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Bacteriological Quality, Phenotypic Virulence Characterization, and Antimicrobial Resistance Profiling of Bacterial Isolates from Packaged Drinking Water in Benin City, Nigeria

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ABSTRACT
Packaged drinking water is consumed by millions of Nigerians, yet the bacteriological quality remains inadequately characterized in many localities. This study investigated the bacteriological quality, phenotypic virulence, and antimicrobial resistance profiles of isolates from packaged water sold in Benin City. Total heterotrophic bacterial counts were determined by membrane filtration for 12 packaged water brands, and bacterial identification was by conventional methods and growth on differential media. DNase activity, gelatinase, hemolysin and lipase production were determined using standard assays. Molecular characterization of representative isolates was performed using 16S rRNA gene sequencing and BLAST analysis. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method, and the multiple antibiotic resistance index (MAR) was calculated. Total heterotrophic bacterial counts ranged from 0.00 to 33.50 CFU/100 mL, with three bottled water brands (OC, MX, UB) and one sachet water brand (LM) exceeding the WHO/NIS permissible limit of 10 CFU/100 mL. Seven bacterial species were identified: Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus pumilus, Proteus vulgaris, Enterobacter cloacae, and Micrococcus luteus. Bacillus pumilus was the most frequently occurring species (27.91%), followed by Staphylococcus aureus (20.93%) and Pseudomonas aeruginosa (13.95%). Molecular identification confirmed all seven species with ≥95% BLAST identity. The isolates produced at least one virulence factor; Escherichia coli and Pseudomonas aeruginosa exhibited the highest overall virulence potential. The MAR index of all isolates exceeded the 0.2 threshold, with Staphylococcus aureus recording the highest index of 0.53, indicating a high-risk environmental origin. The findings reveal the urgency of enhanced water-treatment protocols.

Keywords:
Bacteriological quality,
Antimicrobial
Resistance,
Membrane filtration,
Sachet water,
Virulence factors

INTRODUCTION

In many resource limited communities throughout sub-Saharan Africa, packaged water in sachets and bottles made from polyethylene has become the standard drinking water in cities and towns (Guzman and Stoler, 2018). In Nigeria, the bottled water industry has evolved into a multibillion-naira enterprise overseen by the National Agency for Food, Drug Administration, and Control (NAFDAC); however, there are no documented records that show any consistency in its safety. Benin City is a heavily populated city in Southern Nigeria that has varying qualities of packaged water and several competing brands whose bacteriological content remains unknown to the public.

The bacteriological contamination of packaged water is one route through which various waterborne illnesses such as cholera, typhoid fever, gastroenteritis, and dysentery can be transmitted (Isokpehi *et al*., 2026). Faecal indicator organisms such as *E. coli* and total coliform bacteria indicate faecal contamination and may also suggest the presence of enteric pathogens in water (Ellis *et al*., 2015). Besides the faecal indicators, opportunistic pathogens isolated from the drinking water, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus* species among others, can be hazardous, especially in people whose immune system is weakened, newborn babies, and older individuals (Curutiu *et al*., 2019).

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These pathogens become more pathogenic due to their ability to synthesize several extracellular virulence factors like hemolysins, proteases, DNases, and lipases.

Antibiotic resistance in environmental bacteria poses an ever-growing problem in terms of global health issues. Environmental water systems, as well as bottled water sources, have been found to be the main sources of antibiotic-resistant microorganisms and their corresponding genes (Mulamattathil *et al*., 2015). The MAR (Multiple Antibiotic Resistance) index measures the ratio between the number of antibiotics to which a specific microorganism is resistant. It can be considered the measure of the antibiotic exposure within the environment in which the specific microorganism was isolated. MAR values higher than 0.2 are considered to be the sign of the presence of high levels of contamination (Joseph *et al*., 2017; Ogofure *et al*., 2018; Afunwa *et al*., 2020; Ologbosere and Ogofure, 2020).

Although there have been reports of bacteriological dangers linked to packaged water, scientific research that involves the assessment of the bacteriological characteristics, virulence profile, antibiotic resistance patterns, multiple antibiotic resistance (MAR) index, and the molecular characterization of isolates obtained from packaged water in Benin City is sparse. The current study was thus conceived with the aim of conducting an extensive bacteriological investigation on twelve sachet and twelve bottled water products, the specific aims of which include: (i) enumeration and isolation of bacterial species through membrane filtration procedure; (ii) characterization of isolates through phenotyping, genotyping, and virulence analysis; and (iii) determination of antibiotic sensitivity and calculation of their MAR index (Ologbosere and Ogofure, 2020).

