***Original Research Article***

# Bacteriological Quality Assessment and Antibiogram Profile of Bacteria Associated with Sachet Drinking Water Sold at Zaria, Northern Nigeria


## ABSTRACT

**Aims:** Bacteriological quality assessment and antibiogram profile of bacteria associated with sachet drinking water was carried out with view to determine the bacterial load and fitness of the water sold in the study area for human consumption.

**Materials and methods:** Ten samples of different brands were aseptically collected and analyzed using heterotrophic count and most probable number technique. The isolated bacteria were microscopically and biochemically characterized and finally confirmed using Box™ Easygel® *E. coli* Quantitube™ Test Kit (Micrology Laboratories manufactures, Easygel®, USA) biotyping. Physicochemical analysis of the samples was done using standard methods. The isolated bacteria were screened for antimicrobial susceptibility using Kirby-Bauer disc diffusion technique.

**Results:** The total heterotrophic bacterial count ranged from $1.0\times10^2$ to $3.0\times10^2$ cfu/ml, with MPN/100ml values ranged from <0.03 to 1.2. The temperature ranged from 8 to 18℃, at the pH range of 5.0 to 7.6. The findings showed that, high temperatures and pH was associated with high bacterial counts. There was no statistical relationship between microbial loads and the temperature (p= 0.454) of the analyzed sachet water. Also, there was no statistical relationship between microbial loads and pH (p= 0.446) of the sachet water. The bacteria isolated were *Escherichia coli, Enterobacter aerogenes, Salmonella sp, Citrobacter freundii* and *Proteus vulgaris*. Most 9 (90%) of the water analyzed fell within the statutory limits. While, the remaining 1 (10%) fell within the contamination level, which recorded high bacterial counts beyond the standard of safe drinking water set by water and food regulatory bodies.

**Conclusion:** The isolation of *E. coli* and *Salmonella* sp. in sample brand "A" in this study is an indication that if not promptly checked, an outbreak could occur in the near future. All the isolates are susceptible to variety of antibiotics used, except *Enterobacter aerogenes, Salmonella* sp. and *Citrobacter freundii* which recorded resistance to amoxicillin, and *Proteus vulgaris* which recorded multidrug resistance.



*Keywords: antibiogram, coliforms, contamination, Escherichia coli, sachet water, potability*


## 1. INTRODUCTION

Water is a precious resources and vital to life. Access to safe and affordable supply of drinking water is universally recognized as a basic need for the present generation and pre-condition of the development and care for the next generation. It is also considered as foodstuff consumed in the greater quantity around the world. Therefore it comes as no surprise that the health risks associated with consumption of contaminated water are of greater interest [1]. Water is essential to life and in fact, the basis of life. Water is the

lifeblood of all living organisms. It is increasingly appreciated as a vital resource, which needs attention and care to remain available not only for the present but also for future generation. Water has been one of the commonest and most treacherous vehicles for transmitting diseases [2]. It also serves as habitat for a wide variety of microorganisms such as protozoa, bacteria, algae, fungi, virus and helminths [3]. Contaminated water creates many problems to man and animals, as far as portability of water is concerned because it causes infectious diseases like typhoid fever, salmonellosis, cholera, bacillary and amoebic dysentery [2]. Water intended for human consumption must be free from organisms and chemical substances that may be hazardous to health. The absence of turbidity, colour, or any disagreeable taste or smell is of utmost importance in public drinking water suppliers. The presence of bacteria and pathogenic (disease-causing) organisms is a concern when considering the safety of drinking water. Pathogenic organisms can cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, and other illnesses [4]. Water is a universal solvent which is essential for the lives of plants, animals and humans. Water is a very unusual substance with many strong unique properties that are so important to live on the planet. Based on the properties of water, it is colourless; tasteless; odourless; which dissolves nearly in everything. It exists in three forms liquid, solid and gas and is cycled through the water cycle; it can absorb a large amount of heat; it slicks together into beads or drops; it flows and erode the surface of the earth; it occurs in sediment form which is found in river banks and bars; and it is part of every living organism on the planet [2].

Potable water is the water that is free from any disease causing microorganisms and chemical substances that are deleterious to health. Water contaminated with agricultural, domestic or industrial waste is known as non-potable or polluted water. Water has always been a medium for transmission of human microbial disease. Infectious disease caused by pathogenic bacteria, protozoa and viruses are the most common and wide spread health risk associated with drinking water [2, 4]. Infectious diseases are transmitted primarily through humans and animal excreta, particularly faeces. If there are active cases or career in the community, the faecal contamination of water resources will result in the causative organisms being present in the water. Those pathogens that are present in the drinking water include; *Salmonella* sp, *Shigella* sp, *Vibrio cholerae*, pathogenic *Escherichia coli*, *Yersinia enterolitica* and *Campylobacter jejuni*. Others are viruses such as Norwalk virus, rotavirus, and hepatitis A virus. Parasites like *Giardia lamblia, Entamoeba histolytica* and *Dracunculus medinensis* occur in contaminated drinking water. Other diseases transmitted through drinking contaminated water include bacillary dysentery and schistosomiasis [2].

