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# Microbiological and parasitological study of different brands of sachet water sold within Nekede

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**Abstract**
This study evaluated the microbiological and parasitological study of different brands of sachet water sold within Nekede. A total sample of 10 different brands of sachet water was purchased randomly from hawkers and vendors within Nekede. Standard microbiological methods were adopted in the determination of the microbial load, isolation and identification of the bacterial isolates. Parasites were identified using microscopic examination and parasite atlas. Results revealed that microbial load of the different brands of sachet water sold within Nekede. Total viable bacterial count recorded ranged from 2.0 x $10^1$ cfu/ml to 3.0 x $10^2$ cfu/ml. There was no total coliform and *Salmonella-Shigella* count recorded. Bacterial isolates were; *Pseudomonas, Bacillus* and *Corynebacterium* species. *Bacillus* species 4(57.1%) had the highest bacterial frequency while *Pseudomonas* species 1(14.3%) had the least bacterial frequency. Parasitic load and parasites in the different brands of sachet water sold ranged from 2 to 6. Out of the ten (10) sachet water samples, two (2) had parasites. The parasites were; *Entamoeba histolytica, Entamoeba coli,* and *Entamoeba hartmanni* cysts and trophozoites. The findings from this study suggest that NAFDAC should critically monitor and carry out routine assessment of the site, water source, equipment and chemicals used by water factories in order to avoid drinking contaminated sachet water by the public.

**Keywords:** Coliform, salmonellosis, purification, trophozoites

## Introduction

### 1. Background of study

Water is an essential part of human nutrition, both directly as drinking water or indirectly as constituent of food; in addition to various other applications in daily life. Water is not only essential for life, it also remains a most important vehicle of transmitting disease and infant mortality in many developing countries and even in technologically more advanced countries (Ekelozie *et al.*, 2018) [6]. Good quality water is odorless, colourless, tasteless, and free from fecal pollution. However, it is estimated that about 1.2 billion individuals world-wide do not have access to potable water. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities that depend on non-public water supply system (Ekelozie *et al.*, 2018) [6].

The business of "pure water" (sachet water) business has become lucrative (Ekwunife *et al.,* 2013). Though, sachet drinking water in terms of packaging has improved than the former hand filled, hand tied types of drinking water. Sachet water is a phenomenon that has gained widespread use as an alternative to the insufficient provision of potable water. It is an alternative that is readily available, affordable but not without concerns about its purity.

Due to the shortfall in the provision of adequate safe drinking water for the populace, the private sector, although for profit purposes, has been of increasing significance in the effort to supply the populace with adequate and safe drinking water. They provide alternatives to the erratic municipal pipe-borne drinking water supply system in the form of packaged water of which includes the sachet water popularly known in Nigeria as 'pure water (Omalu *et al.*, 2012) [14]. The production of sachet water has increased tremendously and has found its patronage mainly from the middle and low socioeconomic classes.

Sachet water is not completely sterile; it may not be entirely free of all infectious microorganisms. The potential danger associated with sachet water is contamination, which is a factor of the source of the water itself, treatment, packaging materials, dispensing into packaging materials and closure under prolonged storage of packaged water at favorable environmental conditions, total aerobic heterotrophic can grow to levels that may be harmful to humans (Omalu *et al.,* 2014).

The access to safe drinking water is essential to health, a basic human right and a component of effective policy for health protection. Drinking water is not only a liquid for hydration; it interacts with industrial, agricultural, economic, social and cultural components characterizing the human society (Ancuta, 2012) [2]. Drinking water is an important vehicle for spreading microorganisms that interact with human body.

Water pollution results in transmission of infectious diseases such as dysentery, cholera, diarrhea, typhoid, shigellosis, salmonellosis, and varieties of other bacterial as well as fungal, viral, and parasitic infection (Nwachukwu & Ume, 2013) [10]. The water quality of borehole is generally neglected based on the general belief that it is pure through the natural purification process. For water to be potable, it must be microbiologically and physico-chemically safe and in order to achieve this, an approach that will eliminate pathogenic organisms from the source of water supply must be ensured.

Drinking unsafe and unhygienic water can cause high prevalence of water-borne diseases. In May 2000, 2,300 people became seriously ill and seven died because of water contamination with *E. coli* in Philippines. Despite the abundance of water covering 71% of the earth surface, people spend billions of dollars per year to buy purified water that is pre-bottled. Most people around the world regard bottled water as safe for consumption (Khaniki *et al.*, 2012) [9].