MATERIALS AND METHODS

Study Area and Sample Collection

The study was conducted in Benin City, Edo State, Nigeria (6°20′N, 5°37′E). Twelve sachets and twelve bottled water brands, all representing the same commercial labels, were purchased in triplicate from retail outlets across major commercial districts in the city. Samples were collected aseptically and transported in ice-packed coolers to the Microbiology Laboratory at the University of Benin for immediate analysis.

Membrane Filtration and Bacterial Enumeration

Total heterotrophic bacteria counts were determined by the method of membrane filtration, which involved the use of a filtration apparatus that operates under vacuum pressure. Specifically, 100 mL of each sample of water was filtered through a cellulose nitrate membrane filter with a pore diameter of 0.45 µm (Millipore®). After that, the membrane was carefully transferred to nutrient agar media, where it was incubated at 37°C for 24-48 hours. Colony counts were done and reported as colony-forming units per 100 mL of water (CFU/100 mL) as mean ± SD (Isokpehi *et al*., 2026).

Bacterial Isolation and Phenotypic Identification

Pure cultures were obtained by repeated sub-culturing of distinct colony morphotypes on Nutrient Agar. Phenotypic identification was performed using a combination of cultural and morphological characteristics, Gram staining, potassium hydroxide (KOH) string test, spore staining, and a panel of biochemical tests, including indole, oxidase, catalase, citrate utilization, urease, mannitol fermentation, and Triple Sugar Iron (TSI) reactions. Differential and selective media, such as Sorbitol MacConkey agar with Cefixime Tellurite supplement, Bile esculin agar, *Bacillus cereus* agar base (with polymyxin supplement), Mannitol Salt agar (MSA), *Pseudomonas* agar, *Salmonella-Shigella* agar (SSA), and Eosin Methylene Blue (EMB) agar, were employed to facilitate species-level differentiation. Results were interpreted using Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994; Ngozi *et al*., 2025).

Phenotypic Virulence Characterization

Four virulence-associated properties were evaluated for all isolates, which include hemolysin production (where isolates were streaked onto 5% sheep blood agar plates and incubated at 37°C for 24 hours; with zones of hemolysis being recorded [β-hemolysis (clear lysis) or α-hemolysis (partial greenish lysis] around colonies), DNase activity (where isolates were spot-inoculated onto DNase agar and incubated at 37°C for 24 hours before flooding with 1N hydrochloric acid for clear zones around colonies to show positive DNase activity), Gelatinase production (via stab-inoculating isolates into Nutrient Gelatin tubes and incubate at 37°C for 24 hours, then cooled at 4°C for 30 minutes to observe the presence of liquefaction of gelatin after cooling, indicative of gelatinase production), and Lipase production (via streaking on Spirit Blue agar and incubated at 37°C for 48 hours for a blue halo around colonies to indicate lipase activity).

Molecular Characterization

Representative isolates from each identified species were subjected to molecular characterization of the 16S rRNA gene. Genomic DNA was extracted, and the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR products were resolved on a 1.5% agarose gel, purified by ethanol precipitation, and sequenced using an Applied Biosystems 3130xl Genetic Analyzer. Sequences were subjected to a BLAST search against the NCBI GenBank

database, and those with≥95% identity were considered definitive species-level matches.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. Eight antibiotic discs were tested: Erythromycin (E, 15 mcg), Tetracycline (TE, 30 mcg), Gentamicin (GEN, 10 mcg), Amoxycillin + Clavulanic acid (AG, 20+10 mcg), Clindamycin (CD, 2 mcg), Ciprofloxacin (CIP, 5 mcg), Metronidazole (M, 5 mcg), and Colistin (CS, 10 mcg). Inhibition zone diameters were measured and interpreted as Sensitive (≥60%), Intermediate (40–59%), or Resistant (≤39%) based on the percentage of susceptible isolates across replicate isolates.