More than 1000 people in the world are without safe water supply and proper sanitation. World Health Organization (WHO) in 1993 estimated that up to 80% of medical cases in developing countries is water related, since the international drinking and sanitation decades (1981-1990), when significant progress was made in water supply and sanitation increased due to population growth, inefficient system and lack of training and spares to maintain systems in working order. Also, the World Health Organization (WHO) in co-operation with the World Bank showed that 1, 230 million people are still without safe water supply and also, 1, 350 million people lacked adequate sanitation. Among the rural populations of developing countries, only 22% has reasonable safe water supply and only 15% had waste disposal facilities [4, 5]. Groundwater originates from deep wells, springs and it is virtually free of bacteria due to filtering action of soil, deep sand and rock, as it flows along channels. However, contaminants may enter the water and change its quality. The hygienic quality of drinking water is reduced if pathogenic microbes penetrate water treatment, or if the condition in the water distribution network allows a high level of microbial growth. Most of the microbes in drinking water are heterotrophic; they need organic compound for their carbon and energy sources. Different disinfection agents, such as chlorine, hypochrorite and ozone, control microbial occurrence and growth in drinking water [2, 3].

Sachet water has become an important source of drinking water of every individual in our urban areas in most part of the country, little is known about the problems associated with this sachet water which is so-called “pure water”, because some or even most are not really portable and does not met the standard quality of drinking water, and is responsible for many water-borne diseases. Although, it is now possible to detect the presence of any pathogens in the water, the method of isolation and enumeration is time-consuming and complex. It is therefore impracticable to monitor drinking water for every possible microbial pathogen that might occur with contamination. The need for reliable and simple methods for detection and enumeration has led to the use “indicators” for the bacterial group that include the desired specific species [2, 6]. Certain criteria should exist before an indication organisms can be used as an index for faecal contamination [7]. Some coliform bacteria are harmless to animals including humans. The major concerns include that well known subspecies of coliform bacteria are harmful. The presence of coliform suggests contamination of water, which may include harmful microorganisms like *Giardia* and *Cryptosporidium* and others. Coliform bacteria may not cause disease, but can be indicators of pathogenic organisms that cause diseases [8].

Establishment of sachet water supplies was therefore an important prerequisite for the development and the survival of our contemporary congested urban civilization. The safe drinking water act was enacted by U.S. congress and it was amended in 1996 (United State Public Law, 1996). The standard recommended by the World Health Organization (WHO) that is; 0 MPN/100 ml and 10MPN/100 ml of coliforms for drinking and recreational waters respectively [9, 10]. Examination of bacteriological quality of water to determine whether the quality of the water is acceptable for drinking purposes has traditionally been done by various researchers. The bacteriological quality of water is determined by the presence of bacteria of faecal contamination namely; total coliforms and *Escherichia coli*. According to health Canada’s guidelines for Canadian drinking water quality, drinking water should contain less than one coliform bacterium per 100ml of water. Ideally drinking water should not contain any microorganism known to be pathogenic [5].

The World Health Organization (WHO) guidelines for drinking water quality requires that all water, *Escherichia coli* and thermo-tolerant coliform bacteria should not be detectable in any 100ml sample and also total coliform must be present or detected in the sample, both pipe and un-pipe treated and untreated [4, 5].

Humans are not the only one’s threatened by poor water quality as many ecosystems are sensitive to degraded water. Despite the recent advances in management and modern use in the processing and production of sachet water, the problem of microbial contamination and spoilage remains the most devastating aspect that causes waterborne diseases in consumers. Government has failed to adequately provide safe, pipe-borne water for the increasing population in Nigeria, and this has encouraged the sale of drinking water by private enterprises that have little knowledge about good manufacturing practices. Waterborne disease resulting from consuming improperly manufactured package water contaminated by bacteria species is one of the causes of morbidity and mortality. Therefore, this research study aimed to determine the bacteriological quality of sachet water sold within Zaria, with a view to characterize the commonly isolated bacteria using standard methods and to determine the antimicrobial susceptibility profile of the isolated bacteria.

