highest total bacteria count, Ambrose Alli University (AAU) hostel tank has a total bacteria count of 64 cfu/ml x 10^2, Ambrose Alli University (AAU) bottle water has a total bacteria count of 56 cfu/ml x $10^2$and Ambrose Alli University (AAU) sachet water has a total bacteria count of 56 cfu/ml x 10^2 which has the least total bacteria count in the study.

Antibiotics susceptibility pattern of isolates from the study is shown in table 5. Ciprofloxacin, Augmentin, Pefloxacin, Tarivid and Sparfloxacin showed 100% susceptibility to all isolates. Amoxicillin was intermediate to all the bacteria isolates. Only Streptomycin showed resistivity to streptomycin. Table 6 shows the cultural characteristics and biochemical identification of the isolates from various sources of Water from Ambrose Alli University (AAU) Ekpoma.

Table 1: Presumptive Coliform Test Result: Number of Tubes Inoculated, Number of Tubes Positive and MPN of coliforms per 100ml of Sampled Water

Samples	Number of Tubes Sampled			Number of Positive Tubes			MPN coliform/100ml sample
	50ml	10ml	1ml	50ml	10ml	1ml	
AAU Dam	1	5	5	1	5	5	92
AAU Hostel Tank	1	5	5	1	4	5	54
AAU Bottle Water	1	5	5	1	5	2	43
AAU Sachet Water	1	5	5	1	5	0	24

Table 2: Samples Examined and Percentage Contamination

Samples	No. Examined	No. Contaminated	Percentage (%) Contaminated
AAU Dam	3	3	100
AAU Hostel Tank	3	3	100
AAU Bottle Water	3	3	100
AAU Sachet Water	3	2	66.7
Total	**12**	**11**	**91.7**

Table 3: Bacteria Isolated and Total Bacteria Count (cfu/ml) in Nutrient agar

Sample	Bacteria Isolated	Total Bacteria Count (cfu/ml)
AAU Dam	*Escherichia coli* *Staphylococcus aureus*	8.0 x 10^4/ml
AAU Hostel Tank	*Klebsiella* spp	6.4 x 10^4/ml
AAU Bottle Water	*Proteus* spp	5.6 x 10^4/ml
AAU Sachet Water	*Proteus* spp	3.2 x 10^4/ml

Table 4: Antibiotics Susceptibility Pattern of Isolates

Antibiotics	Organisms				Organisms
Gram Negative Disc	***Escherichia coli***	***Klebsiella* spp**	***Proteus* spp**	**Gram Positive Disc**	***Staph aureus***
Ciprofloxacin	S	S	S	Ciprofloxacin	S
Amoxicillin	I	I	I	Norfloxacin	R
Augmentin	S	S	S	Gentamycin	S
Gentamycin	I	I	S	Amoxil	R
Pefloxacin	S	S	S	Streptomycin	S
Tarivid	S	S	S	Refampicin	S
Streptomycin	I	I	R	Erythromycin	R
Septrin	I	S	I	Chloramphanicol	S
Chloramphenicol	S	S	I	Ampiclox	S
Sparfloxacin	S	S	S	Levofloxacin	R

Key: S – Sensitive (> 20mm); I – Intermediate (5mm – 19mm); R – Resistant (0mm - 4 mm)

Table 5: Cultural Characteristics and Biochemical Identification of the Isolates from different sources of Water from Ambrose Alli University (AAU) Ekpoma.

Isolates	Cultural characteristics				Biochemical analysis						Probable Organism
	Shape	**Elevation**	**Consistency**	**Colour**	**Gram**	**Catalase**	**Indole**	**Motility**	**Oxidase**	**Urease**	
1	Rod	Convex	Buterious	Rose Pink	_	+	+	+	_	_	*Escherichia coli*
2	Rod	Raised	Mucoid	Light Pink	_	+	+	_	_	+	*Klebsiella* spp
3	Rod	Flat and Swamy	Buterious	Light Pink	_	+	_	+	_	+	*Proteus* spp
4	Cocci	Spherical	Moist	Golden yellow	+	+	_	_	_	+	*Staphylococcus aureus*

