

COMPARATIVE ANALYSIS OF MICROBIAL LOAD OF WATER IN SELECTED HOSTELS IN IFITE, AWKA

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ABSTRACT

Water in its natural form contains number of microbes and particulate pollutants. Due to industrialization and other anthropogenic activities; the purity of water is critically threatened. Because of increasing relevance of these factors, the necessity of water analysis is felt desirable. An investigative study was carried out to determine the bacteriological and physical qualities of water samples. Comparative analysis of five samples of water supply were carried out with a view to determine the level of contamination of bacteria and fungi. The study involved five (5) water samples obtained from tap and well water situated at hostels in Ifite Awka, to ascertain the physicochemical property, presence and population of different bacterial and fungal groups influencing the quality of these water sources. The pour plate method was used to examine the water samples. Six genera of bacteria which include *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp*, *Shigella spp*, *Pseudomonas aerogienes* and *Enterobacter spp* were isolated from the water samples. A genera of fungi was also isolated which is *Candida albicans*. Total bacteria count, total coliform count and total fungi count were also analysed on the water samples. Biochemical tests were also carried out which includes; catalase test, citrate utilization, motility, urease and gram-staining were carried out. It was concluded that not all tap and well water are safe for consumption and there is need to sensitize the public on the importance of maintaining clean and hygienic environmental conditions around the water to prevent contamination.

Keywords: Microbial load, Water, Hostel, Awka, Contamination

1. INTRODUCTION

Following the theory of creation, it is clear that water is as old as man. From time immemorial, man has resorted to the use of this unique commodity for domestic and other purposes. Water covers approximately 71% of the Earth's surface, with continents and islands accounting for the remaining 29% (Agu *et al.*, 2014; Agu *et al.*, 2017; Agu *et al.*, 2021; Agu *et al.*, 2023). Most of our water supplies are from surface water which include: rivers, streams, lakes, oceans and seas and there, water bodies are likely to be polluted with domestic and industrial wastes as well as agricultural waste. Although there are no official definitions for the general term river as related to geographic features, in some countries or communities, a river is defined by its size (Agu *et al.*, 2017). As population increases, the problem become more serious and as such, water can endanger the health and life of human beings. Because when polluted by fecal materials, it becomes potential carrier of pathogenic organism (Carpenter, 2018). Water, is of course, absolutely essential to life, not only human life but all life; animals and vegetables. Most of the biochemical reaction that occur in metabolism and growth of living cells involve water, and all take place in water (Camp *et al.*, 2017). Man uses water not only for drinking purposes but also for bathing, washing, laundering, heating, air conditioning, agriculture, stock raising and gardens, industrial processes and cooling water power and steam power, fire protection, fishing, swimming and wild life propagation and navigation. Natural water contain not only the natural flora but also microorganisms from soil and possibly from animals or sewage. Surface waters in streams or pools and stored waters in lakes and large ponds vary considerably in microbial content (Frazier, 2019).

Water is broadly divided into three types and they include: Surface water which include: streams, rivers, lake, seas, and oceans (Kelman *et al.*, 2020). The ground water which include: well, bore hole. Many people have defined the ground water in different ways; Ground water is non-saturated water that occurs where all pores in the soil or rock counting materials are saturated (Pelezer *et al.*, 2015). The atmospheric water, which involves, the rainfall. All water bodies consist of a variety of bacterial and other microorganisms like the Algae, fungi, which inhabit these natural water bodies. Some of these microorganisms are indigenous to natural water while others are transient, entering the water from external environment (Pelezar and Reg, 2012). Leachate from open garbage dump sites frequently enters

groundwater sources and pollute it (Agu *et al.*, 2014). The generality of bacteria are mostly commonly found ordinarily in fresh water some of which include: *Pseudomonas*, *Archaeobacter* and *Vibrio*. These are gram negative. The gram-positive bacterial which are found in water include: *Micrococcus archaeobacter* and *Actinomycetes* (Gebharal, 2015). Tap water, as one of the water sources is mostly used domestically. It is observed that tap water changes, sometimes the water will be clear; this calls for load, in order to be sure of its portability (Bonde, 2011). The increase in drinking water from different sources especially in Anambra State has made it necessary to investigate the microbial content of water. Water is a potential carrier of pathogenic organisms that can endanger human life. Most of drinking water sources are often contaminated with different pollutants like faeces, animal and plant wastes, making such water unfit for drinking if not treated. The pollution of water with pathogenic organisms and other pollutants can only be detected by carrying out microbiological assessment of such water. Most human disease such as typhoid, Paratyphoid Cholera, Amobiasis, Trichinosis, Gastroenteritis, Salmonella, Shigellosis, Diphtheria, Giardia, Dracunculus etc. are known to be water borne disease (Ewington *et al.*, 2018). Water born disease are those disease which have water as their vehicle of transmission. These disease are capable of destroying a whole community if not checked. Therefore, the quickest ways to prevent outbreak of these disease and to determine the portability of such water sources is to determine the microbial load or content if the microbial content is nor within acceptable limit, such water sources should be condemned immediately.