Multiple Antibiotic Resistance (MAR) Index

The MAR index for each bacterial species was calculated as the ratio of the number of antibiotics to which the organism was resistant to the total number of antibiotics tested. A MAR index ≤0.2 was considered indicative of low antibiotic exposure risk, while values >0.2 indicated origin from a high-risk environment characterized by frequent antibiotic use (Afunwa *et al*., 2020; Igbinosa *et al*., 2022).

Statistical Analysis

All statistical analyses and visualization of results were done using R (R Core Team, 2025) in a completely scripted analysis pipeline, with all results presented as mean ± standard deviation. The visualization approach integrates both the grammar of graphics approach and specific microbial science encoding rules, whereby heterotrophs were graphed using grouped bar charts with confidence bands and regulatory cut-off levels. Heatmaps were used to visualize virulence determinant profile patterns, whereas stacked SIR charts were created for antibiogram profiling of the isolates in addition to multiple antibiotics resistance (MAR) indices. Therefore, the entire visualization process adheres to and expands on patterns described in previous scientific papers utilizing R for visualization of microbe- and antibiogram-related data sets (Ogofure *et al*., 2024; Ogofure and Green, 2025; Ogofure *et al*., 2025).

RESULTS AND DISCUSSION

Total Heterotrophic Bacterial Counts

Table 1 shows the total number of heterotrophic bacteria in both twelve-sachet water and twelve bottled water brands. In the case of sachet water brands, the number of bacteria varied between 0.00 ± 0.00 and 12.00 ± 1.41 CFU/100 mL. Sachet water brand LM had the highest bacterial number (12.00 ± 1.41 CFU/100 mL) beyond the WHO/NIS permissible limit (10 CFU/100 mL). Other sachet water brands were within acceptable limits, but sachet water OV had the least bacterial number (1.50 ± 0.71 CFU/100 mL).

Bottled water brands had bacteria numbers ranging from 0.00 ± 0.00 CFU/100 mL (OV bottled water, no bacterial growth) to 33.50 ± 9.19 CFU/100 mL (OC bottled water). Bottled water brands OC, UB, and MX had bacterial counts (33.50 ± 9.19 CFU/100 mL, 22.00 ± 8.49 CFU/100 mL, and 18.00 ± 5.66 CFU/100 mL respectively) beyond the permissible limit. OV bottled water was the only brand with zero bacterial growth, thus the safest among the evaluated brands based on bacteriological quality.

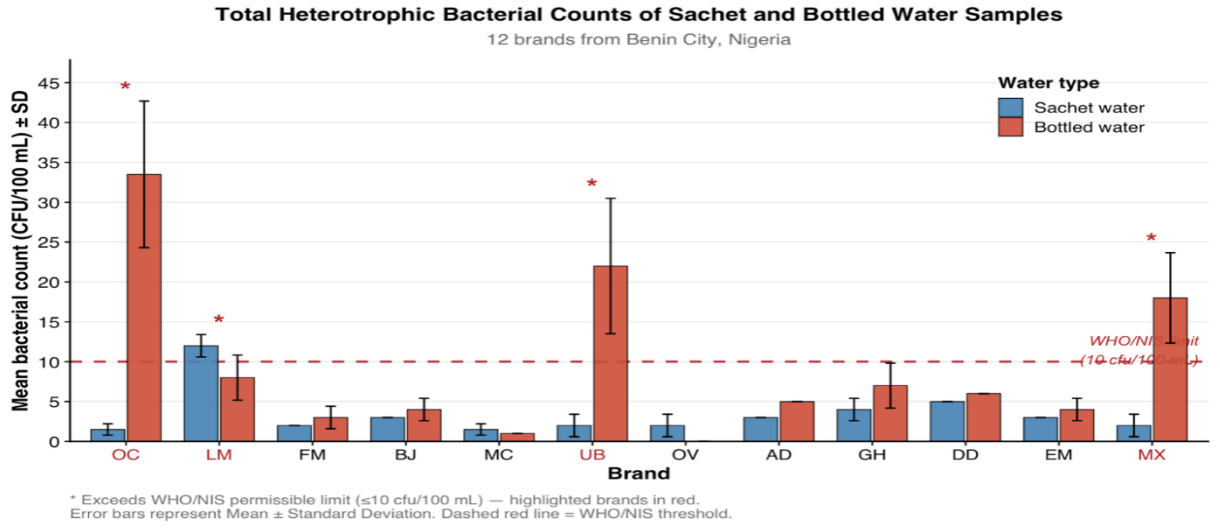

Figure 1. Total Heterotrophic Bacterial Counts (CFU/100 mL) of Sachet and Bottled Water Samples from Benin City