Research findings have revealed that a considerable percentage of all diseases which cause mortality in the developing countries are directly and indirectly related to poor drinking water quality, and over 20,000 children die per day (approximately six million annually) due to water-borne diseases resulting from unavailability of safe drinking water. In these parts of the world, the usual sources of drinking water are streams, rivers, wells and boreholes which are mostly untreated and associated with various health risks (Khaniki *et al.,* 2012) [9]. The quality of water influences the health status of any population, hence, analysis of water for physical, biological and chemical properties including trace element contents are very important for public health studies.

Parasites are organisms that live in a close relationship with other organisms (hosts) and are capable of causing harm to their host. Their routes of transmission are of great importance in the health of many populations in developing countries, where the frequency of infection is a general indication of local hygiene and environmental sanitation levels. Intestinal parasites cause significant morbidity and mortality throughout the world, particularly in developing countries. When water is contaminated by parasites, it can cause varieties of illness, pain, disability and even death (Omolade & Zanaib, 2017) [15].

Packaged sachet water is an alternative source of drinking water in Nigeria, hence the need to regularly assess their microbial quality. The packaging materials used in packaging water, production room and handling by workers could be sources of contamination of sachet water packs. In most sachet water production companies, sachet water packs are seen displayed on cement floors and tiles after production. In most cases, the workers use bare hands in packaging of the produced water while sweating profusely. When this happens, the sachet water packs get contaminated and serve as means of transmission of pathogenic organisms to those who will use mouth to open the sachet water. The need to examine sachet water packs for their microbiological and parasitological quality is therefore necessary. Hence the need for this study which is to determine and investigate into the microbiological and parasitological quality of sachet water sold within Nekede in order to know if they are of good microbiological quality and as well determine the microorganisms that could be associated with the consumption of this sachet water.

## Materials and Methods

### 1. Collection of samples

A total sample of 10 different brands of sachet water was purchased randomly from hawkers and vendors within Nekede. They were conveyed to Biology/Microbiology Laboratory, Federal Polytechnic Nekede, Owerri.

### 2. Sterilization of materials

The method described by Cheesbrough (2010) was adopted in the sterilization of media and glasswares that was used for this study. All the glasswares used for the experiment was sterilized using the laboratory hot air oven at a temperature of 160 $^0$C for 1 hour. Wire loop was sterilized over burning flame and allowed till it was red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame. The media to be used in this study; nutrient agar, peptone water, eosin methylene blue agar, Sabouraud dextrose agar, triple sugar iron agar and Simmon's citrate agar was prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121 $^0$C at 15 psi for 15minutes and was allowed to cool to a temperature of 45 $^0$C. About 20 milliliters was poured into sterile petri-dishes. The plates were allowed to cool and set for inoculation.

### 3. Parasitological examination

The method described by Omolade and Zanaib (2017) [15] was adopted in the parasitological examination of the sachet water samples. Each of the sachet water surfaces were carefully cleaned using cotton wool soaked in ethanol. Ten (10) ml of each sample was vigorously shaken and dispensed into ten 15ml sterile centrifuge tubes. The test tubes and its content were centrifuged at 2500 revolutions per minutes. The resultant sediment was stirred with a clean applicator stick and a drop will be placed on a clean grease free slide mixed with a drop of Lugol's iodine solution. The mixture was covered with a cover slip and examined under an Olympus binocular microscope using x4 and x10 objective lenses. Only viable eggs and cysts will be identified.

### 4. Determination of the microbiological quality of the sachet water

The method described by Bello *et al.* (2013) [3] was adopted in the determination of the microbiological quality of the sachet samples. 0.1 milliliter aliquot of the samples was dropped into the different media in the plates. A sterile bent glass rod was used to spread the aliquot evenly on the media. The plates were labeled accordingly. The inoculated plates were inverted and incubated at a temperature of 37$^0$C for 24 hours for bacteria and 28$^0$C for 3-7 days for fungi.

### 5. Microbial plate count

After the incubation of the different plates, the different colonies formed on the media were counted using the digital colony counter. The total population of the colonies was expressed as colony forming unit per milliliter (cfu/ml).

### 6. Colonial morphology identification

The method described by Cheesbrough (2010) was adopted in the colonial morphology identification. Presumptive identification of the colonies was done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation.

