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Redefining Bacteria Resistant Pattern in Vended Sachets of Water from Mgbuoduahia, Port Harcourt, Nigeria

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Abstract

Bacterial resistance to water purification processes reflects the capacity of microorganisms to adapt to changing environments, necessitating a critical re-evaluation of bacterial resistance patterns in drinking water. Although sachet drinking water often contains detectable microbial loads, the potential role of inadequate disinfection procedures within quality control units is frequently overlooked. This study investigated ten samples from each of four popular brands of sachet drinking water (Teenerx, Danchiz, Wisdom, and Fountino table water) purchased within the study area in Nigeria. Isolation and enumeration of bacteria were performed using the spread plate technique, while morphological and biochemical characterizations were conducted to identify specific bacterial genera. The results revealed detectable concentrations of non-fastidious bacteria across the samples, with mean total viable counts of 1.0×10^2, 7.0×10^1, and 4.5×10^2 CFU/mL for Teenerx, Danchiz, and Fountino table water, respectively, showing statistically significant differences ($p < 0.05$). The isolated bacteria were identified as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and Proteus spp., all of which exhibited sensitivity to chlorine alongside distinct biofilm-forming features. While chlorine susceptibility indicates effective protein denaturation, the expression of biofilm structures serves as a cellular defense mechanism that enhances bacterial persistence in water systems. Although these levels may technically comply with specific baseline colony-forming thresholds, the presence of opportunistic pathogens poses potential public health concerns that warrant strict regulatory oversight. Therefore, this study recommends a comprehensive reassessment of bacterial resistance traits and optimization of disinfection protocols in sachet water production to ensure consumer safety.

Keywords: Sachet drinking water, bacterial contamination, antibiotic/chlorine resistance, biofilm, public health.

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Introduction

Access to safe drinking water remains a critical determinant of public health and socio-economic well-being globally. Sachet drinking water, secondary or derived water, is produced and packaged by means of technological processes from base sources, namely underground water and surface water supplies (Gross et al., 2022). Popularly and locally referred to as "pure water," sachet water serves as a primary, affordable, and accessible hydrating option for millions of individuals in developing regions. In all of these challenges, the public is constrained to resort to and depend on sachet drinking water to satisfy their daily hydration needs and control their thirst. Standard industrial production processes for sachet water are designed to ensure microbiological safety through sequential treatment stages, including coagulation, filtration, disinfection using chlorine, ultraviolet (UV) light, and the adjustment of hydrogen ion concentration (Rosemiarti et al., 2026). These multi-barrier treatment systems are engineered to eliminate pathogenic microbes and certify the potability of sachet water before it reaches the consumer market (Onyebuchi Okafor et al., 2024). When executed stringently, these processes significantly reduce biological hazards, instilling public confidence in commercially packaged drinking water.

However, the reality of sachet water distribution often diverges significantly from regulated standards. Sachet drinking water producers, either in good faith or through purposefully sharp and dishonest practices, frequently package raw or untreated water into sachets and sell them to unsuspecting consumers. Such malpractices, combined with quackery and inadequate quality control among local manufacturers, severely compromise the biological safety of the final product. Consequently, studies have continued to implicate sachet drinking water with bacterial contamination, highlighting a persistent failure in maintaining sanitary standards throughout the supply chain (Ali et al., 2026). Bacteria gain entry into sachet drinking water through multiple pathways, including contaminated raw water sources, faulty processing equipment, compromised packaging materials, and unhygienic handling during sealing and transportation (Rossi et al., 2024); . Bacteria are ubiquitous, microscopic organisms ranging from approximately 0.5 to 5 µm, possessing a remarkable capacity to adapt to changing and harsh environmental conditions. The presence of these microorganisms in packaged water cannot be overemphasized, as even relative hygienic handling fails to guarantee sterility when systemic operational flaws exist within manufacturing facilities.