** Exceeds the WHO/NIS permissible limit of ≤10 cfu/100 mL. Values represent Mean ± Standard Deviation.*

Biochemical Identification of Bacterial Isolates

A total of seven bacterial species were recognized in the sachet and bottled water samples through culture, morphology, and biochemical testing (Table 1). These include *Proteus vulgaris, Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia coli, Bacillus pumilus, Staphylococcus aureus* and *Micrococcus luteus*. The Gram-negative rods are *Proteus vulgaris* (flagellated and positive for urease production and H_2S production); *Enterobacter cloacae* (flagellated and positive for citrate utilization and gas production); *Pseudomonas aeruginosa* (oxidase-positive and produces a fluorescent pigment in Pseudomonas agar); and *Escherichia coli* (positive for indole production and acid-gas production from glucose fermentation in EMB agar with a metallic-green sheen). The Gram-positive bacteria include *Bacillus pumilus* (spores-producing, mannitol fermenter); *Staphylococcus aureus* (Gram-positive cocci in clusters, positive for urease production and yellow colonies in MSA); and *Micrococcus luteus* (oxidase-positive and non-flagellated Gram-positive cocci).

Table 1: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates from Sachet and Bottled Water Samples in Benin City

Biochemical Test	*Proteus vulgaris*	*Enterobacter cloacae*	*Pseudomonas aeruginos*	*E. coli*	*Bacillus pumilus*	*S. aureus*	*Micrococcus luteus*
Gram staining	**GN**	**GN**	**GN**	**GN**	**GP**	**GP**	**GP**
KOH test	+	+	+	+	-	-	-
Spore staining	-	-	-	-	+	-	-
Indole	+	-	-	+	-	-	-
Catalase	+	+	+	+	+	+	+
Citrate	+	+	-	-	+	+	+
Oxidase	-	-	+	-	-	-	+
Motility	+	+	+	+	+	-	-
Urease	+	-	+	-	-	+	+
Mannitol	-	-	-	-	+	-	-
TSI (Slant/Butt)	**K/A H_2S**	**K/AG**	**K/K**	**A/AG**	**A/A**	**A/A**	**K/K**

Key: (+) = positive; (−) = negative; GN = Gram-negative; GP = Gram-positive; K = alkaline; A = acid; G = gas; H_2S = hydrogen sulphide. Species are abbreviated for clarity.

Frequency of Occurrence of Bacterial Isolates

In total, 43 bacteria were obtained from the sampled water sources. The predominant bacteria isolated was *Bacillus* (27.91%; n = 12), followed by *Staphylococcus aureus* (20.93%; n = 9) and *Pseudomonas aeruginosa* (13.95%; n = 6). The occurrence of *Proteus vulgaris* and *Micrococcus luteus* in the sampled water was 11.63% (n = 5), while that of *E. coli* was 9.30% (n = 4). The rarest of the isolates was *Enterobacter cloacae* (4.65%; n = 2), although its presence in drinking water poses serious health risks due to its categorization under the Enterobacteriaceae family.

Phenotypic Virulence Properties

The phenotypic virulence profiling of all identified bacterial species is presented in Figure 2. All seven species produced at least one virulence factor, demonstrating the potential pathogenic significance of their occurrence in packaged drinking water.

The maximum amount of hemolysin was observed in *S. aureus* (100.00%) and *Bacillus* (75.00%), signifying that these bacteria have the potential to break down red blood cells and induce damage in vivo. Hemolysin was produced in *Micrococcus luteus* (60.00%), *Proteus vulgaris* (80.00%), and *Enterobacter cloacae* (50.00%). DNase activity was maximal in *Escherichia coli* (100.00%), *S. aureus* (100.00%) and *Pseudomonas aeruginosa* (83.33%). DNases help in the degradation of host DNA to evade the immune response and aid in biofilm dispersion. High amounts of gelatinase, enzymes that help in degrading host protein of connective tissue, were found in *Pseudomonas aeruginosa* and *Staphylococcus aureus* (more than 60.00%), while in *E. coli* and *Proteus* sp., the percentages were 50% and 40%, respectively. Interestingly, gelatinase and lipase activity were absent in *Enterobacter cloacae*, while *Escherichia*

coli was positive for 100.00% lipase activity. Of particular concern is the high virulence score recorded for *Escherichia coli* (62.50%) and *S. aureus* (91.27%) across all four categories are particularly concerning, given the WHO's zero-tolerance stance on bacterial in drinking water.