2. MATERIALS AND METHODS

The medium and the reagents used in this work and the methods of their preparation are presented in this appendix.

Study Area- The study was conducted using a water samples from different hostels and lodges in Ifite Awka, Anambra State.

Sample Collection- Water samples were collected from five different sources comprising of tap water and well water. Sterile universal sampling bottles were used to collect the samples. The water sources were differentiated using alphabets to label the bottles. This helped in not mistaking one for another. The water in the bottles were then sterilized. The samples were examined within two hours of collection.

3. PREPARATION OF CULTURE MEDIUM

Five different agars were prepared. Each for its purpose.

- **Nutrient Agar:** 3.6g was measured out and diluted with 100ml of water. This agar is used in Total Bacterial Count or Total Heterotrophic Bacteria Count.
- **Salmonella-Shigella Agar:** 3.6g was measured out and diluted in 100ml of water. This agar is used in the identification of the organisms, *Shigella* and *Salmonella*.
- **Cetrimide Agar Base:** 3.6g was measured out and 99ml of water and 1ml of glycerol was used in its dilution. This is used in identification of *Pseudomonas spp.*
- **Eosin Methylene Blue Agar:** 3.6g was measured out and diluted 100ml of water. It is used in Total Coliform Count and Fecal Coliform Count.
- **Sabouraud Dextrose Agar:** 3.6g was measured out and diluted in 100ml of water. It is used in identification of fungi.

4. SAMPLE ANALYSIS

One millilitre (1ml) of the water samples were pipetted out aseptically and introduced into 9ml of sterile distilled water for bacteria and fungi, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using distilled water as the diluents. 0.1ml of appropriate dilutions (10^{-1}) of the sample were pour plated in sterile plates of Nutrient agar (NA), Eosine Methylene Blue Agar (EMB), Sabouraud Dextrose Agar (SDA), Cetrimide Agar, and *Salmonella-Shigella* agar plates for the culture of bacteria and fungi. The culture plates were incubated at 37°C aerobically for 24-48hours for bacteria and 48-72hrs for fungi. Developing colonies on Nutrient agar and SDA were counted to obtain total viable. Discrete colonies for the bacteria and fungi were obtained by sub-culturing into Nutrient agar and SDA plates and were subsequently identified using standard methods.

Total Bacterial/fungal Count and Total Coliform count was calculated thus:

$$TBC = \frac{(N)}{VD}$$

Where TBC: Total Bacterial Count

V: Volume Plated

D: Dilution Factor

N: Number of Colonies

5. CHARACTERIZATION AND IDENTIFICATION OF BACTERIA

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 2016). The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manual of Determinative Bacteriology, 9th edition (2013).

GRAM REACTION- Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope ($\times 100$). Purple color indicated Gram-positive organisms while red or pink color indicated Gram-negative organisms.

CATALASE TEST- Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of the 24 hours pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

6. MOTILITY TEST HANGING DROP METHOD

A loopful of 18-24 hours broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interpret "drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile.

OXIDASE TEST

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does not mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport. Whatmann No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observe the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*).

Positive was indicated by development of dark purple color (indophenols) within 10 seconds.

Negative: Absence of colour.

UREASE TEST USING CHRISTENSEN'S UREA AGAR

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive *Proteeae* from other *Enterobacteriaceae*. Heavy inoculum from an 18- to 24-hours pure culture was used to streak the entire Christensen's Urea Agar slant surface. Adequate care was taken not to stab the butt as it will serve as a color control. The tubes were incubated loosened caps at $35^\circ C$. The slants were observed for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production would be indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive *Proteeae* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative.