## 2. MATERIAL AND METHODS

### 2.1 Sample Collection

A total of ten samples of different brands of sachet water were aseptically and randomly collected by direct purchase from the retail points in various locations within Zaria, Kaduna state, Nigeria. The samples were kept in a sterile and non-pyrogenic polythene bag to avoid post-collection contamination, and immediately transported to the Microbiology Laboratory, Nigerian Institute of Leather and Science Technology, Zaria for analysis. Physicochemical parameters such as pH and temperature were measured by standard methods. The samples collected were designated with laboratory codes, and the brand names of the sachet water samples were concealed for ethical reasons.

## 2.2 Physicochemical Analysis of Sachet Water

### *2.2.1 Determination of Temperature*

The temperatures were determined instantly using calibrated mercury-in-glass thermometer and recorded as described by Sabah *et al*. [11].

### *2.2.2 Determination of pH*

The pH of these samples was determined immediately the samples were brought to the laboratory. A potable pocket-sized pH meter (Hanna Instrument with specification of 0.0 to 14.0ph range, 0.1pH resolution and ±0.1pH accuracy) was used as adopted by Sabah et al. [11]. Ten milliliters of the sample was placed in a beaker. A buffer solution was used to zero and calibrate the pH meter. Then the electrode of the pH meter was inserted into each of the samples and the pH readings were taken as adopted by Umar *et al*. [12].

## 2.3 Sample Processing

The collected samples were diluted serially on arrival to the laboratory. The samples were mixed gently, and a quantity of 1ml was aspirated using a sterile pipette and dispensed into a test tube containing 9ml buffered peptone water (Oxoid™, UK). The solution was mixed to make a dilution of 1:10 or $10^{-1}$. A volume of 1ml from the dilution of 1:10 was aspirated and transferred into another test tube containing 9ml of buffered peptone water using a different sterile syringe to make a dilution of 1:100 or $10^{-2}$. The procedure was repeated for the third test tube containing 9ml buffered peptone water to make a dilution of 1:1000 or $10^{-3}$ as adopted by APHA [13].

### *2.3.1 Total Heterotrophic Aerobic Mesophilic Count*

An aliquot of 0.1ml of the dilution from each diluent was transferred into each of the correspondingly marked duplicate Petri dishes. About 15ml of an already prepared molten nutrient agar was poured into each correspondingly marked Petri dish within 15 minutes from the time of original dilution. The molten nutrient agar (Oxoid™, U.K) was mixed with the inoculums uniformly, after which it was allowed to solidify. The prepared Petri dishes were incubated in inverted position at 35℃ for 24 hours. Following incubation, the number of colonies was counted using colony counting chamber, and the colony forming unit per ml was calculated. The colonies formed on nutrient agar plates were determined by multiplying the number of colonies with a reciprocal of the dilution factor divided by the volume of the inoculum to quantify the total number of the organisms using the Eqn (1):

$$cfu/ml = \frac{average\ number\ of\ colonies\ counted \times \frac{1}{dilution\ factor}}{volume\ of\ inoculum} \quad (1)$$

*2.3.2 Coliform Enumeration by Most Probable Number (Multiple Tube Fermentation) Technique*

The most probable number (MPN) method was used to analyze the sample for the presence of microorganisms. A volume of 10ml of water was dispensed into three test tubes of single strength lactose broth (CM0137, Oxoid®, U.K), 1.0ml was inoculated into three test tubes of double strength lactose broth and 0.1ml into three test tubes of single strength lactose broth. All the tubes were incubated and observed after 24–48 hours for production of gas that indicates positive presumptive test of coliform bacteria. The number of positive tubes from each set was recorded and using the standard table of MPN, the number of bacteria present was estimated. This method was repeated for each sample [13]. The result values obtained are based on statistical probability which specify that the number of organisms present in the original culture has a 95% chance of falling within a particular range [14].

*2.3.2.1 Presumptive Test*

The inoculated presumptive tubes were incubated for 48 hours. Yellow coloration and gas production in Durham's tubes indicated positive results. For the completed test, positive test tubes were streaked on Eosin Methylene Blue (CM0069, Oxoid®, U.K) agar. All the tubes were then incubated at 40±1℃ for 48 hours to check for the production of gas in the Durham tube [13]. Formation of gas in any tube within 24 hours in the fermentation tubes constitutes a positive presumptive test. The appearance of air bubble was not confused with actual gas production, the medium become turbid and active fermentation was indicated by a continuous stream of the gas bubbles throughout the medium when the tube is gently shaken. The absence of gas formation at the end of 24 hours incubation constitutes a negative test. The fermentation tubes showing any amount of gas production at the end of 24 hours incubation was subjected to confirmed test (Figure 1). Coliform enumeration Most Probable Number (MPN) table was used as reference index to quantify the microbial load of water samples [14].