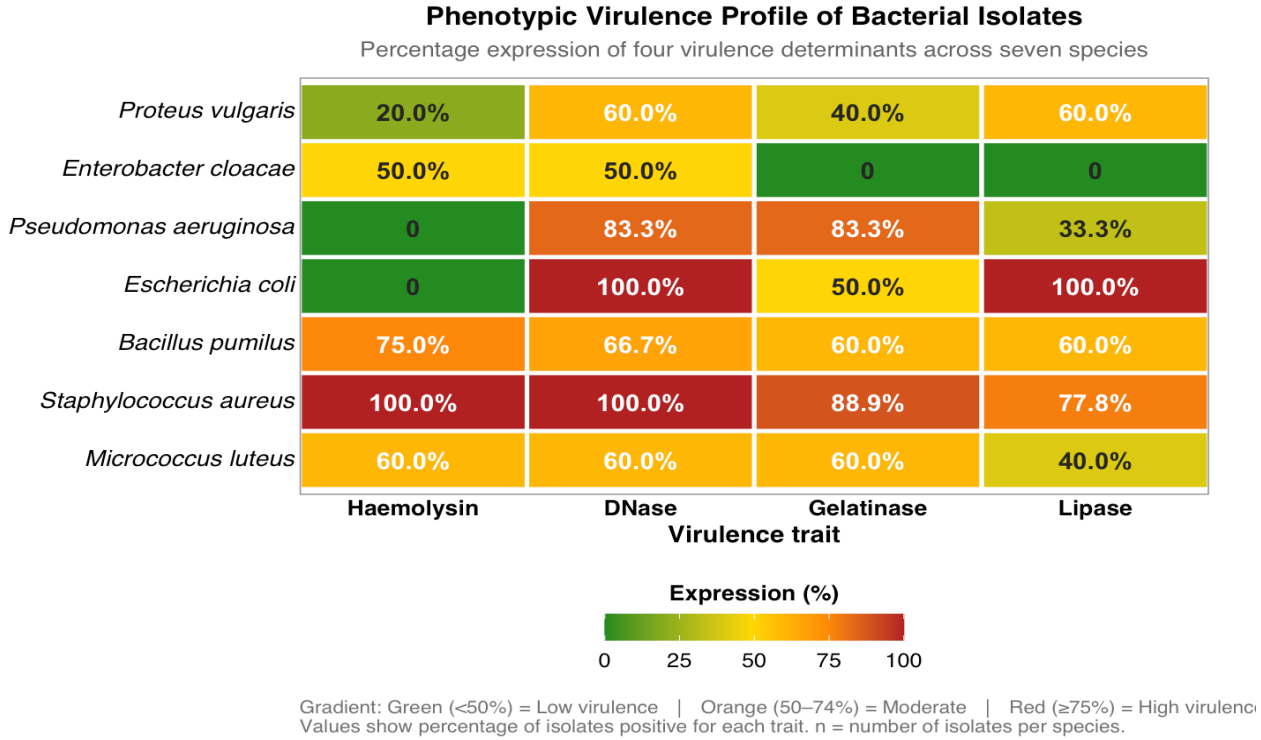

Figure 2: Phenotypic Virulence Properties (%) of Bacterial Isolates from Sachet and Bottled Water Samples.
Colour key: Red = high virulence (≥75%); Orange = moderate (50–74%); Green = low (<50%).

Molecular Identification by 16S rRNA Gene Sequencing

Identification of the representative strains using molecular techniques such as 16S rRNA gene sequencing and BLASTn search proved to be accurate for all seven bacterial strains (Table 2). *Escherichia coli* (strain from LM) and *Micrococcus luteus* (strain from UB) had 100% query coverage to the GenBank records, whereas the rest had 99% query coverage. The percent identities varied from 95.00% to 99.87%, with all seven bacterial strains falling within the 95% cutoff percentage that allows species-level classification. The importance of 16S rRNA sequencing in accurately identifying species of bacteria, especially *Bacillus* genus, is highlighted in this study where the *Bacillus subtilis* isolate was actually identified as *Bacillus pumilus* by molecular analysis, underscoring the value of 16S rRNA sequencing in resolving closely related *Bacillus* taxa.

