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Detection of Klebsiella variicola, Escherichia coli and Providentia staurti in sachet water sold in Okada metropolis, Ovia North LGA,Edo state Nigeria

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Abstract:

Availability and access to safe drinking water is very important for public health development. Although water is essential, it is the most common way for infectious diseases to spread. Sachet water from 4 different brands were collected during products distribution in Okada town. Four (4) sachets were randomly collected from each brand to make a total of 16 sachet, and labelled A-D. They were observed for cloudiness or particles,taste,color and odor. Colony forming unit counts and coliform count/100ml present in the water of sample A and B have average count of 1.5 x 10^4 cfu/ml and 1.8x 104cfu/ml respectively. Sample B had the highest colony count and coliform count among the selected samples with 1.8 x10^4 cfu/ml and 3.0 X 10^3CC/100ml respectively. All the sample water A-D were prospect of acid forming organisms. Morphological and Microscopy revealed the presence of gram-negative rods. Molecular analysis using 16S rRNA amplification revealed the presence w1= Klebsiella sp, w2= *Escherichia coli* and w3= *Providencia* sp., contaminating the sachet water A -D. Sequencing and blasting computed using Jukes-Cantor method showed isolate W1 within the *Klebsiella* sp and revealed a closely relatedness to *Klebsiella variicola,* W2 and W3 were closely related to *Escherichia coli* and *Providencia staurti* , than other *Escherichia* respectively. *Klebsiella variicola,* showed strong resistance to all the antibiotics except for Ciprofloxacin. While *Providencia staurtii* showed resistance to all antibiotics. The public health impacts of drinking contaminated water cannot be over emphasized, especially in this part of Edo state.

Keywords: Sachet-water, molecular analysis , resistance , organism

INTRODUCTION:

Packaged drinking water refers to water packaged in cans, plastic bags and pouches specifically for drinking purposes. Save drinking water and income is more common in countries where economic and social conditions are poor. However, many studies have shown that packaged drinking water is unsafe to drink due to the presence of viruses (Ahmed *et al.,*2013; Onuguh,*et al*., 2025). Access to safe drinking water is very important for health and development (Adetunde *et al.,*2014). Due to water scarcity and the government's inability to provide adequate drinking water, most small water producers package drinking water in bags and sell it to the factory (Thliza *et al.,*2015). These are small nylon bags containing 0.5 liters of electrically heated water and sealed at both ends as reported Adegoke *et al.,* in Onuguh, *et al.,*(2025). This is believed to be cheaper and less expensive than bottled water and also safer, cleaner and better than hand-packed, hand-tied water in polyethylene bags that was previously widely marketed (Oyedeji *et al.*, 2010; Akinde *et al*.,2010). As a result, bottled water has become the most used water by both the rich and the poor (Akinde *et al.,*2011; Onuguh, *et al.,*2025).

Although water is essential for health, it is the most common way for many infectious diseases to spread. Therefore, it is important to verify the quality of water before drinking and to ensure that the water we drink is safe. Safe drinking water is defined as water whose microbial, chemical and physical properties meet the standards of the International Water Health Organization (World Health Organization,2005). From a microbiological perspective, drinking water must be free of all kinds of viruses and opportunistic microbes. Although there are many microorganisms in water that may be harmful to health, such as Salmonella, Shigella, coliforms and mycobacteria, coliforms are used to evaluate water quality (Shiaka, *et al.,*2020). Coliforms are Gram-negative bacteria that grow in a high salinity environment and can ferment lactose at 35-37°C, producing acids, gases and aldehydes within 24-48 hours. It is a weak oxidizer and does not form traces. Microorganisms in water can cause various diseases such as typhoid, cholera, diarrhea, dysentery and hepatitis (Shiaka, *et al.,*2020; Onuguh, *et al.*,2025). According to the World Health Organization (2005), insecurity is a major problem and pollution of water resources is an ongoing problem in the world. 1 billion people worldwide depend on safe drinking water from lakes, rivers and open sources. Most of these are located in Asia (20%) and sub-Saharan Africa (42%) (Ribeiro et al., 2006). The use of these unhealthy resources helps explain why 90% of human infections in underdeveloped countries are caused by waterborne diseases (WHO,2005). According to the World Health Organization, up to 80% of diseases worldwide can be caused by poor

sanitation, pollution or lack of water. For this reason, water must be purified and disinfected before it can be used as drinking water.

According to Oyedeji *et al.,*(2010) waterborne diseases are one of the major health issues in developing countries such as Nigeria. Most bottled drinking water manufacturers in Nigeria mainly obtain their raw water from sources such as local, municipal tap water or well water, and therefore fail to meet set standards due to lack of drinking water technology (Oluyege *et al.,* 2014).