### 7. Purification and preservation of isolates

The method described by Ochei and Kolhatkar (2010) [11] was adopted in the purification and preservation of the isolates. After the various colony counts, bacterial isolates were pick with a wire loop based on their cultural and morphological characteristics. The picked colonies were sub-cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24hrs at 37$^0$C. After incubation pure cultures were stored in McCartney Bottle in a refrigerator. Fungal isolates were sub cultured onto freshly prepared Sabouraud dextrose medium using needle tease method.

### 8. Gram staining

The Gram staining technique described by Cheesbrough (2010) was adopted. The procedure will be as follows: A smear of each of the bacterial isolates were made and fixed by air drying. The smears were then covered with crystal violet stain for 60 seconds and rapidly washed off with water thereafter. The smears were covered with Lugol's iodine for 60 seconds and washed off with water. The smears were decolorized with acetone alcohol and washed off after 10 seconds. The smears were finally flooded with safranin for 2minutes and washed- off with clean water. The back of the slides was wiped and placed in a draining rack for the smear to dry before they were viewed with x 40 and x 100 oil immersion objective lens. Gram positive bacteria gave purple coloration while Gram negative bacteria gave pinkish coloration.

## 9. Biochemical tests with the bacterial isolates

The method described by Cheesbrough (2010) was adopted in the biochemical characterization of the bacterial isolates. The biochemical tests that were carried out included; catalase, oxidase, coagulase, citrate utilization, indole production, sugar fermentation and motility test.

### 9.1 Catalase test

Three milliliter (3ml) of freshly prepared hydrogen peroxide was poured in a test tube. A colony of test organism was taken with sterile wooden stick and immersed into hydrogen peroxide solution. Generation of bubbles indicated oxygen production. If bubbles were produced, the organism was catalase positive. However, if bubbles were not produced, the organism was catalase negative.

### 9.2 Oxidase test

A piece of filter paper was placed in Petri-dish and 3 drops of freshly prepared oxidase reagent were added. Using a sterile glass rod, a colony of test organisms was removed from a culture plate and smeared on the filter paper. Oxidase-positive organisms gave blue color within 5-10 seconds, and in oxidase-negative organisms, color did not change.

### 9.3 Citrate utilization test

A bacterial colony was inoculated in Simmons citrate agar and incubated at 37°C for 24 hours. Thereafter, development of blue color was observed. Citrate positive showed that growth was visible on the slant surface and the medium became an intense blue while Citrate negative showed trace or no growth was visible and no color change occurred.

### 9.4 Indole test

Test bacterial colony were inoculated in peptone water and incubated at 37°C for 24-28 h. Thereafter, 0.5 ml of freshly prepared Kovac’s reagent was added. Positive test showed pink colored ring was observed after addition of reagent. Negative test showed no color change after reagent addition.

### 9.5 Motility test

The semi-solid agar of nutrient agar was used for this study. The media was prepared in slants and the organisms were inoculated by stabbing technique. Zig-zag growth along the line of stabs indicated a positive result while none indicated a negative result.

### 9.6 Coagulase test

A drop of distilled water was placed on each end of a slide for each of the test organisms. Thereafter a colony of each of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then-added to one of the suspensions and mixed gently for each of the test organism. Clumping within 10 seconds was an indication of positive test while none was an indication of a negative test.

### 9.7 Sugar fermentation test

Each colony of the different test organisms were inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated, agar slopes were incubated at 37°C for 24 hours. The different colors of the slopes and butts in addition to the presence of gas production and hydrogen sulphide ($H_2S$) blackening was indicative of the type of bacteria present.

## 10. Identification of the fungal isolates

The method described by Cheesbrough (2010) was adopted in the identification of the fungal isolates. The fungal isolates were identified by morphological characteristics on Sabouraud dextrose agar (SDA) and microscopic examination after lacto-phenol cotton blue staining technique. Each of the fungal isolates were separately collected with a sterile wooden stick and teased out on a drop of lacto-phenol cotton blue stain on a clean glass slide. The wet mount preparations were then viewed under the microscope for branched and unbranched hyphae.

## Results and Discussion

### 1. Results

Table 4.1 showed the microbial load of the different brands of sachet water sold within Nekede. Total viable bacterial count recorded ranged from $2.0 \times 10^1$ cfu/ml to $3.0 \times 10^2$ cfu/ml. There was no total coliform and *Salmonella-Shigella* count recorded. Growth was recorded in seven (7) out of the ten (10) sachet water samples used in this study.