Key: + = Positive; - = Negative

DISCUSSION

The aim of this work was to ascertain the prevalence of bacteria present in different water in Ambrose Alli University (AAU) Dam water, AAU hostel tank, AAU bottle water and AAU sachet water. It has been observed that most of the water sampled in AAU is not free from microbial content containing some level of coliforms. This study showed that all water sampled were contaminated by some microorganisms possibly coliforms. This finding agreed with that of Olanipekun *et al.* (2024), Agbasi *et al.* (2024) in which these organisms are not in conformity with NAFDAC standard except for AAU sachet water which was close to NAFDAC standard of microbial content NAFDAC (2002). Although physical examination of the water sample analysed did not show any particulate object or discolouration of any type yet the presence of any pathogenic bacteria in them calls for a serious concern. International standards for drinking water state that portable water should not contain 100cells of heterophilic bacteria per 100ml of water (WHO, 2022). According to Agbai *et al.* (2024), bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these organisms may pose a potential health risk to consumers.

The multiple tube test study showed the presence of coliform bacteria which is an indicator bacterium that is used to evaluate the quality of drinking water and the absence of faecal coliform (AAU Dam) which indicates the contact of water with sewage or inadequate treatment or post treatment contamination. World Health Organisation (WHO) standards for treated water says that no sample of 100ml should contain more than three coliform organisms and *Escherichia coli* should not be detected in any sample of 100ml (WHO, 2022). Tenebe *et al.* (2023) in their study on bacteriology of sachet water sold in Lagos reported that organisms contained in the wastewater were inevitably the source of contaminants on the sachet surface. The water vendors and their patrons contributed to the overall contamination of hawked sachet water in Lagos.

None of the water samples studied in this work met up with this standard which could be attributed to the effective water treatment and proper location of water source away from sewage tanks. To protect public health and ensure that water is safe for public use, any water intended for drinking treated or untreated, piped or unpiped must meet certain microbiological standards. A violation of set standards warrants treatment of the present source or the need for an alternative water supply.

The total bacteria count range of 3.2×10^1 to 8.0×10^1 recorded in this work (Table 3) is above the value recommended by the WHO. This might be unconnected to the improper sterilization, poor handling of the products during production, transportation, and sales of the products (Iyevhobu *et al.*, 2023; Iyevhobu *et al.* 2024). This supports the earlier views of Agbasi *et al.* (2024) that the sachet water, Dam water, bottled water and hostel water are of questionable quality. The implication therefore was that the bottle and sachet water investigated carried NAFDAC (Registration) approval numbers and the products are popularly and freely served at open parties and social functions. Also, the possible contamination of sachet water at the point of production has been confirmed by Dam (1996) and Yongyod, Phusomya & Chopjitt (2022) and in which they reported that pure water vending machine may not be so pure, after all, because investigations found bacteria like Escherichia coli in the machine. However, it was gratifying to note that the sachet and bottled water analysed in this study were not from bacterial contamination which possibly showed that the manufacturers (AAU) are defaulting from the guidelines set up by NAFDAC and SON.

The results obtained so far highlight the fact that communities in urban areas suffer from acute portable water shortages. Also, Ambrose Alli University hostels are exposed to disease conditions posed by the organisms isolated from the hostel tanks. To AAU bottle and sachet water producers, there is no need to rush to get into business and as a result quality control has been compromised. Therefore, packaged water other than those in company sealed bottles could pose as a source of waterborne infection as this study has shown that the bottled water is obviously of better quality than the popular sachet water. Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet standard for portable water. There is, therefore, a need to monitor all those involved in water business to comply with the guidelines to avert outbreaks of water-borne diseases because of consumption of contaminated water.