Studies by Parashare *et al.,*2003; Banu and Menakulu,2010; Ibimesim,2014; Akinde *et al.,*2011; Thliza *et al.*2015; Opara and Ennodine,2014; Maduka *et al.,*2014), and currently by Shiaka, *et al.,*(2020) and Onuguh, *et al.*,(2025), have studied the quality of sachet and bottled water in Nigeria and have discovered that water is the most common route of transmission for many infectious diseases. Thus, they resolved that ensuring water quality is imperative. Based on these reports the study aims at specifically screening, isolating and using molecularly characterization to detect the presence of bacterial isolates associated with sachet and bottled water sold in Okada, Edo State.

METHODS:

Study Design

This research was conducted in Okada town situated in Ovia North East Area of Edo State Nigeria. Okada is a Sub Urban Community with an area of about 2,301km^2 and an estimated population of about 155,000 (One Hundred and fifty-five thousand) people as at the 2006 census. The people rely on water from sources such as boreholes, bottled and sachet water, and ponds for their daily use. Okada is also a town where the Premier Private University (Igbinedion University Okada) is located.

Sample collection and Labelling

In a sample of 80 sachet water from 4 different brands (a bag contains 20 sachets per brand). A total of 16 sachet were randomly collected from the bags (16 sachets from 4 brands). The samples were collected from different local water vendors in Okada town for analysis. Samples collected were coded to aid the identification through Alphabets A-D. the 16-sachet water were used for the analysis.

Physico-chemical analysis

Turbidity/Clarity, Traceability, parameters and Organoleptic determination

Water samples were observed for any sign of cloudiness or particles. The taste of the water samples was observed as well as color, odor of the water samples as described by Shiaka, *et al.,*(2020).

Test for odour: A wide mouthed glass bottles were rinsed with 4M Hydrochloric Acid (HCl) and then cleaned with distilled water. The bottles were half-filled with each sample about 50 mls of the water, stoppered and were shaken vigorously for 2 to 3 seconds. The stoppers were then removed and observed for odour using the nostril (Shiaka, *et al.,* 2020).

Test for taste: The test were carried out according to Shiaka, *et al.,*(2020).An aliquot each of the water samples (1ml) were taken and the list of a 5(five) human panel tasted it by dropping the samples of water each in their mouth. The taste were immediately recorded accordingly. The procedures were repeated until the whole samples were tasted.

Test for color: The sachet and bottle water samples each were poured into clean grease free beaker and were viewed using a bench-top multipurpose photometer. This is done using distilled water for the blank, and calibrate the spectro-photormeter to zero as described by Shiaka, *et al.,*(2020), before placing the water sample on the cuvette ,and then inserting into the spectro-photometer for results. This is based on American Public Health Standard method (APHA).

External features of the various packages were assessed such as NAFDAC number, date of manufacture, expiry date and batch number. These are requirement for products that meet standards as spelt out by the National Agency for food and Drug Administration and Control (NAFDAC).

Determination of pH

The pH of the water samples was determined using a compact pH meter. The pH meter was standardized with pH7 and pH4 buffer solutions in other to adjust the device. The pH probe electrode ball is then immersed in the beaker containing the sachet water sample and the measurement was taken (infiteck, USA, PH-B200/PHB200EM). The values were expressed in mean and standard deviations. The turbidity and organoleptic assay were described based on visibility.

Enumeration of bacteria and molecular identification

An aliquot (1 ml) of each water sample (A-D) was aseptically inoculated on each of the sterilized agar (Plate count agar for AMC and MacConkey agar (Biotech, England) using the pour plate method. They were inoculated in duplicate and incubated at 37°C for 24 hours. The plates were carefully observed for microbial growth and distinct colonies enumerated and expressed in CFU/mL. The isolates were further identified using 16SrRNA sequencing after the gene was purified on agar gel as (Winsley, *et al*.,2012). The 16S rRNA primer was used to locate the internal transcribed spacer gene (Winsley, *et al*.,2012).

Antibiogram test of the isolates

The organisms were screened for antimicrobial activity and were carried out by the single disc agar diffusion method as described in Onyenwe, *et al.*(2011). Using sterile pipette, 0.1ml of 10^{-2} dilution of an overnight broth culture of each test bacterium we analyzed. Selected antibiotics discs from different groups of antibiotics such as the Cephalosporin, Penicillin, Fluoroquinolones and Aminoglycosides were used for the test.

Data analysis

The packaged water/ bottled water statistical analysis were carried out using simple descriptive data analysis like mean and standard deviation.