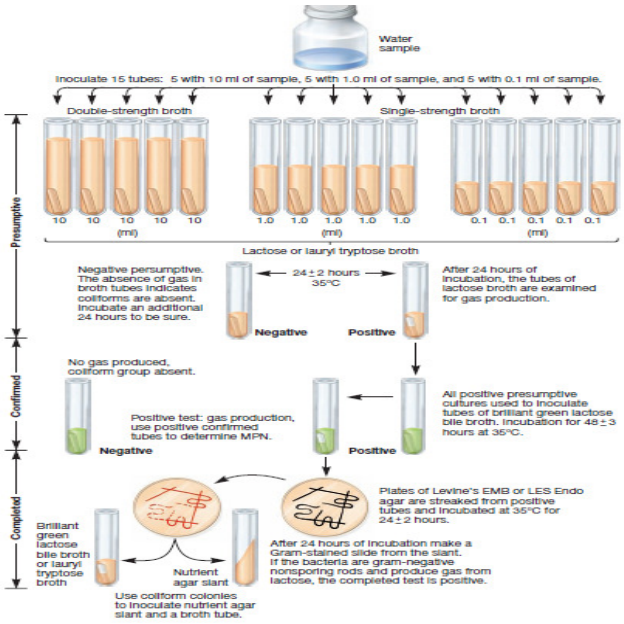


**Figure 1: Multiple Tube Fermentation Technique for Water Testing**
*Source: Prescott et al. [7]*

*2.3.2.2 Confirmed Test*

Due to the gas production by certain non-coliform bacteria, additional testing was necessary to confirm the presence of coliforms. Brilliant Green Lactose Bile broth (CM0031, Oxoid®, U.K) was prepared in test tubes with inverted Durham's tubes inside them according to manufacturer's instruction. One of the tubes showing positive presumptive test was used for the confirmatory test. A loopful inoculum was transferred to the tube containing the brilliant green bile broth and was shaken gently so as to mix well with the media. After the inoculation, the tubes were then incubated at 35℃ for 24 hours. The presence of any amount of gas in the inverted Durham's tubes after the incubation period signifies a positive confirmed test for coliform bacteria. While the tubes without any gas production signifies negative result and no need of going further to the next test [14].

*2.3.2.3 Completed Test*

After 24 hours of incubation of the plates, distinct growth of colonies was seen on the line of streak, some of which showed metallic sheen, and some were showing pinkish and dark-centered colonies. Discrete colonies from Eosin Methyl Blue (CM0069, Oxoid®, U.K) agar plates were picked and transferred to an already prepared nutrient agar slant and incubated at 35℃ for 24hours, and then stored in the refrigerator for Gram's staining and biochemical tests. All the colonies on Eosin methylene blue plates were Gram stained to identify the Gram reaction of the isolates [13, 14].

## 2.4 Microscopic Identification

Microscopy was carried out on the smear and was examined with 40× objectives to check the staining and to see the distribution of material, then with oil immersion objective to report the bacteria and the cells as gram positive or gram negative [8].

## 2.5 Biochemical Characterization and Identification of the Isolates

IMViC set of biochemical tests which encompass Indole utilization, Methyl red, Voges Proskauer, Motility and Citrate utilization tests were carried out to identify the isolates up to their species level. Pure cultures of bacteria isolated were presumptively identified on the basis of their morphological and biochemical characteristics. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt *et al*. [15], and Oyeleke and Manga [16]. Following these tests, the isolates were identified [17]. The isolated coliforms were further confirmed by using Laboratory In A Box™ Easygel® *E. coli* Quantitube™ Test Kit (Micrology Laboratories manufactures, Easygel®, USA) in accordance with manufacturer's specifications.

### 2.6 Antimicrobial Susceptibility Pattern of the Isolated Bacteria

Antimicrobial susceptibility trend of the isolated bacteria was carried out using Kirby-Bauer disk diffusion method as adopted by Umar *et al*. [12]. This was by transferring the pure isolates into sterile normal saline to obtain bacterial density of $3\times10^8$ organism per milliliter McFarland standard. The culture was streaked uniformly on Muller Hinton agar, and discs of antimicrobials were mounted on the surface of the streaked inoculum. The plates were incubated at 37℃ for 24 hours. Each of the cultures was examined for zone of growth inhibition using micrometer. The following antimicrobial agents (size of zone of inhibition) were used: amoxicillin (≥ 17 mm), tetracycline (≥ 15 mm), chloramphenicol (≥ 18 mm), streptomycin (≥ 15 mm), and ciprofloxacin (≤ 31 mm) [18].