Microbiological assessments of various sachet water brands across diverse residential neighborhoods have revealed varying levels of microbial loads (Asril et al., 2023; Izah & Ogwu, 2025). Perhaps the most commonly known bacterium in sanitary works and services is *Escherichia coli*, a common fecal coliform utilized globally as a definitive indicator of the bacteriological quality of water (Dimpor et al., 2025). In addition to *E. coli*, pathogenic and opportunistic bacteria isolated from vended sachet water include *Staphylococcus* sp. (Ahiabor & Donkor, 2025) and *Vibrio cholerae* (Adesakin et al., 2022). The isolation of such diverse microbial species underscores the severe biological risks posed to populations reliant on packaged water. Alarmingly, even after passing water through conventional treatment models, specific bacterial strains have been shown to survive chlorination and UV radiation (Gomes, 2024). Bacteria in a supposedly treated or chlorinated sachet water environment can exhibit physiological mechanisms that resist chlorination during the sachet water production process. This survival suggests that routine industrial disinfection may select for resistant bacterial phenotypes or facilitate the

formation of protective biofilms within processing pipes, rendering standard treatment regimens ineffective against persistent strains.

The incidence of bacterial resistance to water purification processes during water production calls for an urgent review (Koumassa et al., 2025). While information on the general bacteriological quality of vended sachet water is enormous, existing literature on the specific bacterial resistance patterns within sachet drinking water remains surprisingly scarce and underreported. This gap in scientific knowledge conceals the true extent of microbial adaptation in commercial water systems, leaving public health authorities without critical data required to formulate targeted regulatory interventions. Already, several widespread effects of relying on sachet water as a major drinking source have been studied and documented (Sharma et al., 2024). The potential consumption of resistant bacterial pathogens directly threatens public health by elevating the risk of waterborne disease outbreaks that are difficult to treat with standard antimicrobial measures. Sequel to the challenges noted above, there is a strong and pressing need to redefine bacterial resistance features and patterns in vended sachets of water within communities that heavily depend on these products (Umoafia et al., 2023). Therefore, this study aims to evaluate, characterize, and redefine the bacterial resistance profiles in vended sachet water to safeguard public health and restore biological integrity to consumer water supplies.

Methodology

Study area

The area covered in this research work is the Mgbuoduahia Community of Port Harcourt, Nigeria. The study area is a sub-hub of Obio/Akpor Local Government metropolis in Rivers State, Nigeria. The demographic status of Obio/Akpor makes it one of the largest cities in Nigeria at large. Over the years, they have not felt government presence, although features interesting economic activities. As several road construction projects have been awarded and commissioned. Basically, the sachet water sold is attributed to hot climatic weather condition of the area, hence, the need for drinking water. 2.2 Experimental Design/Collection and Preparation of Samples for Bacteriological Analysis. The study adopted Four (4) brands of sachet water namely: Teenerx table water, Danchiz table water, Wisdom table Water and Fountino table water, all of which were purchased from the study area and coded as AAA, DDD, FFF and LLL respectively. A total of forty quantities (samples) of sachet drinking in units of 50cl were purchased from different sale points (hawkers, open shops and kioks). The samples, thus were comprised of ten (10) quantities for each brand; as they were purchased, they were placed in an iced cooler and then transported to the Microbiology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni. Port Harcourt. Rivers State. In the Laboratory, the sealed nylon bag (sachet bag) surface containing the water was first sterilized by wiping with cotton wool soaked with ethanol before the bag was teared open with the aid of sterile blade to get the water content out, with a sterile 1ml graduated pipette aid.

Preparation of media

Nutrient agar was adopted for the study; hence the media was prepared based on manufacturer's instruction and used for the isolation and enumeration of non-fastidious bacteria. Following manufacturers instruction for the use, the media was sterilized in an autoclave at 121^{o}C for 15 minutes and after sterilization, the media were dispensed in a sterile petri dish.

Isolation and enumeration of non-fastidious bacteria

For the isolation and enumeration of the bacteria in the different sachet drinking water, the spread plate technique was adopted. The sachet drinking water samples were pipetted and plated

on the petri dishes containing the freshly prepared Nutrient agar medium and thereafter a sterile spreader (a bent glass rod) was used to evenly spread the inoculum onto the media. The media plates were then incubated at 37º C for 24 - 48 hours. Discrete colonies that developed on the plate were counted and recorded as colony forming unit per mill (CFU.ml). Following primary isolation, pure cultures of the isolates were obtained by repeated sub-culturing on fresh Nutrient agar using the streak plating technique as done. Following the sub culture for pure cultures, the isolates recovered from the sachet water samples were preserved in 10% glycerol in a sterile bijou bottle and stored in a refrigerator for further research.