RESULTS

Summary of Physicochemical properties of the water samples

The pH of the samples ranged from 6.06 – 6.90 for the entire sample analyzed, all the sample were void of particles and their color were clear, tasteless and odorless (Table 1).

10 Sample Source	PH	Temp (C)	Particles	Colour	Odor	Taste
A	6.09±0.	36.00±0.18	Absents	Clear	Absent	Absent
B	6.06±0.16	36.00±0.15	Absents	Clear	Absent	Absent
C	6.50±0.04	35.00±0.11	Absents	Clear	Absent	Absent
D	6.10±0.23	35.00±0.18	Absents	Clear	Absent	Absent

Table1: Physico-chemical properties of the isolates

pH *– Concentration of acidity or alkalinity;* ***Tem****p-Temperature*

Presumptive and microbial counts analysis

The colony forming unit counts and coliform count/100ml present in the water of samples A and B have average count of 1.5 x10^4 cfu/ml and 1.8x 104cfu/ml respectively as shown in Table 2. Sample B had the highest colony count and coliform count among the selected samples with 1.8 x10^4cfu/ml and 3.0X10^3CC/100ml respectively. Also, all isolates were Gram-negative rod-shaped organisms. Some had smooth, moist or glistering colonies and mucoid as seen in samples A and B while other colonies were grayish white and non-sporing as seen in samples B and C (see tables 3 and 4). Only sample D produced gas. Samples A and B show the prospect of acid forming microorganisms which reflects in the chemical features of the water by having the mean lowest pH of the samples.

N&A – Name and address; cL–Centilitre; Reg. No – NAFDAC registration number; D.O.M – Date of Manufacturing date D.O.E –Date of Expiring ; N. Val –Nutritional value, A =Absent, P = Present

Table 2; Traceability parameters of the water samples

Sample source	*GP*	*AP*	Colony count(cfu/ml) mean	Coliform count(cfu/ml) mean
A	*Absent*	*Present*	1.5 X 10^4	3.0 X 10^3
B	*Absent*	*Present*	1.8 X 10^4	3.6 X 10^3
C	*Absent*	*Absent*	1.02 X 10^4	2.0 X 10^3
D	*Present*	*Absent*	1.4 X10^4	2.8 X 10^3

Table 3: Presumptive and microbial counts analysis

GP *– Gas Production;* ***AP*** *– Acid production*

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Samples Source val	N&A	Volume (cL)	D.O.M	D.O.E	Batch	N.
A	P	75	A	A	A	A
B	P	60	A	A	A	A
C	P	60	A	A	A	A
D	P	60	A	A	A	A

Table 4: Morphological/Microscopic and Molecular Identification of Isolates

Morphological/Microscopic Examination	Molecular identificatio based on 16SrRNA	Samples source
Gram-negative mucoid and non- motile rods Gram negative rods, Greyish-white rods on agar	*Klebsiella variicolla, E. coli, Providencia stuartii*	A and B (n=8
Gram-negative and mucoid shape rods Gram negative circular rods, Greyish-white rods on agar	*Providencia stuartii E. coli, Klebsiella variicolla*	C and D (n=8

Molecular identification

PCR Amplification of 16S rRNA

The result of 16S rRNA amplification showed the bands in lane I= b1, while that of lane 2= L Molecular ladder of 100 bp (base pairs), Lane 3 = b2, and the lane 4= b3 shown Figure 1.

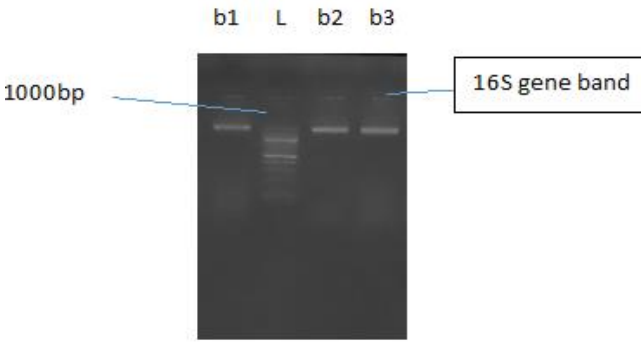

Figure 1: Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes b1, b2, 3 represent the 16SrRNA gene bands (1500bp), lane L represents the 1000bp molecular ladder of sample A and B

16s rRNA sequence of the isolate

The 16s rRNA sequence from the isolate produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate W1 had 100% similarity to other species. The evolutionary distances computed using the Jukes-Cantor method showed isolate W1(c1and b1) within the *Klebsiella* sp and revealed a closely relatedness to *Klebsiella variicola,* W2(b2and c2) and W3(b3and c3) were closely related to *Escherichia coli* and *Providencia staurti* than other *Escherichia* respectively (Fig. 2 and Fig. 3) (Winsley *et al.,*2012).