Table 2: Molecular Identification (16S rRNA Gene Sequencing) of Bacterial Isolates from Packaged Drinking Water in Benin City

Code	Bacterial Identity	Query Cover (%)	Percent Identity (%)	GenBank Accession
LM	*Escherichia coli*	100.00	99.70	CP000247.1
MX	*Proteus mirabilis*	99.00	99.79	KF051776.1
UB	*Micrococcus luteus*	100.00	95.00	HQ220044.1
GH	*Pseudomonas aeruginosa*	99.00	99.41	MN490072.1
BJ	*Staphylococcus aureus*	99.00	99.86	EF463060.1
DD	*Bacillus pumilus*	99.00	99.71	EU660365.1
FM	*Enterobacter cloacae*	99.00	99.87	NR_118011.1

Antibiotic Susceptibility Patterns and MAR Index

Antibiotic susceptibility pattern of bacteria with their MAR index values is illustrated in Figure 3. All seven types of bacteria showed resistance to at least two out of eight different antibiotics, and MAR index values were greater than the critical value of 0.2. The susceptibility of *Proteus vulgaris* to erythromycin, tetracycline, gentamicin, and ciprofloxacin was found to be 100%, but its resistance to metronidazole and colistin was 100%. Its intermediate susceptibility to clindamycin was 40%. *Enterobacter cloacae* was 100% resistant to gentamicin, amoxycillin-clavulanic acid, and ciprofloxacin, and showed intermediate resistance to erythromycin, tetracycline, clindamycin, and metronidazole The MAR index for both the organisms was 0.38. *Pseudomonas aeruginosa* showed sensitivity to erythromycin and gentamicin while showing resistance to amoxycillin-clavulanic acid, metronidazole and colistin, leading to a MAR index of 0.46. *Escherichia coli* was found sensitive to erythromycin, tetracycline, gentamicin and clindamycin whereas it was found resistant to amoxycillin-clavulanic acid, metronidazole and colistin, resulting in a MAR index of 0.44. The resistance pattern shown by these organisms, which are of great significance clinically and epidemiologically, is very troubling in light of the contamination of drinking water sources. *Bacillus pumilus*, which was found as the most common organism, showed the lowest MAR index at 0.25 due to its sensitivity to erythromycin, amoxycillin-clavulanic acid, clindamycin and ciprofloxacin. *Staphylococcus aureus* was found to be sensitive only to gentamicin and ciprofloxacin while being resistant to clindamycin, metronidazole and colistin, resulting in the highest MAR index of 0.53. *Micrococcus luteus* showed susceptibility to erythromycin, gentamicin, clindamycin, and ciprofloxacin but was resistant to metronidazole and colistin, yielding a MAR index of 0.48.