**Table 1:** Microbial load of the different brands of sachet water sold within Nekede

| Sachet water samples | Total viable bacterial count (cfu/ml) |
|---|---|
| A | $2.0 \times 10^1$ |
| B | NG |
| C | $9.0 \times 10^1$ |
| D | $2.4 \times 10^2$ |
| E | NG |
| F | $1.4 \times 10^2$ |
| G | $2.8 \times 10^2$ |
| H | NG |
| I | $1.4 \times 10^2$ |
| J | $1.6 \times 10^2$ |

**Key:** A – J = Different sachet water samples
cfu/ml = Colony forming unit

Table 2 shows the cultural morphology and biochemical characteristics of the bacterial isolates from the different brands of sachet water sold within Nekede. They were; *Pseudomonas, Bacillus* and *Corynebacterium* species

**Table 2:** Cultural morphology and biochemical characteristics of the bacterial isolates from the different brands of sachet water

| Morphological Characteristics | Gram reaction | Oxidase test | Indole test | Spore test | Catalase test | Citrate test | Coaguase test | Motility test | S | FT | G | H2S | Possible bacteria |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milkish, flat, rhizoid-like dry-surface colonies | Gram positive rods in short chains | - | - | + | + | - | - | - | Y | Y | + | - | Bacillus species |
| Bluish-green, flat, non-mucoid colonies | Gram negative rods in diploids | + | - | - | + | - | - | + | R | R | - | - | Pseudomonas species |
| Milkish, raised, non-mucoid regular shaped colonies | Gram positive rods | - | - | - | + | - | - | - | No Reaction | - | - | | Corynebacterium species |

**Key: - =** Negative + = Positive S = color of slope B = color of butt G = Gas production $H_2S$ = Hydrogen sulphide production blackening) R = Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

Table 3 shows the frequency and percentage occurrence of the bacterial isolates from the different brands of sachet water sold within Nekede. *Bacillus* species 4(57.1%) had the highest bacterial frequency while *Pseudomonas* species 1(14.3%) had the least bacterial frequency.

**Table 3:** Frequency and percentage occurrence of bacterial isolates from the different brands of sachet water

| Bacterial isolates | Frequency | Percentage |
|---|---|---|
| Pseudomonas species | 1 | 14.3 |
| Corynebacterium species | 2 | 28.6 |
| Bacillus species | 4 | 57.1 |
| Total | 7 | 100.0 |

Table 4 shows the parasitic load of the different brands of sachet water sold within Nekede. The count ranged from 2 to 6. Out of the ten (10) sachet water samples, two (2) had parasites. The parasites were; *Entamoeba histolytica, Entamoeba coli,* and *Entamoeba hartmanni* cysts and trophozoites.

**Table 4:** Parasitic load of the different brands of sachet water sold within Nekede

| Parasites | Parasitic load | Frequency | Percentage |
|---|---|---|---|
| Entamoeba coli cysts | 2 | 2 | 50.0 |
| Entamoeba coli trophozoites | 6 | 2 | 50.0 |
| Total | | 4 | 100.0 |

## Discussion

Water is an essential resource required for human survival on earth since many activities of man require the use of water. Table 1 showed the microbial load of the different brands of sachet water sold within Nekede. Total viable bacterial count recorded ranged from $2.0 \times 10^1$ cfu/ml to $3.0 \times 10^2$ cfu/ml. There was no total coliform and *Salmonella-Shigella* count recorded. There was no coliform growth recorded in all the water samples used in this study. Omalu *et al.* (2010) [14] in their study on contamination of sachet water reported microbial load of $1.51 \times 10^2$ to $1.54 \times 10^2$ with sachet water sold within Lagos metropolis, Lagos State, Nigeria. Bello *et al.* (2013) [3] reported total bacterial count in borehole waters and well water samples from Ijebu-Ode, Ogun State to range from $1.2 \times 10^2$ cfu/ml to $2.5 \times 10^2$ cfu/ml.