CONCLUSION

Tap water, bore-hole water, and publicly sold sachet and bottled water should be adequately treated before use and NAFDAC should ensure and enforce strict compliance with the standards as regards the production and sales of packaged sachet water. Packaged water consumers should be aware of a danger of consumption of poorly packaged water, especially the sachet water and the potential health risk associated with such. Also, NAFDAC should apart from educating the consuming public on the dangers of patronizing sachet pure water that does have NAFDAC approved numbers; producers should also be educated on how to maintain Good Manufacturing Practice (GMP) and companies that fail to maintain the standard should be properly sanctioned either by stipulating adequate fines to be paid or out rite withdrawal of their production Licenses.

Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet portable water standard. There is, therefore, a need to monitor all those involved in water manufacturing business to comply with the guidelines. The national regulatory bodies and Ministries of Health, Water Resources as well as those of Trades and Industries should exercise more stringent surveillance programmes and educate the producers and the consumers alike on the need to look for water quality, proper labelling, and certification. To achieve this goal the manufacturers, the consumers and government should work together to achieve this common goal for the betterment of all.

ETHICAL APPROVAL AND INFORMED CONSENT

Ethical clearance for the study was obtained from the Health Research and Ethics Committee of Ambrose Alli University, Ekpoma, Edo State.

AVAILABILITY OF DATA AND MATERIALS

The authors declare consent for all available data present in this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

FUNDING

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTIONS

The entire study procedure was conducted with the involvement of all writers.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the management, laboratory, and technical staff of Saint Kenny Diagnostic and Research Centre, Ujoelen, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing and support in accordance with Good Publication Practice (GPP3) guidelines.

REFERENCES

Agbasi, J. C., Ezugwu, A. L., Omeka, M. E., Ucheana, I. A., Aralu, C. C., Abugu, H. O., & Egbueri, J. C. (2024). More about making profits or providing safe drinking water? A state-of-the-art review on sachet water contamination in Nigeria. *Journal of Environmental Science and Health, Part C*, *42*(4), 255-297. DOI: https://doi.org/10.1080/26896583.2024.2319009

Aziegbe, F. I. (2006). Sediment Sources, Redistribution and Management in Ekpoma, Nigeria. *Journal of Human Ecology, 20*(4):259-268.

Bell, R. L., Kase, J. A., Harrison, L. M., Balan, K. V., Babu, U., Chen, Y., ... & Brown, E. W. (2021). The persistence of bacterial pathogens in surface water and its impact on global food safety. *Pathogens*, *10*(11), 1391. DOI: https://doi.org/10.3390/pathogens10111391

Chesbrough, M. (2006). Water-related disease and testing of Water. In District Laboratory practice in Tropical countries part 2. Cambridge University Press, London. Pp. 143-155.

Dan, R. (1996). Pure water vending machines may not be so pure, In Medical Microbiology. Volume11, Churchill Livingstone, Edinburge, London. Pp 273-285

Edo state of Nigeria (1992). General profile. A one-year Edo pictorial special attraction. In a Guide. Pp 12-15.

Islam, M. M., Iqbal, M. S., D'Souza, N., & Islam, M. A. (2021). A review on present and future microbial surface water quality worldwide. *Environmental Nanotechnology, Monitoring & Management*, *16*, 100523. https://doi.org/10.1016/j.enmm.2021.100523

Iyevhobu, K. O, Adewuyi, G. M., Ken-Iyevhobu, B. A, Obohwemu, K. O, Asibor, E., Aliemhe, C. A., Adeji, A. J., Ozurumba-Dwight, L. N., Oseni, D. I., & Momodu, K.O. (2024). Evaluation of the Bacteria Found on Students' Washed and Unwashed Hands at a South-South Nigerian Tertiary University. *Journal of Microbes and Research, 3*(2), 1-6. DOI: https://doi.org/10.58489/2836-2187/026

Iyevhobu, K. O., Ken-Iyevhobu, B. A., Omolumen, L. E., Aliemhe, C. A., Ozurumba-Dwight, L. N., & Oseni, D.I. (2023). Bacteria Load Assessment from Hands of Students in a Tertiary University in South-South Nigeria. *Journal of Biotechnology Research, 9*(1), 44-49. DOI: https://doi.org/10.32861/jbr.91.44.49