Morphological characterization of the bacteria isolates gram reaction

In this stage of the analysis, the isolates were subjected to staining. Gram staining differentiates the isolates into Gram-positive and Gram-negative bacteria based on the differences in the nature of their cell wall. It involved heat fixing smear preparations of the test bacterium on a microscope slide and then staining with crystal violet for 60 seconds. The slide was then rinsed with water for 2 seconds. Gram's iodine was then applied for 60 seconds and rinsed with water again for 2 seconds. Thereafter, ethanol was applied for 10-30 seconds and rinsed with water again for 2 seconds. Finally, safranin was flooded on the slide for 30-60 seconds and rinsed with water for 2 seconds. The slide was then viewed under a light microscope. Gram positive bacteria showed purple blue colour while gram negative bacteria showed red/pink colour. Shapes and descriptions of the isolates were also considered and reported.

Motility test

The motility test was done to spot the presence of flagella in the isolates. The process as required preparation and dispense of Nutrient agar media into test tubes and with the aid of a sterile needle to inoculate the isolates on the medium by piercing. The tubes were then incubated for 24 hours at 37^0C. Positive results were noted by cloudy growth moving away from the line of stab while negative results were noted by growth only along the line of stab.

Biochemical characterization of bacterial isolates

The isolated non fastidious bacteria isolates were further classified biochemically using the classification schemes Hence, the isolates were subjected to Gram staining and several biochemical tests such as Methly Red, Voges Proskauer, Citrate utilization, Indole, Catalase, Glucose, Oxidase, Urease and Coagulase tests.

Catalase test

To spot the presence of catalase, an enzyme which catalyzes the breakdown of hydrogen peroxide to water and oxygen, a strile wire loop was used to pick the isolate onto a clean dry glass slide. thereafter a drop of 3% hydrogen peroxide was introduced onto the slide and the composition smeared. Catalase-positive isolates were observed to produce bubbles of oxygen while catalase-negative isolates did not produce oxygen.

Citrate utilization test

Citrate test was used to determine the ability of the isolate to convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle). Involved the preparation and dispence of a Simion's citrate agar media into test tube slants and with the aid of a sterile wire loop to inoculate into the agar tube. The tube was then incubated for 24 hrs at 37ºC. A change in the colour of the media from green to blue pinpointed a reaction while a reverse indicated no reaction.

Methyl red-vogues Proskauer (MRVP) test

This is a two-phase test, where the MR (methyl-red) fraction was used to determine if glucose can be transformed to acidic products like lactate, acetate and formate while the VP (Vogues Proskauer) fraction was used to determine if glucose can be transformed to acetoin. The sterilization of MR/VP broth that have already been dispensed into test tubes, then using a sterile wire loop to inoculate the isolates into the medium. The medium was then incubated for 48 hours at 37^{o}C. After incubation, half of the culture was transferred to another set of sterilized tubes and then used for the VP test, while the other half used for MR test. The MR test involved adding few drops of freshly prepared methyl red solution onto the MR tubes, the formation of a red colour indicated a positive result while a yellow colour indicated negative result. In a similar procedure, ten drops of freshly prepared Barrityt's reagent A was added to the (Voges Proskauer) test tube and the tube shaken for 30 seconds. A positive test was spotted by a gradual formation of a red colour, while the formation of a yellowish colour indicated a negative result.

Glucose test

Glucose test was conducted to detect the ability of the isolates to utilize the glucose sugars. Involved a compounded broth, that was dispensed into test tubes inserted with durham tubes. The media was then sterilized and after sterilization, a sterile wire loop was used to inoculate isolates into the broth cultures. Thereafter, the isolate was incubated for 24-48 hours at 37^{0}C. There after observation and results were recorded as: AG - acid and gas produced, A - acid produced and no gas, N – acid not produce and no gas.

Oxidase test

Oxidase test was used to classify which of the isolates would produce Cytochrome C Oxidase, an enzyme of the bacterial electron transport chain. A sterile wire loop was then used to pick the isolate and smear onto the filter paper. The filter paper was then observed for colour change from deep blue colour to purple within10-30 seconds signifying positive while no colour change signifies negative.

Coagulase test

Coagulase test was used to identify the bacterium, *Staphylococcus aureus* specifically. It differentiates *Staphylococcus aureus* which produce the enzyme, coagulase from other species of *Staphylococcus.* In this testing procedure, slide coagulase test method was adopted. placing a drop of normal saline at the end of a clean dry glass slide with the wire loop used to emulsify a portion of the isolate on the slide, followed by adding rabbit plasma and mixing gently. After 10 seconds, a positive result was indicated by clumping of the organism while a negative result shows no clumping.