Note: All the organism recovered in the 4 **water samples** brands (n=16), labelled A-D were later labelled as b1, b2 b3 for A and B, while sample C-D were labelled as c1,c2,c3 respectively for PCR and molecular analysis.

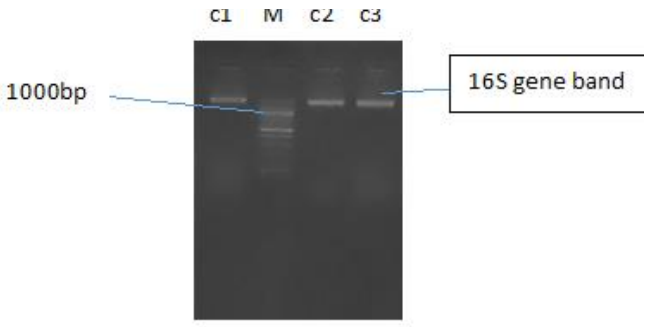

Fig2: Phylogenetic tree showing the evolutionary distance between the bacterial isolates w1(c1 and b1), w2(b2 and c2) and w3(b3 and b3) represent the 16SrRNA gene bands (1500bp), lane M represents the 1000bp molecular ladder of sample Cand D.

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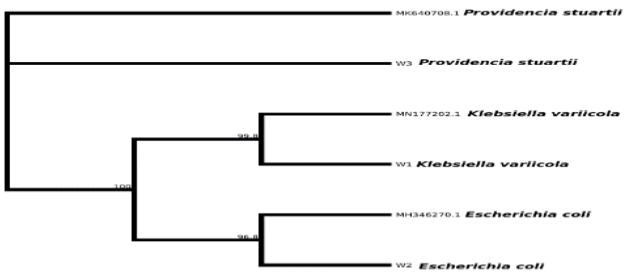

Figure3: Phylogenetic tree showing the evolutionary distance between the bacterial isolates w1(c1 and b1), w2(b2 and c2) and w3(b3 and b3)

Table 5: Antibiogram test of the isolates detected

Antibiotics	w1	w2	w3
CAZ	**R**	R	R
CRX	**R**	R	R
GEN	**R**	R	30mm
CXM	R	R	R
OFL	R	30mm	R
AUG	R	R	R
NIT	R	R	R
CPR	25mm	20mm	R

R – Resistant, **S** - Susceptible; **CAZ** – Ceftazidine, **CRX** – Cefuroxime; **GEN** – Gentamycin; **CXM** – Cefixime; **OFL** – Ofloxacin; **AUG**- Augmentine (Clavulanic acid &Amoxicillin); **NIT** – Nitrofurantoin; **CPR** – Ciprofloxacin; ***1***-*Klebsiella variicola;* ***2***-*Escherichia coli;* ***3***-*Providencia staurtii*

Table 6: Phenotypic resistance pattern of isolates

CAZ – *Ceftazidine,* ***CRX*** – *Cefuroxime;* ***GEN*** – *Gentamycin;* ***CXM*** – *Cefixime;* ***OFL*** – *Ofloxacin;* ***AUG-***Augmentine, **CPR** – Ciprofloxacin, NIT-Nitrofurantoin

ORGANISM	*NUMBER OF ANTIBIOTIC*	*RESISTANCE PHENOTYPIC PATTERN*
Klebsiella varricola	5	CAZ,CRX,CXM,AUG,NIT
E coli	*5*	CAZ,CRX,CXM,AUG,NIT
Providencii staurtii	*8*	CAZ,CRX,CXM,AUG,NIT,GEN OFL,CPR

Antibiogram test of the isolates

The analysis of the antibiogram in Table 5 and 6 respectively shows the susceptibility/resistant pattern and Phenotypic resistance of the microorganisms found in 4 brands of the water samples. The result indicates that the isolates were strongly resistant to the various antibiotic drugs used against them. *Klebsiella variicola* showed strong resistance to all the antibiotics used except for Ciprofloxacin, *E coli* showed susceptible features to Gentamycin, Ofloxacin and Ciprofloxacin; while, *Providencia staurtii* showed resistance to all antibiotics used against it.