Iyevhobu, K. O., A. O. Alleh, A. I. Airefetalor, E. K. Osagiede, R. E. Ikede, I. O. Babatope, E. O. Edo and L. N. Ozurumba-Dwight, L. N. (2022). Evaluation of carrier of methicillin resistance/sensitive Staphylococcus aureus producing penicillinase from health workers. *Journal of Pharmacology and Toxicology*, *17*: 43-49. DOI: https://doi.org/10.3923/jpt.2022.43.49

Iyevhobu, K. O., Obodo, B. N., Usoro, E. R., Airefetalor A. I., Turay, A. A., and Ken-Iyevhobu, B. A. (2021). Isolation, Identification and Antibiotics Susceptibility Pattern of Bacteria Present in the Palms of Fresh Fish Sellers. *Addaiyan Journal of Medical Research Journal of Biotechnology Research, 1*(1), 14 - 20.

Joshi, S. S., Kothuri, M., & Yadav, M. M. (2024). Enhancing Water Quality of Panchaganga River in Kolhapur, Maharashtra: A Study. *Advances in Environmental Sustainability, Energy and Earth Science*, 261.

Lin, L., Yang, H., & Xu, X. (2022). Effects of water pollution on human health and disease heterogeneity: a review. *Frontiers in Environmental Science*, *10*, 880246. DOI: https://doi.org/10.3389/fenvs.2022.880246

NAFDAC., (2002). Guidelines for Registration and Production of Package water in Nigeria.website:www.NAFDAC.org.Retrieved 2017-07-25.

National Population Commission of Nigeria (NPCN) (2012). Esan West (Local Government of Nigeria) Population. National Population Commission of Nigeria. http||www.citypopulation.de. Retrieved in July 2024.

Ochei, J. and Kolhatkar, A. (2000). Bacteriological Examination of Water, Milk, Food and Air. In: Medical Laboratory Science Theory and Practice. Tata McGraw-Hill Publishing Company Limited New Delhi. Pp. 818-830.

Olanipekun, A., Idusuyi, D., Aiyelokun, O., Saka, Q., & Cole, O. (2024). Determination of Shelf Life of Packaged Water in Selected Water Packaging Factories, Ota, Ogun State, Nigeria. *European Journal of Applied Science, Engineering and Technology*, *2*(5), 88-100. DOI: https://orcid.org/0009-0003-1833-1166

Tenebe, I. T., Babatunde, E. O., Eddy-Ugorji, C. C., Etu, E. E. E., Ogarekpe, N. M., Ekeanyanwu, C. V., ... & Ezeudu, O. B. (2023). Bacterial contamination levels and brand perception of sachet water: a case study in some Nigerian urban neighbourhoods. *Water*, *15*(9), 1762. DOI: https://doi.org/10.3390/w15091762

Troeger, C. E., Khalil, I. A., Blacker, B. F., Biehl, M. H., Albertson, S. B., Zimsen, S. R., ... & Seyedmousavi, S. (2020). Quantifying risks and interventions that have affected the burden of diarrhoea among children younger than 5 years: an analysis of the Global Burden of Disease Study 2017. *The Lancet Infectious Diseases*, *20*(1), 37-59.

Venkatesha, R., Rao, A. B., & Kedare, S. B. (2020). Appropriate household point-of-use water purifier selection template considering a rural case study in western India. *Applied Water Science*, *10*(5), 1-15.

Wen, X., Chen, F., Lin, Y., Zhu, H., Yuan, F., Kuang, D., ... & Yuan, Z. (2020). Microbial indicators and their use for monitoring drinking water quality—A review. *Sustainability*, *12*(6), 2249. DOI: https://doi.org/10.3390/su12062249

World Health Organization. (2022). *Guidelines for drinking-water quality: incorporating the first and second addenda*. World Health Organization.

Yongyod, R., Phusomya, P., & Chopjitt, P. (2023). Microbiological Quality and Sanitation of Food Stalls and Drinking Water Vending Machines: 10.32526/ennrj/21/20230014. *Environment and Natural Resources Journal*, *21*(4), 312-321. https://doi.org/10.32526/ennrj/21/20230014