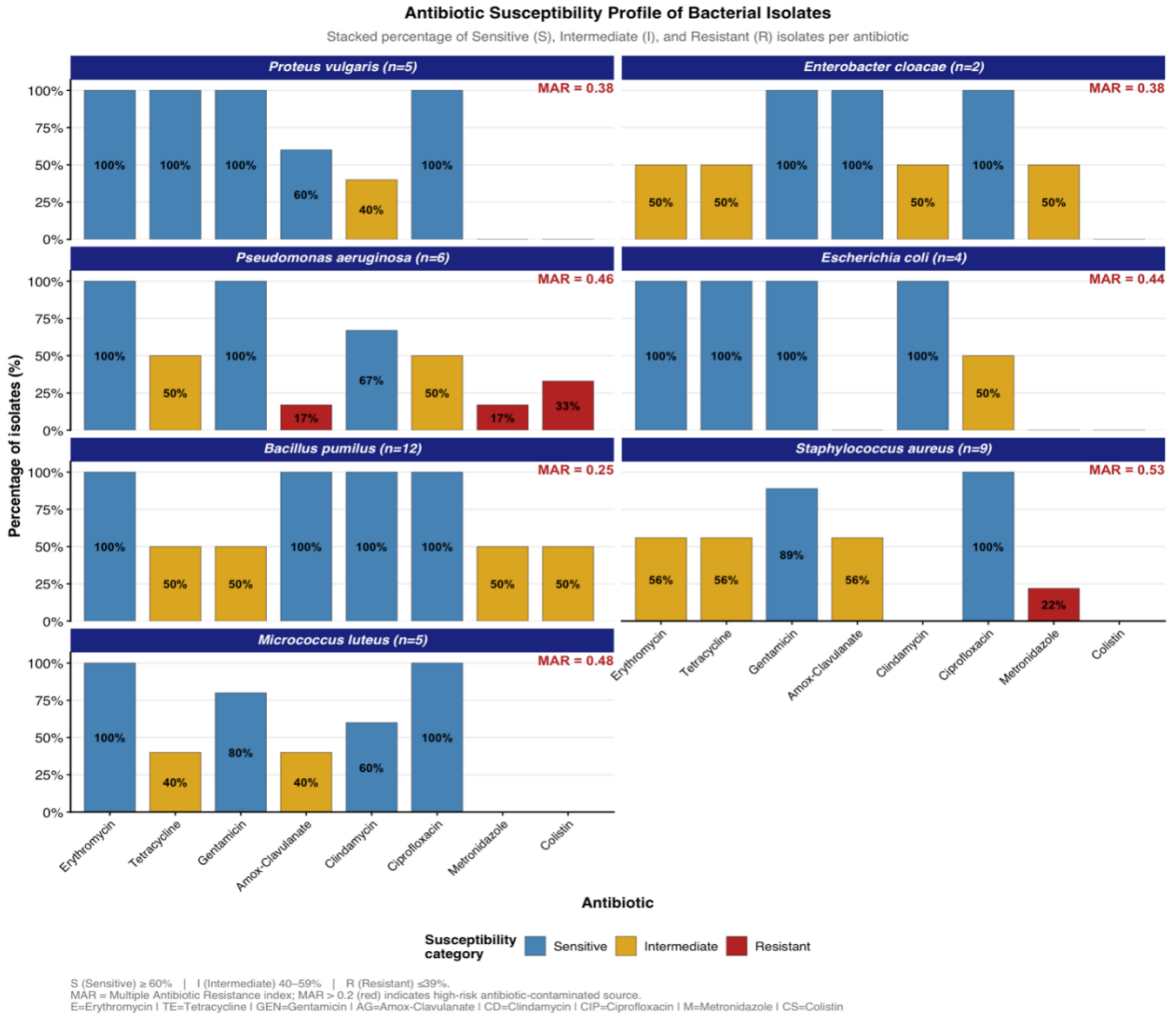

Figure 3: Percentage Antibiotic Susceptibility (%) and Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

S = Sensitive (≥60%); I = Intermediate (40–59%); R = Resistant (≤39%). MAR index >0.2 indicates high-risk antibiotic-contaminated source (shown in red). E = Erythromycin; TE = Tetracycline; GEN = Gentamicin; AG = Amoxycillin+Clavulanic acid; CD = Clindamycin; CIP = Ciprofloxacin; M = Metronidazole; CS = Colistin.

The findings from the bacteriological quality assessment conducted in this study reveal an apparently contradictory and concerning situation with respect to the bacteriological safety of packaged drinking water in Benin City. Although the vast majority of the sachet and bottled water samples analyzed in this study recorded a total heterotrophic bacterial count below the maximum allowable concentration of ≤10 cfu/100 mL as per the guidelines of the WHO/Nigeria Industrial Standards, there were three brands of bottled water and one of sachet water whose findings exceeded this level.

It is paradoxical that there were higher counts in bottled water brands compared to sachet water brands in this research due to the common perception that bottled water goes through more elaborate processes. This has also been observed by Okoye *et al*. (2022) in Nnewi, Anambra State and was caused by contamination after treatment during bottling, inadequate sealing of bottles, and also during distribution and storage. The OV brand of bottled water that had no growth is the perfect example of bacteriological purity and shows that this is possible in Nigeria.