Urease test

Urease test was done to identify certain genera and species of *Enterobacteriacea.* The procedure for the test according to Charlse et al. (2026) involved the inoculation of the urease broth medium with the isolate by streaking. This was followed by incubation for 35^{o}C for 18 to 24hrs. A positive result was indicated by a colour change of the medium whereas a negative result retains its original colour.

Indole test

Indole test was done to find out if the isolates could split tryptophan. The procedure, which was adopted by Charlse et al. (2026) required the inoculation of the isolates into tryptophan broth. Incubation was followed at 37^{o}C for 24-28 hours and after incubation, 0.5ml of Kovac's reagent was added to the tryptophan broth culture. Pink colored ring signified a positive result whereas the reverse indicated a negative result.

Determination of the resistant features of the bacterial isolates from the sachet drinking water biofilm formation features

At this phase of the study, the bacteria were screened for its biofilm capacity. The analysis adopted the conco red agar method, were the congo red agar media was prepared according to the directives. The congo red agar was freshly prepared and dispensed into petri dishes for the inoculation of the bacteria. Following the inoculation, the petri dish plates were incubated at a temperature of 37 degrees centigrade for 22-24 hours. At the end of incubation, the plates were observed for black colonial morphology which indicated biofilm formation and an absence indicated no biofilm property.

Screening for disinfectant effect

In these phases of screening for disinfectant effect on the bacteria for resistant and sensitivity character, the bacteria isolated were screened for their responses to chlorine, which is adopted in the sachet water industry for water treatment. The chlorines' sensitivity and resistant tests were carried out using the disc diffusion technique carried out by Clinical and Laboratory Standards Institute (CLSI) (2010). Hence, in carrying out the disinfectant effect of the chlorine on the bacteria, a freshly prepared Nutrient agar was dispensed into Petri dishes, allowed to solidify and thereafter, a sterile wire loop was used to pick/ introduce the bacteria onto the freshly prepared media plate. The plate was adequately smeared with a glass spreader and a chlorine treated disc was impregnated on the media. The plate was then incubated at 37^{o}C for 24 hours. Zones of inhibition of growth around discs indicated resistant while the clearings or no visible sign of the bacteria indicated sensitivity.

Results

Determination of non-fastidious bacteria population in the water samples

Table 4 showed the non-fastidious bacteria counts in the different sachet table water samples as coded as AAA, DDD, FFF and LLL. The counts as analyzed, varied with the different sachet water samples. The samples contained detectable numbers of non-fastidious bacteria. Non fastidious bacteria were found in all the brands of the table water except the sample coded FFF. The mean counts obtained showed 1 x 10^2, 7 x 10 and 4.5 x 10^2cfu/ml for Table water sample coded AAA, DDD and LLL respectively with statistical analysis indicating a significant ($p < 0.05$) variation.

Table 1. Mean counts of non-fastidious bacteria population in the water samples

Sachet drinking water samples	Non-fastidious bacteria count (cfu/ml$^{2)}$
AAA	1x 10^2
DDD	7 x 10
FFF	0
LLL	4.5 x 10^2
p-value	< 0.05

Key: AAA = Teenex table water, DDD = Danchiz table water, FFF=Wisdom table water, LLL=Fountino table water, cfu/ml= Colony forming unit per mil

Prevalence of non-fastidious bacteria loads in the sachet water samples

Table 2, showed the frequency of occurrence of non-fastidious bacteria. The result showed that the *Staphylococcus epidermidis* had 25% occurrence in the sachet water samples, while

Proteus spp. had the highest level of occurrence with a percentage of 64 and *Staphylococcus aureus* had the least with 11 percent prevalence.