### 2.7 Data Management and Statistical Analysis

All the statistical analyses were done using the statistics software SPSS (Statistics Package for Social Sciences, Chicago, USA), version 20.0 for Windows.

## 3. RESULTS AND DISCUSSION

Table 1 showed hysicochemical properties of sachet drinking water sold in Zaria. Samples G and I recorded highest temperatures of 16℃ and 18℃ respectively, while low temperatures were recorded in J (8℃). Slightly alkaline pH was recorded in samples A, D and G, whereas samples B, E, F, J and I recorded slightly acidic pH. Samples C and H were found to fell within neutral or nearly neutral pH readings. There was no statistical relationship between microbial loads and the temperature ($p= 0.454$) of the analyzed sachet water. Also, there is no statistical relationship between microbial loads and pH ($p= 0.446$) of the sachet water.
The study showed that, high temperature readings and pH above neutrality were found to be related to the high total heterotrophic bacterial counts of the sachet drinking water analyzed (Table 1). This may be due to the fact that most of the slightly acidic drinking waters are not suitable for growth by many alkalophilic bacterial species owing to the corrosive and antimicrobial nature of such water samples. This concurs with a previous study, which reported that the high acidic contents of the water samples could be attributed to the delay of the outbreak of cholera in the community because acidic environment do not support the growth of *Vibrio cholerae*. This supports the finding of Charles and Angelo [20] that *Vibrio cholerae* grows best under alkaline condition. Also, the findings agreed with the study of Terna *et al.* [21] and Adeyemi and Umar [22], who reported pH of 4.5–5.0 and documented that this pH (4.5–5.0) is effective in keeping the population of microbial contamination low, making the risk of waterborne infection or intoxication low. However, even a slight adjustment in the optimum temperature may inhibit the growth of some bacterial species. A temperature range of 26℃ to 30℃ was recorded in a similar study and attributed it to the insulating effect of increased nutrient load resulting from industrial discharge [23]. Prescott *et*

*al*. [7] documented the optimum temperature for growth and proliferation of Enterobacteriaceae as 10 to 37℃.

**Table 1: Physicochemical Analysis of Sachet Drinking Water Sold in Zaria**

| Physicochemical parameters | Samples | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | |
| Temperature (℃) | 15 | 14 | 14 | 15 | 10 | 12 | 18 | 12 | 16 | 8 | ($\chi^2$ = 0.454; df =9) |
| pH | 7.6 | 5.8 | 6.9 | 7.3 | 5.9 | 5.0 | 7.5 | 6.7 | 5.1 | 6.0 | ($\chi^2$ = 0.446; df =9) |

Table 2 showed the total heterotrophic count of sachet drinking water sold at Zaria, Nigeria. The result showed that samples A and H are contaminated because of their total heterotrophic counts which ranged from $1.2\times10^2$ to $3.0\times10^2$ cfu/ml. These values are slightly more than the recommended value of $1.2\times10^2$ cfu/ml by WHO [4] and FAO [19].
Based on the total heterotrophic bacterial counts, two samples (20%) A and H recorded high bacterial counts beyond the standard set by WHO/FAO of $1.2 \times 10^2$ cfu/ml, whereas, the remaining eight samples (80%) recorded low bacterial counts within the standard limit of safe drinking water set by WHO/FAO (Table 2). This may be due to the improvement of the hygienic level of the sachet water resulting from recent raid by regulatory bodies to commercial sachet water producers in the study area. This low counts deviates from the findings of Okonko *et al*. [24]; and Anyanwu and Okoli [25] who reported higher heterotrophic plate counts from water samples analyzed from various water sources.

**Table 2: Total Heterotrophic Bacterial Counts of Sachet Drinking Water Sold in Zaria**

| Batches | Average range of heterotrophic bacterial counts | | | WHO/FAO standards | Level of contamination | Level of safety |
|---|---|---|---|---|---|---|
| | 0 — 1.0 ($\times10^2$ cfu/ml) | 1.1 — 2.0 ($\times10^2$ cfu/ml) | 2.1 — 3.0 ($\times10^2$ cfu/ml) | | | |
| A | 0 | 1* | 1** | $1.2\times10^2$ | 1 (100%) | 0 (0%) |
| B | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| C | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| D | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| E | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| F | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| G | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| H | 0 | 1* | 1** | $1.2\times10^2$ | 1 (100%) | 0 (0%) |
| I | 1 [β] | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| J | 0 | 0 | 0 | $1.2\times10^2$ | 0 (0%) | 1 (100%) |
| **Total** | | | | | **2 (20%)** | **8 (80%)** |