Discussion:

According to the World Health Organization, diarrheal diseases constitute 4.1% of the world's total daily burden and affect 8 million people annually. It is estimated that 88% of this burden is caused by inadequate water, sanitation and hygiene (WHO, 2005). 50% (8/16) of the water samples analyzed in this study had a drinking water pH lower than WHO 6.5- 8.5. These sachet water has passed basic water tests and is tasteless, odorless, colorless and does not contain particles. This demonstrates that water purification standards that meet WHO standards are of good practice. In this study, the presence of organisms indicates that the water is contaminated with

pollutants, while their absence indicates the general safety of the water. Although, coliform organisms are not always directly related to contamination or the presence of contamination in drinking water, coliform testing is still useful in monitoring the microbial quality of drinking water (Magda *et al.,*2008; Shiaka, *et.al.,*2020; Onuguh*, et al.,*2025). The analysis also found that the presence of these organisms in water samples may be due to inefficient or ineffective treatment methods. Therefore, appropriate treatment methods must be used to obtain safe and clean packaged water (Oyedeji, *et al.,*2010; Shiaka*, et. al.,*2020). Coliforms are not pathogenic organism, rather they are organism found in the intestinal tract of warm-blooded animals; therefore it is considered an indicator of fecal contamination. Its presence is therefore indicative of contamination by human waste or animals. As reported by Shiaka, *et al*.,(2020) and Onuguh, *et al.,* (2025), the presence of *E. coli* in water is almost always associated with recent pollution and is indicative of organisms selected for this purpose. In this study, sachet water samples were analyzed for microbial quality and antibiotic resistance among isolated bacteria, with the aim of raising awareness about the public health risks of drinking this type of water. Although some of the samples B and C ed sachet / bottled water. Drinking water containing antibiotic-resistant organisms may prolong the treatment of waterborne diseases. showed an average value of more than 100 cfu / ml, it was still less than 100 cfu / ml. According to WHO,(2005) guidelines, no *Escherichia coli* or any coliform bacteria should be present in a 100 ml samples of water, This is contrary to what we found in this study as shown in the results of the molecular analysis (Fig,1 and 2), and it is indicative of possible sachet water contamination. Thus, most of the sachet water sampled were unsafe for human consumption, according to Shiaka *et al.,*(2020), other bacteria, such as *Klebsiella variicola* isolated from the water samples, may have entered the water during packaging or processing because the organisms are part of the normal flora of human skin or through sewage (Hunter, 1993). However, the presence of organisms in drinking water is of great importance for public health (Shiaka, *et al.,*2020). Antibiotic test results showed high antibiotic resistance in bacteria isolated from packaged water. The presence of antibiotic-resistant bacteria in drinking water is important because of the risk of developing more antibiotic-resistant organisms in humans. The prevalence of resistant organisms has created a serious problem for physicians. Nevertheless, this study provides guidelines for antibiotic-resistant bacteria that can manifest on individuals as result of drinking contaminat

The molecular results of this study showed the presence of *Klebsiella varicolla*, *Providenciia staurtii* and *Escherichia coli* in water samples.

Previous studies in other parts of the country have shown that similar bacteria characterize water quality (Shiaka, *et al.,*2020; Oludairo *et al*.,2015), but not *Providenciia staurtii*, with 4.5% of samples containing the total coliform and 2.3% had feces (Shiaka, *et al.,*2020). Another study on packaged drinking water in Ibadan, Nigeria, found that higher proportion of bagged water was statistically better than bottled water (Ajayi *et al.,*2008). Less than 7.0% of water pollution occurs after production, while 40 to 45% of the product were detected among markets and road transporters (Omalu *et al.,*2010). The results of this study revealed the presence of some microorganisms in sachet water samples which pose a public health concern. Access to good drinking water has been reported to be on the number six of the sustainable development goals (SDGs) according to Adewale, *et al*.,(2023), but until now, is still quite unfortunate that access to portable drinking water is still an issue, especially in this part of Okada, Benin city in Nigeria, and its also of global concern.

Conclusion:

It has been observed that many people are engaged in the production and sale of bottled water as a source of income. Therefore, health authorities need to ensure that producers comply with government regulations, as some of this sachet / bottled water may be produced without hygiene and therefore may not meet the legal standards or requirements of the World Health Organization. Therefore, the public health impacts of drinking contaminated water in this part of the country cannot be over emphasized based on the results found in this study. Additionally, lack of knowledge about clean sachet water storage for as long as a years from the time of production, and high ambient temperature may be the reasons for the high bacterial contamination of sachet or bottled water in Okada City. It is well known that sachet water should be treated before human consumption and microbiological tests should be performed regularly to prevent waterborne diseases from sachet water.

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Conflicts on interest

Authors declare no conflict of interest

Informed consent

Ethical approval number was issued to this study; IUO/Ethics/016/22

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