Table 2. Distribution and frequency of occurrence of the bacterial isolates

Probable bacteria	AAA (n = 10)	DDD (n = 10)	FFF (n= 10)	LLL (n = 10)	Frequency of occurrence	Frequency of occurrence (%)
Staphylococcus epidermidis	3	3	0	1	7	25
Proteus spp.	8	6	0	4	18	64
Staphylococcus aureus	1	2	0	0	3	11

Key: AAA = Teenex table water, DDD = Danchiz table water, FFF=Wisdom table water, LLL=Fountino table water, n = number of sachet bags, spp = species

Morphological characterization of bacteria isolates

Table 3 showed the isolation and colonial characterization of the bacteria isolates recovered. Two (2) common bacteria genera were recovered from the samples. The bacteria *Staphylococcus epidermidis* appeared gray white with large size and round shape. Similarly, *Proteus* spp. was noted with a white color, irregularly shaped and its elevation, flat. *Staphylococcus aureus* appeared light yellow in colour with a large size and round shape. the elevation is round and also smooth in surface

Table 3. Colonial characterization of the bacteria isolated

Edge	Colour	Size	Shape	Elevation	Surface	Probable bacteria
Curved	Gray white	Large	Round	Convex	Rough	*Staphylococcus epidermidis.*
	White	Large	Irregular	Flat	Smooth	*Proteus* spp.
Curved	Yellow	Large	Round	Flat	Smooth	*Staphylococcus aureus*

Biochemical characterization of the bacteria isolated

Table 4 showed *Staphylococcus epidermidis* and *Staphylococcus aureus* had slightly similar biochemical results, except for the coagulase test which distinguished *Staphylococcus aureus* with a coagulase positive result. *Proteus* spp. were observed with a differed result with respect to positive for citrate, Vogues Proskauer and Methyl Red reactions but negative for oxidase, motility and urease.

Table 4. Biochemical characterization of the bacteria isolated

Cal	Oxi	Cit	Ind	Mot	MR	VP	Ure	Spore	Probable Bacteria
									Staphylococcus epidermidis. Staphylococcus aureus
-	-	+	-	-	+	+	-	+	*Bacillus* sp.

Keys: CAL-Catalase; OXI-Oxidase; CIT-Citrate; IND-Indole; MOT-Motility; MR-Methyl Red VP-Vogues Proskauer; URE-Urease; += Positive; - = Negative

3.6: Biofilm formation and Disinfectant Features of the Bacteria Isolated

Table 5 showed that the bacteria *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* sp. are all biofilm formers and also sensitive to chlorine treatment. Consequently, there is a likely possibility of defense with respect to the biofilm feature offered by the bacteria and an offensive attack from external factor towards the cell resistance.

Table 5. Biofilm formation and disinfectant features of the bacteria isolated

Biofilm formation	Chlorination effect	Bacteria
+	+	*Staphylococcus epidermidis.*
+	+	*Staphylococcus aureus*
+	+	*Bacillus* sp.

Discussion

The diversity in non-fastidious bacteria counts in the sachet water samples varied across the brands. Although, the brands have different addresses and location of production. Moreover, the varied counts in bacteria in different brands have also been reported in diverse study including study by (Mosi et al., 2019). Varied counts in bacteria may be due to different sampling locations, so also, they stated water production processes may be faulty as inadequate treatment sometimes observed given room to such abnormal contamination. The high incident of non-fastidious bacteria counts in a specific sachet water, over the other showed clearly, the hygienic deficiency. Consequently, the isolation and enumeration of non-fastidious bacteria in satchet water cannot be overemphasized as the sachet drinking water have always had bacteria contained in it as seen in studies by (Umoessien et al., 2019). The bacteria *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* sp. recovered and identified in this study are classified as disease causing agent according to (Kudakwashe et al., 2020). Furthermore, the said bacteria have been isolated from the palm of fast food handler (Tenebe et al., 2023).

Similarly, an overview of bacteria contaminants in drinking water excluded *Staphylococcus epidermidis* so isolated but identified *Staphylococcus aureus* and *Bacillus* sp. as a contaminated of drinking water (Dissasa et al., 2022). Chlorine is a non-antibiotic agent however has a bactericidal effect. Its effect on *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* sp. was evident. Basically, sensitivity of these recovered bacteria to chlorine has severally been reported (Botondi et al., 2021). Chlorine which act a disinfectant in the sachet water production process aimed at controlling bacteria It is also noted that some bacteria such as *Escherichia coli, Salmonella* and some other *Enterococcus* species not isolated in this study possess catalase enzyme that breaks down chlorine (Rodríguez-Melcón et al., 2024). Consequently, the recovered isolate; *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* sp. showered features of biofilm which may have resisted the effect of the chlorine (Popoola et al., 2024). According to (A. Singh et al., 2019) the biofilm formation expressed by the bacteria so isolated may have enable the bacteria remain in the sachet water, and hence develops resistant features.