*[β] = the threshold is fit for human consumption; * = slightly contaminated and not fit for human consumption; ** = highly contaminated and absolutely not fit for human consumption; cfu = colony forming unit*

Table 3 showedthe coliform count of the analyzed sachet drinking water showed significant coliform count was recorded in sample A. While, sample C, E, G and I recorded least coliform counts. The overall occurrence of coliforms beyond the standard in the study area was found to be 1 (10%).
From the results obtained, most 9 (90%) of the sachet drinking water analyzed meet up with the standard of safe drinking water set by World Health Organization of less than 1 coliform bacteria per 100ml of treated water [4]. The number of total coliform observed in the samples using the MPN technique ranged from <0.03 MPN/100ml to 1.2 MPN/100ml. The highest

count was recorded in sample A as 1.2 MPN/100ml accounting for 1 (10%) contamination. This slightly conforms to the study of Umaru *et al.* [26], who reported the contamination of sachet drinking water as 8.3%. While the lowest MPN counts were recorded in samples C, E and I (0.03 MPN/100ml) each, and sample G recorded (<0.03 MPN/100ml) (Table 3). The high density of coliforms observed in sample A may be due improper process of manufacturing, which may include poor processing, packaging or storage of the sachet drinking water by violation of human safety practices such as sneezing, coughing, unwashed hands, use of unclean overall and head gears by the personnel which involved in the manufacturing process. The total sum of which can lead to bacterial contamination, and can provide an avenue for coliforms to grow.

**Table 3: Total Coliform counts of packaged water samples using MPN Technique**

| Batches | Presence of gas in the tubes | | | MPN/100ml |
|---|---|---|---|---|
| | 1st set of tubes | 2nd set of tubes | 3rd set of tubes | |
| A | 3 | 1 | 2 | 1.2 |
| B | 2 | 2 | 1 | 0.28 |
| C | 0 | 1 | 0 | 0.03 |
| D | 2 | 1 | 2 | 0.27 |
| E | 0 | 1 | 0 | 0.03 |
| F | 2 | 1 | 2 | 0.27 |
| G | 0 | 0 | 0 | <0.03 |
| H | 2 | 2 | 2 | 0.35 |
| I | 0 | 0 | 1 | 0.03 |
| J | 2 | 2 | 1 | 0.28 |

*MPN = Most Probable Number*

Figure 2 showed the heterotrophic distribution of coliform bacteria in the sachet water analyzed. The predominant isolates were *Escherichia coli* (60%) followed by *Enterobacter aerogenes* (20%), while the remaining 20% were found to be non-coliform isolates.

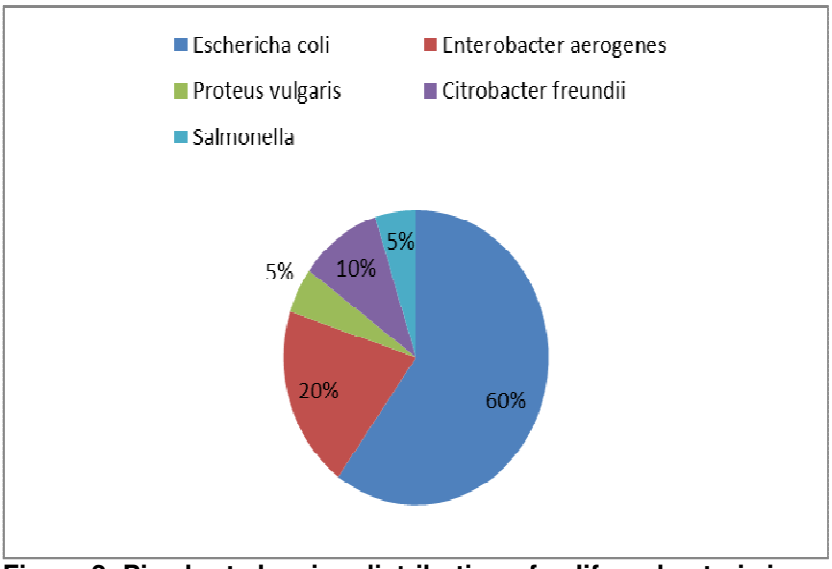


**Figure 2: Pie chart showing distribution of coliform bacteria in sachet water**

Figure 3 showed the distribution of the overall heterotrophic bacterial species isolates in the sachet water analyzed. The predominant isolates are *Escherichia coli* and *Enterobacter aerogenes* (29%), with least occurrence recorded by *Citrobacter freundii Salmonella* sp and *Proteus vulgaris* (14%) each.