Table 2 showed the cultural morphology and biochemical characteristics of the bacterial isolates from the different brands of sachet water sold within Nekede. They were; *Pseudomonas, Bacillus* and *Corynebacterium* species. In Anambra, Nigeria, Ezeugwunne *et al.* (2009) [8] isolated bacteria *Escherichia coli* (36%), *Streptococcus faecalis* (19.4%), *Klebsiella pneumonia* (19.4%) and *Staphylococcus aureus* (25%) in sachet water samples analysed. Oladipo *et al.* (2009) [13] worked on the microbial analysis of some vended sachet water in Ogbomoso, Nigeria. The isolates characterized were identified as *Bacillus subtilis, Bacillus alvei, Pseudomonas putida, Pseudomonas fluorecens, Bacillus cereus, Enterobacter aerogens and Proteus mirabilis.* Assessment of quality of packaged water sold in Ibadan, Nigeria showed that 5% of 78 samples (Type A), and 28% of 30 samples (Type B) tested positive for coliform counts. The dominant bacteria were *Klebsella* species, *Streptococcus faecalis* and *Pseudomonas aeruginosa*.

Adegoke et al. (2012) [1] reported the isolation of *Staphylococcus, Klebsiella, Pseudomonas, Proteus, Enterobacter* species from sachet water samples. Aroh *et al.* (2013) reported the isolation of *Staphylococcus* species, *Enterobacter* species, *Salmonella* species, *Klebsiella* species, *Micrococcus* species from sachet water. The results of this study are similar to the report.

Table 3 showed the frequency and percentage occurrence of the bacterial isolates from the different brands of sachet water sold within Nekede. *Bacillus* species 4(57.1%) had the highest bacterial frequency while *Pseudomonas* species 1(14.3%) had the least bacterial frequency.

Table 4 showed the parasitic load of the different brands of sachet water sold within Nekede. The count ranged from 2 to 6. Out of the ten (10) sachet water samples, two (2) had parasites. The parasites were; *Entamoeba histolytica, Entamoeba coli,* and *Entamoeba hartmanni* cysts and trophozoites. A study carried out in Ebonyi state, Nigeria by Chollom *et al*. (2013) [5] to evaluate the parasitic contamination of local sources of drinking water, recorded high parasitic contamination of the different water sources in the rural villages. Parasites in sachet water indicate contamination of water or packaging materials used in sachet water production.

Ekwunife *et al.* (2010) [7] in Akwa, South-eastern Nigeria, ascertained the parasites associate with sachet drinking water. The samples collected met the W.H.O recommended standard. That finding corroborated the result of this work. In that study, no parasites were found within the sachets but outside the sachets, which is partly in contrast with this work because parasites were found within some sachet water in this present study. That study also reported that the sachet water purchased from hawkers had the highest number of parasites. Identified parasites detected were similar with that of this present study except the eggs of *Trichruris trichuria which were not detected in this present study.*

It can also be due to many reasons which include: feacal contamination, poor sewage disposal, and poor personal hygiene, lack of basic sanitation, bad storage facilities, and poor treatment of water. Apart from environmental contaminants, improper storage and handling by vendors also poses a serious threat to the health of the ignorant consumers (Okwa & Gbadamosi, 2014) [12]. According to Omalu *et al.* (2010) [14] assessment of water quality at some important stages of production and post-production stages at the factories is important in order to ensure their quality and safety.

## Conclusion and Recommendations

### 1. Conclusion

The results of this study revealed the presence of some bacteria and parasites in the different brands of sachet water analyzed. From the result, bacterial isolates occurred in seven (7) out of the ten (10) sachet water samples analyzed. Parasites occurred in two (2) out of the ten (10) sachet water samples analyzed. The findings from this study suggest that NAFDAC should critically monitor and carry out routine assessment of the site, water source, equipment and chemicals used by water factories in order to avoid drinking contaminated sachet water by the public.

### 2. Recommendations

a. Assessment of water quality at some important stages of production; pre-production, production and post-production stages at the factories is therefore suggested in order to ensure their quality and safety.
b. Standard Organization of Nigeria (SON) should be actively involved in the regulation of the quality of packages used in packaging water.
c. NAFDAC and the Ministry of Health need to get the producers of ‘packaged water’ to comply with the national drinking water guidelines. All water that fails NAFDAC and WHO regulations should be withdrawn from the market.
d. Hawkers should be educated and enlightened on the proper way to handle and sell sachet water in other not to get it contaminated and should also be encouraged to maintain proper hygiene.

## Conflict of Interest

The authors declared that there is no conflict of interest regarding the publication of this manuscript.

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