The diversity in non-fastidious bacteria counts in the sachet water samples varied significantly across the different analyzed brands. Although the brands originate from different production addresses and geographical locations, the fluctuation in microbial load highlights widespread inconsistencies in quality control within the local packaging industry. Varied bacterial counts among distinct commercial drinking water brands have been similarly documented in previous literature, including the research conducted by (Nwaiwu et al., 2020). According to (Espina et al., 2021), such variations are primarily attributable to differences in environmental sampling locations as well as systemic faults in water production processes, where inadequate

treatment regimens create opportunities for abnormal microbial contamination. The high incidence of non-fastidious bacterial counts observed in specific sachet water brands compared to others clearly reflects severe hygienic deficiencies during handling and packaging. Consequently, the routine isolation and enumeration of non-fastidious bacteria in vended sachet water cannot be overemphasized, as commercial packaged water has persistently been shown to harbor bacterial populations across various empirical studies (Lee et al., 2017). The bacterial species recovered and identified in this study—namely *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus* sp.—are well-documented disease-causing agents (Saeed et al., 2023). Furthermore, these specific organisms are notorious opportunistic pathogens, having also been frequently isolated from the hands and palms of commercial food handlers in public sanitation assessments (El-Nemr et al., 2019).

While broader overviews of drinking water bacterial contaminants sometimes exclude *Staphylococcus epidermidis* from primary waterborne monitoring lists, they consistently identify *Staphylococcus aureus* and *Bacillus* sp. as dangerous contaminants in municipal and packaged water supplies (Fijan et al., 2024). The presence of *S. aureus* in drinking water is particularly alarming due to its capacity to produce heat-stable enterotoxins, which can lead to acute gastroenteritis upon ingestion. Conversely, *Bacillus* sp. represents a resilient genus capable of forming endospores, allowing it to endure severe physicochemical stresses during water treatment that easily eliminate vegetative bacterial cells. Chlorine functions as a non-antibiotic chemical agent widely utilized for its potent bactericidal effects in municipal and industrial water treatment facilities. Standard chlorination protocols aim to disrupt bacterial cell membrane integrity and oxidize essential cellular enzymes, thereby preventing microbial proliferation. The antimicrobial effect of chlorine on *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus* sp. has been thoroughly established in historical water treatment studies, where sensitivity to chlorine disinfection was routinely observed under controlled laboratory conditions (Mehdipour et al., 2023). As a primary disinfectant in sachet water manufacturing, chlorination is intentionally applied to eliminate pathogenic loads prior to final sealing. However, the efficacy of chlorine can be significantly hindered by specific bacterial defense mechanisms. It has been noted that certain bacterial pathogens—such as *Escherichia coli*, *Salmonella*, and various *Enterococcus* species—possess active catalase enzymes that functionally degrade chlorine and oxidative compounds (C. K. Singh et al., 2025). Although these specific catalase-producing species were not isolated in the current sample set, the surviving isolates exhibited alternative physiological strategies to withstand chemical exposure.

The bacterial isolates recovered in this study (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus* sp.) demonstrated distinct phenotypic features associated with biofilm production, which likely mediated their survival against chlorine exposure (Pereira et al., 2026). Biofilms consist of complex extracellular polymeric substances (EPS) that encapsulate bacterial communities, creating a physical and chemical barrier that limits the penetration of free chlorine molecules. According to (Hu et al., 2020), the expression of biofilm structures enables isolated bacteria to adhere to internal packaging equipment and pipe networks, allowing them to persist in sachet water and progressively develop resistance traits. The formation of biofilms within industrial processing pipes, storage tanks, and filling nozzles presents a critical engineering challenge in sachet water manufacturing. Once a biofilm establishes itself on stainless steel or plastic tubing, routine chemical flushes with chlorine fail to sterilize the lower cellular layers within the matrix (Tenebe et al., 2024). As treated water flows through these compromised delivery channels prior to sachet sealing, bacterial cells periodically detach from the biofilm matrix and recontaminate the final product, directly contributing to the variable microbial counts reported across different production batches (Volf et al., 2025). In addition to internal treatment failures, secondary contamination during the packaging phase substantially elevates bacterial loads in finished sachet water (Anwar et al., 2024). The frequent recovery of *Staphylococcus* species—

which naturally colonize human skin and nasal passages—points directly to unhygienic practices among factory personnel (Nishmitha et al., 2025). When production staff handle filling apparatuses or manual sealing machines without appropriate personal protective equipment, commensal microflora are easily transferred to the water or the inner lining of the plastic sachets, effectively bypassing prior filtration and chlorination stages.