The predominant isolates were *Escherichia coli* (60%) followed by *Enterobacter aerogenes* (20%), while the remaining 20% were found to be non-coliform isolates (Figure 2). This concurs with the previous studies that recorded high prevalence of *Escherichia coli* whose presence in drinking water is a strong indication of recent sewage or human/animal waste contamination [27]. However, the presence of coliform bacteria signifies the presence of other enteric organisms. Therefore, in addition to coliforms, other enteric pathogens such as *Salmonella* sp, *Citrobacter freundii* and *Proteus vulgaris* were isolated from the samples, though in small proportions of 14% each (Figure 3). This concurs with the characteristics of coliforms that makes them suitable for water testing as stated by Prescott *et al*. [7], that indicator bacterium should be present whenever enteric pathogens are present.

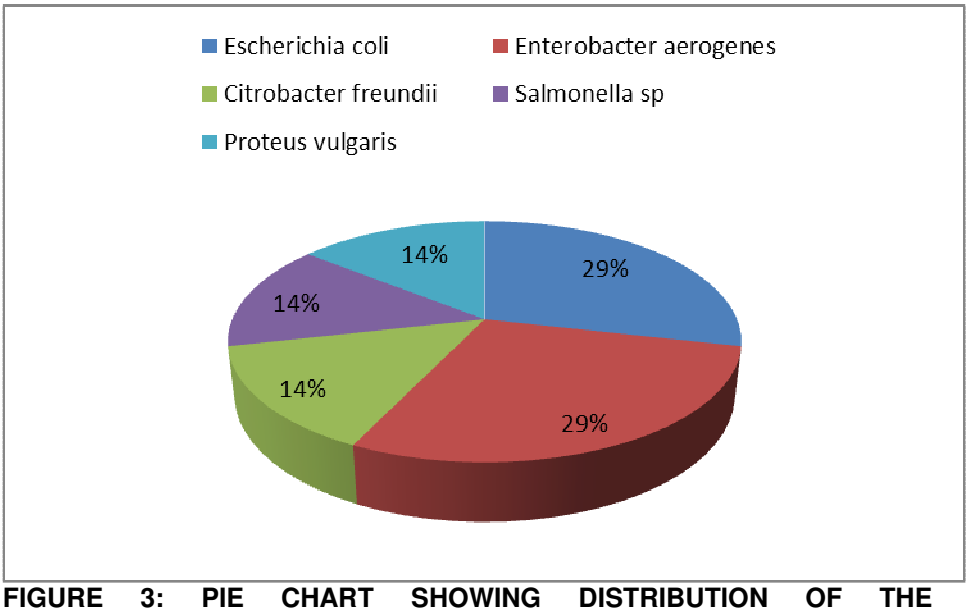


**FIGURE 3: PIE CHART SHOWING DISTRIBUTION OF THE OVERALL HETEROTROPHIC BACTERIAL SPECIES IN SACHET WATER**

**TABLE 4 SHOWED THE BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATED BACTERIA. THE BACTERIA ISOLATED WERE *ESCHERICHIA COLI, ENTEROBACTER AEROGENES, SALMONELLA* SP, *CITROBACTER FREUNDII* AND *PROTEUS VULGARIS*.**

**Table 4: Biochemical characterization and identification of the isolated bacteria**

| Cultural Appearance on EMB agar | Biochemical reactions | | | | Inference ** |
|---|---|---|---|---|---|
| | Indole | Citrate | Methyl red | Voges-Proskauer | |
| Large mucoid, dark centered and pinkish colony with green metallic sheen | + | – | + | – | *Escherichia coli* |
| Round with dark mucoid and the pinkish colony with no metallic sheen | – | – | – | + | *Enterobacter aerogenes* |
| Swarming growth pattern | – | + | + | – | *Proteus vulgaris* |
| * | + | – | + | – | *Citrobacter freundii* |
| * | – | – | + | – | *Salmonella* sp. |

** = not applicable, + = positive, – = negative, **= All the isolates were preliminarily identified as motile, Gram negative, non-spore forming, non-capsulated bacilli*

Table 5 showed the antimicrobial susceptibility pattern of the isolated bacteria. All the isolates were highly sensitive to chloramphenicol (100.0%), ciprofloxacin (100.0%) and streptomycin (100.0%). *Proteus mirabilis* showed exclusive resistance to tetracycline (0.0%). Each of *Enterobacter aerogenes*, *Citrobacter freundii* and *Salmonella* sp recorded relative resistant to amoxicillin (0.0%) respectively.