The seven types of bacteria identified in this study include *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus pumilus, Proteus vulgaris, Enterobacter cloacae* and *Micrococcus luteus.* These seven bacterial types include a range of gram positive and negative organisms of different levels of public health importance. Of special concern is the presence of *E. coli* in any sample of drinking water. According to the guidelines put forth by WHO, there must be zero tolerance of *E. coli* in potable water since this bacterium acts as an indicator of faecal contamination of water samples. The isolation of *E. coli* in LM sachet water sample suggests that there could be insufficient treatment or even faecal contamination of treated water. Also of note is the isolation of *P. aeruginosa* from GH bottled water in the study. An environmental opportunist known to thrive and grow even after the water has been treated, *P. aeruginosa* was found at a high level of occurrence (13.95%), consistent with reports by Agbendeh and Okonkwo (2021) and Curutiu *et al*. (2019), who identified *P. aeruginosa* as a common contaminant in packaged water environments.

The virulence profiles from this research are particularly relevant. The fact that there is high prevalence of haemolysin, DNase, gelatinase, and lipase production among these bacteria, especially *E. coli* (100% in each case), and *P. aeruginosa* (100% haemolysin and gelatinase), implies that not only are they present in the water samples, but can also cause complete virulence reactions once consumed by an appropriate host. Hemolytic toxins help to breakdown erythrocytes and obtain iron as a nutrient. Gelatinases and lipases enable them to penetrate through tissues and get nutrients in the process. DNases allow them to break down host DNA, thereby evading their immune response of releasing extracellular traps. Thus, these virulent capabilities of the bacterial isolates show the possibility of serious infections caused by these organisms when even slightly contaminated packaged water is consumed.

These results regarding the antibiotic resistance patterns obtained in the present study can be considered consistent with, as well as a continuation of, the work of researchers in other Nigerian studies (Igbinosa *et al*., 2016). In all the bacterial species examined, MAR indices greater than the critical 0.2 value were noted, with *S. aureus* having the highest value among them at 0.53, considering that this bacterium is known to be resistant to a wide array of antibiotics in both clinical and environmental samples in Nigeria (Igbinosa *et al*., 2016). As mentioned in a study conducted by Afunwa *et al*. (2020), a MAR index equal to or greater than 0.2 shows that the bacteria had previously lived in an area where extensive use of antibiotics had been done, implying that the sources of water used in these factories are heavily contaminated by antibiotic residues. A study conducted by Joseph *et al*. (2017) also documented high MAR indices among *E. coli* strains isolated from a tertiary hospital in southwestern Nigeria, which highlights the epidemiological link between clinical and environmental resistance sources.

Resistance to metronidazole and colistin among various isolates is of considerable importance from a clinical perspective. In the case of resistance to colistin, for example, resistance can be considered as a "last resort" form of drug resistance since colistin is used as a treatment method in the case of MDR Gram-negative bacteria such as carbapenem-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Resistance to colistin found among various isolates in packaged drinking water that is used by the general population marks an important milestone in the one-health AMR narrative.

The molecular identification of all seven organisms using the sequence of the 16S rRNA gene and BLAST further confirms these results. It is shown how the differentiation of *Bacillus pumilus* from *Bacillus subtilis*, which appear to be closely related based on morphology and physiology, proves the need for molecular methods in environmental microbiology. The percentage identities were all above 95%, which is the criterion set forth by Chakravorty *et al*. (2007).

CONCLUSION

This research has provided knowledge on the bacteriological properties, phenotypic virulence characteristics, and multidrug resistance profiles of bacteria isolated from packaged drinking water in Benin City, Nigeria. Seven bacterial species were isolated and characterized from the packaged drinking water samples using membrane filtration and bacteriological enumeration techniques, which include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus pumilus*, *Proteus vulgaris*, *Enterobacter cloacae*, and *Micrococcus luteus*. While most of the water brands meet the World Health Organization and National Institute of Standards bacteriological criteria, four water brands exceed the acceptable limit, suggesting that there may be issues with water treatment, packaging, or post-processing hygiene. Bacterial identification was achieved through phenotypic and biochemical analyses coupled with the 16S rRNA gene sequencing technique. Furthermore, phenotypic virulence tests showed that the selected isolates produce important virulence factors, such as hemolysin, DNase, gelatinase, and lipase. Based on the findings, the isolated bacteria appear to be virulence positive with the ability to produce important virulence factors, signifying the possibility of pathogens residing in packaged drinking water samples. In addition, the multidrug resistance analysis revealed that all the tested bacteria had MAR indices above 0.2, suggesting the existence of high-risk conditions related to continuous antibiotic use or misuse.

CONFLICT OF INTEREST

The authors declare that no known conflict of interest that could have influenced this research exists in this paper

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