The isolation of *Bacillus* sp. warrants specific concern due to the intrinsic survival mechanisms characteristic of spore-forming bacilli. Unlike non-spore-forming vegetative cells, bacterial spores possess a dense, multi-layered coat that resists chlorine oxidation, UV irradiation, and thermal fluctuations commonly encountered during storage and distribution (Baranzoni et al., 2017). When sachet water bags are stored under sub-optimal ambient conditions, surviving *Bacillus* endospores can germinate back into vegetative, metabolically active cells, thereby compounding the microbial hazard for end consumers over time. The presence of pathogenic bacteria that exhibit biofilm-mediated chlorine tolerance in widely consumed "pure water" poses a tangible threat to public health. Immunocompromised individuals, children, and the elderly are particularly susceptible to opportunistic infections arising from the ingestion of *S. aureus* and *Bacillus* toxins (Foote et al., 2017). The consumption of contaminated sachet water can manifest as recurrent gastrointestinal distress, food poisoning, and systemic infections, which are increasingly difficult to manage when the implicated bacterial strains demonstrate multi-drug or multi-chemical tolerance. Interestingly, classic indicator organisms such as *Escherichia coli* and fecal coliforms were not detected among the isolates in this specific evaluation.

While the absence of *E. coli* might initially suggest an acceptable level of primary source protection, the high counts of non-fastidious bacteria and opportunistic pathogens demonstrate that the absence of coliforms alone does not guarantee microbiological safety (Ya'aba et al., 2021). Monitoring frameworks must therefore expand beyond basic coliform testing to incorporate general heterotrophic and biofilm-forming bacterial counts to accurately assess water potability. Addressing the public health risks identified in this study requires an immediate overhaul of regulatory oversight and sanitation standards within the sachet water industry. Regulatory bodies must mandate strict biofilm monitoring protocols, routine replacement of processing conduits, and enforced hygienic training for factory workers. Furthermore, drinking water standards should integrate rigorous checks for chlorine-resistant bacterial phenotypes to ensure that commercial water treatment processes effectively neutralize both free-floating and biofilm-associated pathogens before products reach the public market.

Conclusion

Ensuring the microbiological safety of sachet drinking water relies heavily on effective disinfection procedures within production facilities; however, the persistence of bacterial contamination indicates potential gaps in quality control and highlights the adaptive capacity of microorganisms in water treatment systems. This study investigated sachet water samples across multiple brands, revealing detectable bacterial loads ranging from 7.0×10^1 to 4.5×10^2 CFU/mL with statistically significant variations ($p < 0.05$), and successfully recovered opportunistic pathogens including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus* sp. Although these isolates demonstrated sensitivity to chlorine disinfection, their capacity to express biofilm features serves as a vital cellular defense mechanism enabling bacterial regrowth and persistence, thereby pointing to inadequate treatment efficiency or suboptimal disinfectant application during production. Consequently, these resistant traits redefine contamination risks in packaged water systems, which poses potential public health concerns and necessitates stricter regulatory oversight and improved water purification standards despite microbial concentrations technically aligning with baseline thresholds. Nevertheless, this study

was constrained by a limited geographical scope and sample size across selected brands, focusing primarily on short-term enumeration without evaluating broader antimicrobial resistance profiles. To address these gaps, future research should expand the geographical sampling framework, incorporate molecular characterization techniques such as gene sequencing for biofilm and antibiotic resistance, and conduct longitudinal evaluations of disinfection efficacy to safeguard public health in sachet water consumption.

Conflicts of interest

There were no conflicts of interest

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Author contributions

A.I.C.N: Conceptualization, methodology, software, data curation, writing-original draft preparation. visualization, investigation, supervision, software, Validation, writing-reviewing, and editing.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used Gemini in order to improve English grammar, readability, and academic phrasing of the manuscript. After using this tool/service, the authors reviewed, edited, and verified the content as necessary and took full responsibility for the content of the published article.

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