**Table 5: Antimicrobial susceptibility profile of the bacterial isolates**

| Bacteria / Antimicrobial agent | Potency (µg) | Number of isolates | Sensitive | Resistance |
|---|---|---|---|---|
| ***Escherichia coli*** | | 2 | | |
| Amoxicillin | 10 | | 2 (100.0%) | 0 (0.0%) |
| Tetracycline | 30 | | 2 (100.0%) | 0 (0.0%) |
| Chloramphenicol | 30 | | 2 (100.0%) | 0 (0.0%) |
| Streptomycin | 10 | | 2 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | | 2 (100.0%) | 0 (0.0%) |
| ***Enterobacter aerogenes*** | | 2 | | |
| Amoxicillin | 10 | | 0 (0.0%) | 2 (100.0%) |
| Tetracycline | 30 | | 2 (100.0%) | 0 (0.0%) |
| Chloramphenicol | 30 | | 2 (100.0%) | 0 (0.0%) |
| Streptomycin | 10 | | 2 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | | 2 (100.0%) | 0 (0.0%) |
| ***Proteus vulgaris*** | | 1 | | |
| Amoxicillin | 10 | | 1 (100.0%) | 0 (0.0%) |
| Tetracycline | 30 | | 0 (0.0%) | 1 (100.0%) |
| Chloramphenicol | 30 | | 1 (100.0%) | 0 (0.0%) |
| Streptomycin | 10 | | 1 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | | 1 (100.0%) | 0 (0.0%) |
| ***Salmonella* sp** | | 1 | | |
| Amoxicillin | 10 | | 0 (0.0%) | 1 (100.0%) |
| Tetracycline | 30 | | 1 (100.0%) | 0 (0.0%) |
| Chloramphenicol | 30 | | 1 (100.0%) | 0 (0.0%) |
| Streptomycin | 10 | | 1 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | | 1 (100.0%) | 0 (0.0%) |
| ***Citrobacter freundii*** | | 1 | | |
| Amoxicillin | 10 | | 0 (0.0%) | 1 (100.0%) |
| Tetracycline | 30 | | 1 (100.0%) | 0 (0.0%) |
| Chloramphenicol | 30 | | 1 (100.0%) | 0 (0.0%) |
| Streptomycin | 10 | | 1 (100.0%) | 0 (0.0%) |
| Ciprofloxacin | 5 | | 1 (100.0%) | 0 (0.0%) |

Based on the antimicrobial susceptibility profile of the isolated bacterial species, all the isolates are susceptible to most of the antibiotics used, except *Enterobacter aerogenes*, *Salmonella* sp. and *Citrobacter freundii* which recorded resistance to amoxicillin. This may be as a result of ß-lactamase secretion by most of the members of the family Enterobacteriaceae. This is in line with the studies of Gaibani *et al*. [28]; Trivedi *et al*. [29]; López-Martin *et al*. [30]. *Proteus vulgaris* recorded multidrug resistance against amoxicillin and tetracycline. This also agreed with the findings of Trivedi *et al*. [29] who reported multidrug resistance potentiality of *Proteus* biotypes to tetracycline and derivatives of penicillin.

## 4. CONCLUSION

From the results obtained, it can be deduced that 1 (10%) of the sachet drinking water samples analyzed is not potable for human consumption, and have not meet the required standards set by World Health Organization [4] for faecal coliform counts, which should be zero per 100ml of sample (0/100ml) in all water supplies. High temperature and pH were found to be related to the bacterial counts of the sachet drinking water analyzed. Two samples (20%) did not meet with the standard of heterotrophic bacterial count of $1.2 \times 10^2$ cfu/ml as set by WHO/FAO. Coliforms isolated include *Escherichia coli, Enterobacter aerogenes, Salmonella* sp*, Citrobacter freundii* and *Proteus vulgaris.* This may be due to lack of knowledge in sachet water production, and use of unclean production

equipment and lack of potable water for cleaning purposes, which may be some of the factors that might have contributed to the poor hygienic quality of sachet drinking water at production and at retailing centers in the study area. All the isolates are susceptible to variety of antibiotics used. *Enterobacter aerogenes*, *Salmonella* sp. and *Citrobacter freundii* are resistant to amoxicillin. *Proteus vulgaris* recorded multidrug resistance.

The government should therefore privatize the water manufacturing firms by encouraging them to undergo training on good manufacturing practices as well as on the need for safe drinking water production.
Water distribution systems should be checked at regular interval for contamination of sewages, and operators of the water system should be skilled in the design and functioning of those equipment.
Government agencies such as National Agency for Food and Drug Administration Control (NAFDAC) should frequently carryout quality control exercise in the study area in order to regulate and control the sale and production of this packaged water also known as “pure water”.

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