INDOLE TEST

A loopful of an 18-24 hours culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation was done at $35-37^\circ C$ first for 24 hours and further for up to 48 hours. Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

METHYL RED TEST

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR–VP) broth. The production of a bright red colour indicates a positive test while yellow color indicates a negative test after vigorous shaking.

VOGES-PRAUSKER TEST

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR–VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5% α -naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

CITRATE TEST

A 24 hours old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

SUGAR FERMENTATION TEST

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

FUNGAL IDENTIFICATION

Isolation and Characterization of the Fungi: This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope (Agu and Chidozie, 2021); with reference to the Manual of Fungal Atlases (Barnett and Hunter, 2000; Ellis *et al.*, 2007).

7. RESULTS

Five samples were collected from different lodges and hostels in Ifite, Awka. Seven isolates were isolated from the samples. They include six bacteria isolates and one fungal isolate. The fungal isolate which is *Candida albicans*. The bacteria isolates include: *Klebsiella pneumoniae*, *Shigella flexneri*, *Enterobacter aerogenes*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Escherichia coli*. *Candida albicans* is dominant in most samples and also the predominant bacteria isolate is the *Klebsiella pneumoniae*. The tables below show the count and biochemical tests of the isolates.

Table 1: Shows the result of physical analysis carried out on the water samples.

Sa	Samples	Source	O	Odour	T	Taste	Co	Colour
Sa	Sample A	W	Chinagolum Lodge	-		Moldy		Colourless
S	Sample B	T	Girls Hostel	-		-		Colourless
Sa	Sample C	W	Boys Hostel	-		-		Cloudy
Sa	Sample D		Address Lodge		Moldy		Stale	Ash-like
Sa	Sample E	Str	Joy Lodge	-		-		Colourless

KEY:

Negative = -

Table 2: Total Bacterial, Coliform and Fungal Count

Samples	Total Bacteria Count (Cfuml ⁻¹)		Total Coliform Count (Cfuml ⁻¹)		Total Fungi Count (Cfuml ⁻¹)	
	No. of Bacterial Colonies on Plate	Total Bacterial Count (cfuml ⁻¹)	No. of Coliform Colonies on Plate	Total Coliform Count (cfuml ⁻¹)	No. of Fungi Colonies on Plate	Total Fungi Count (cfuml ⁻¹)
Chinagolum Lodge	35	3.5x10 ³	80	8.0x10 ³	38	3.8x10 ³
Girls Hostel	TFTC	TFTC	TFTC	TFTC	147	1.47x10 ⁴
Boys Hostel	TFTC	TFTC	NG	NG	TFTC	TFTC
Address Lodge	210	2.1x10 ⁴	TNTC	TNTC	TNTC	TNTC
Joy Lodge	40	4.0x10 ³	TFTC	TFTC	NG	NG

KEY:

TFTC - Too few to count.

TNTC - Too numerous to count.

NG - No growth.

Table 3: Bacterial Isolates Found in the Samples

Samples	Bacterial Isolates
Chinagolum Lodge	B
	C
	D
	E
	G
	H
Girls Hostel	B
	D
	G
Boys Hostel	B
Address Lodge	B
	D
	F
	G
	H
Joy Lodge	C

KEY:

A - *Penicillium crysogenum*

B - *Candida albicans*

C - *Pseudomonas aeruginosa*

D - *Klebsiella pneumoniae*

E - *Shigella flexneri*

F - *Enterobacter aerogenes*

G - *Salmonella enterica*

H - *Escherichia coli*

Table 4: Fungal Morphology and Microscopy

S/N	COLONIAL MORPHOLOGY	MICROSCOPY	PROBABLE ISOLATE
A	On SDA, it forms colonies that are usually blue-green in color, odorless and have a velvety texture. The colonies can range in size from small to large and can be circular or irregular in shape. It produces conidiophores, which are structures that bear the conidia. It is also a fast-growing fungus that can typically form visible colonies within 5-7 days of incubation on SDA.	Has septate hyphae and also produces conidiophores, which are specialized structures that bear the asexual spores called conidia which are oval in shape and range in size from 2-4 microns in diameter. The conidiophores are usually long and branched, with the tips bearing clusters of conidia	<i>Penicillium crysogenum</i>
B	On SDA, colonies are typically smooth, soft, creamy or white in color, and may have a slightly convex or umbonate shape. Some species may also produce pigmented colonies, such as <i>Candida krusei</i> , which produces purple colonies. <i>Candida</i> species grow rapidly on SDA, with colonies appearing within 24 to 48 hours of incubation at 25°C.	They are oval to round shaped yeasts that typically measure 3-6 micrometers in diameter. They are gram positive organisms and have a characteristic "budding" growth pattern. In some species, such as <i>Candida albicans</i> , yeast cells may form chains or clusters known as pseudohyphae, which are elongated structures that result from incomplete cell separation during budding.	<i>Candida spp</i>

Table 5: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
C	Irregular	Glistening	Cream	irregular	Flat	Opaque	- Rod	+	+	-	-	-	+	-	-	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>
D	Irregular	Glistening	Cream	Entire	Raised	Opaque	-Rod	+	-	-	+	-	+	+	+	+	(-)	+	-	+	<i>Klebsiella pneumoniae</i>
E	Circular	Smooth	Greyish/colourless	Entire	Convex	Translucent	-Rod	+	-	Var	+	-	-	-	+	-	+	var	-	-	<i>Shigella flexneri</i>
F	Circular	Shiny	White	Entire	Convex	Moist	-Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	<i>Enterobacter aerogenes</i>
G	Circular	Smooth	Greyish/white	Lobate	Low convex	Translucent	-Rod	+	+	-	+	-	+	-	+	-	+	+	-	-	<i>Salmonella enterica</i>
H	Circular	Smooth	Whitish	Entire	Convex	Translucent	-Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	<i>Escherichia coli</i>

Keys:

Gram: Gram reaction

Cat: Catalase test

Mot: Motility test

Ind: Indole test

MR: Methyl-red test

VP: Voges-Proskauer test

Cit: Citrate Utilization test

Sugar Fermentation Tests:

Lac: Lactose Fermentation

Glu: Glucose Fermentation

Suc: Sucrose Fermentation

Fru: Fructose Fermentation

Mal: Maltose Fermentation

Oxi Oxidase

Ure: Urease

8. DISCUSSION

The result showed that all the Water samples were contaminated with microorganisms that have public health implications. The total bacterial count recorded a highest count of 2.1×10^4 cfu/ml, in sample D and lowest in sample B and C. Growth was recorded in all the samples. The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water

environments and pathogenic pathogens such as *Salmonella* spp, *Shigella* spp and pathogenic *E. coli* may be present (Grabow, 2016). These pathogens and opportunistic microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis to consumers. The probable bacterial isolates isolated includes; *Escherichia coli*, *Salmonella* & *Shigella*, *Klebsiella*, *Pseudomonas* and *Enterobacter*. The study shows the morphology characterization of bacterial isolates, the morphological features were grouped based on their color, shape, margin and elevation on the plates as shown in Table 4.2. Gram stain was done as a presumptive form of identification of bacterial isolates. This provided view of the microscopic morphology of the bacteria from all samples as gram negative rods. Biochemical tests were carried out to aid in identification of the bacteria. Bergrey's manual was used to trace the probable organisms. The carbohydrate fermentation patterns of the isolates and their characteristics were also studied as shown in Table 4.5. Many of the fungi most frequently isolated from treated drinking water, includes *Penicillium* spp., *Aspergillus* spp., *Candida albicans* and *Cladosporium* spp were reported by (Langfelder *et al.*, 2013) are in line with this research. The total fungal count ranges from the highest in Sample D which is the Address lodge to the least with no growth which is Sample E (Joy Lodge) as shown in Table 4.2. The fungi isolated was identified morphologically and microscopically in Table 4.4. The morphological features were grouped based on their color, shape, margin and elevation on the plates as shown in the Table 4.4.

Some physical tests were performed for testing of its physical appearance such as colour, odour and taste which is shown in Table 4.1.

9. CONCLUSION

This study was centered on ascertaining the microbiological load and physicochemical quality of different samples of water. The study has been able to determine the population of microorganisms (Total heterotrophic bacteria, Total Coliform counts and Total Fungal counts) of water at the institution hostel and lodges with prevalence of some enteric bacteria such as *E. coli*, *Shigella*, *Salmonella* and others. The microbial populations especially for coliform bacteria encountered for some of the samples analyzed were above the limits for drinking water and portable water set by the world health organization. These organisms are opportunistic organisms that can be harmful to individuals with underlying health conditions and immune compromised individuals. This calls for improved sanitary conditions of the water sources in these locations. In addition, community participation through protection of water sources from contamination could help improve the water situation in the area thereby ensuring a health environment.

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