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Antibiotic Susceptibility Patterns and Detection of Enolase and Antibiotic-Resistance Genes in Bacterial Isolates from Packaged Table Water in Port Harcourt, Nigeria

Ekeke, I., J., Wemedo, S., A., Sampson, T., Nrior, R., R.
Department of Microbiology, Rivers State University,
Nkpolu Oroworukwo, Port Harcourt, Nigeria.
***Corresponding Author:** *joy.ekeke@rsu.edu.ng*

ABSTRACT

Packaged table water is an important source of drinking water, but bacterial contamination and antimicrobial resistance may compromise its microbiological safety. This descriptive cross-sectional study evaluated the antibiotic susceptibility patterns and the occurrence of genetic determinants for enolase (*eno*) and antibiotic resistance in bacterial isolates recovered from commercially packaged table water sold in Port Harcourt, Nigeria. The study was conducted over a 12-month period. Bacteria were isolated using standard bacteriological techniques and presumptively identified based on their cellular and biochemical and molecular characteristics. Antibiotic susceptibility was determined using the Kirby–Bauer disc-diffusion method, while polymerase chain reaction (PCR) was performed on ten selected isolates to detect the *eno* (enolase) gene, as well as the *bla*TEM and *aac(6')-Ib* antibiotic-resistance genes. A total of ten bacterial groups were identified from the packaged table-water samples. The identified bacteria included *Staphylococcus* spp., *Lactobacillus* spp., *Bacillus* spp., *Proteus* spp., *Micrococcus* spp., *Salmonella* spp., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Shigella* spp. Antibiotic susceptibility varied among the bacterial groups and tested antibiotics. Among Gram-positive isolates, the highest reported susceptibility varied by genus, with relatively higher values recorded for selected fluoroquinolones (pefloxacin, 40.3% in *Staphylococcus* spp.; ciprofloxacin, 52.2% in *Lactobacillus* spp.) and gentamicin (47.4% in *Bacillus* spp.). Among Gram-negative isolates, *Salmonella* spp. showed the highest reported susceptibility to amoxicillin (85.7%), whereas *Proteus* spp. generally exhibited low susceptibility across tested agents, with maximum susceptibility not exceeding 30.8% (for amoxicillin) and complete resistance (0.0% susceptibility) to amoxicillin–clavulanic acid, ofloxacin, and cefixime. Molecular analysis detected *eno* (enolase gene) in 7 of 10 (70.0%) isolates, *bla*TEM (beta-lactam resistance marker) in 4 of 10 (40.0%), and *aac(6')-Ib* (aminoglycoside resistance marker) in 5 of 10 (50.0%). Three of these isolates (30.0%) carried all three genetic targets, while one isolate (10.0%) was negative for all three markers. The findings demonstrated varied antibiotic susceptibility profiles and the presence of genetic determinants for enolase and antibiotic resistance in bacterial isolates from packaged table water. These results highlight the importance of routine microbiological surveillance and strengthened quality-control measures in packaged-water production.

Keywords: Packaged Drinking Water, Antibiotics, Bacteria, Antimicrobial Resistance Genes, Virulence-Associated Genes.

Introduction

Access to safe drinking water is essential for public health. However, the microbiological quality of commercially packaged water may be compromised during treatment, packaging, transportation, storage or handling. Inadequate hygiene and poor production practices may introduce bacteria into packaged water, raising concerns about consumer safety (World Health Organization [WHO], 2022; Cabral, 2010).

Although the detection of bacteria does not necessarily indicate the presence of pathogenic organisms, identifying recovered bacteria is important for evaluating microbiological quality and potential public-health concerns [American Public Health Association (APHA) 2023]. Antibiotic resistance among environmental bacteria is an additional concern because water systems may provide environments in which resistant organisms persist and interact with bacteria from different sources.

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Resistance can arise through chromosomal mutations or the acquisition of genetic determinants encoding mechanisms such as antibiotic inactivation, target site modification, reduced permeability and active efflux. Some resistance genes are associated with mobile genetic elements, including plasmids, transposons and integrons, which may facilitate their dissemination among bacterial populations (Davies & Davies, 2010; Partridge et al., 2018; Carattoli, 2009). Antibiotic susceptibility testing provides phenotypic information about bacterial responses to antimicrobial agents, whereas molecular techniques such as polymerase chain reaction (PCR) can identify selected genetic determinants associated with resistance. Combining these approaches provides complementary information for characterising antibiotic susceptibility patterns and investigating the occurrence of specific resistance-associated genes (Clinical and Laboratory Standards Institute [CLSI], 2024). Virulence-associated genes are also relevant to the characterisation of bacteria recovered from drinking water. These genes may encode factors involved in bacterial adhesion, colonisation, host interaction and persistence. Enolase, encoded by the *eno* gene, is a metabolic enzyme that can also function as a surface-associated protein and participate in interactions with host proteins, including laminin and plasminogen, in certain bacterial species. Such functions may contribute to bacterial adhesion and colonisation. However, the detection of a virulence-associated gene does not independently establish gene expression or pathogenicity. Studies of packaged drinking water in Nigeria have reported variations in bacteriological quality and the recovery of potentially undesirable microorganisms, demonstrating the need for continued microbiological surveillance (Igbeneghu & Lamikanra, 2014; Odeyemi, 2015). However, conventional bacteriological assessment alone does not establish the antibiotic susceptibility profiles or genetic characteristics of recovered organisms. Integrating bacterial isolation, antibiotic susceptibility testing and molecular detection of selected virulence-associated and antibiotic-resistance genes can provide a broader understanding of the microbiological characteristics of commercially packaged water. Although previous studies have examined the bacteriological quality of packaged drinking water in Nigeria, information integrating antibiotic susceptibility patterns with molecular detection of selected genetic determinants in recovered bacterial isolates remains limited.

This study investigated antibiotic-resistance patterns and the occurrence of selected virulence-associated and antibiotic-resistance genes in bacterial isolates recovered from commercially packaged table water sold in Port Harcourt, Rivers State, Nigeria.

Materials and Methods

Study area

The study was conducted in Port Harcourt, Rivers State, Nigeria, a major urban centre in the Niger Delta region of Nigeria. The city is characterised by substantial commercial activity, high population density and dependence on alternative drinking-water sources, including commercially packaged table water.

Samples were obtained from four locations: Mile 1, Iwofe, Aba Road and Mile 3, located at approximately 4°49′27″N latitude and 7°02′01″E longitude. It is one of Nigeria's largest metropolitan centers. The map of the study area showing the sampling locations is presented in Figure 1.

Study design and Sample collection

A descriptive cross-sectional study was conducted over a 12-month period (January to December 2025), involving 480 packaged table-water samples representing ten different brands, collected from retail outlets across four selected locations within the study area. Ten brands were sampled from each of the four locations monthly throughout the 12-month sampling period, giving 40 samples per month and 480 samples in total. Samples were purchased from supermarkets, street vendors, wholesale distributors and retail shops to capture variations in brand availability and microbiological quality. Only bottles with intact seals and identifiable production and expiry dates were included.

Information recorded for each sample included brand name, batch number, production date, expiry date, manufacturer's location and National Agency for Food and Drug Administration and Control (NAFDAC) registration number, where available. Each bottle was appropriately labelled and transported to the laboratory under aseptic conditions. Samples were processed within 24 hours of collection to minimise changes in the bacterial population before analysis.

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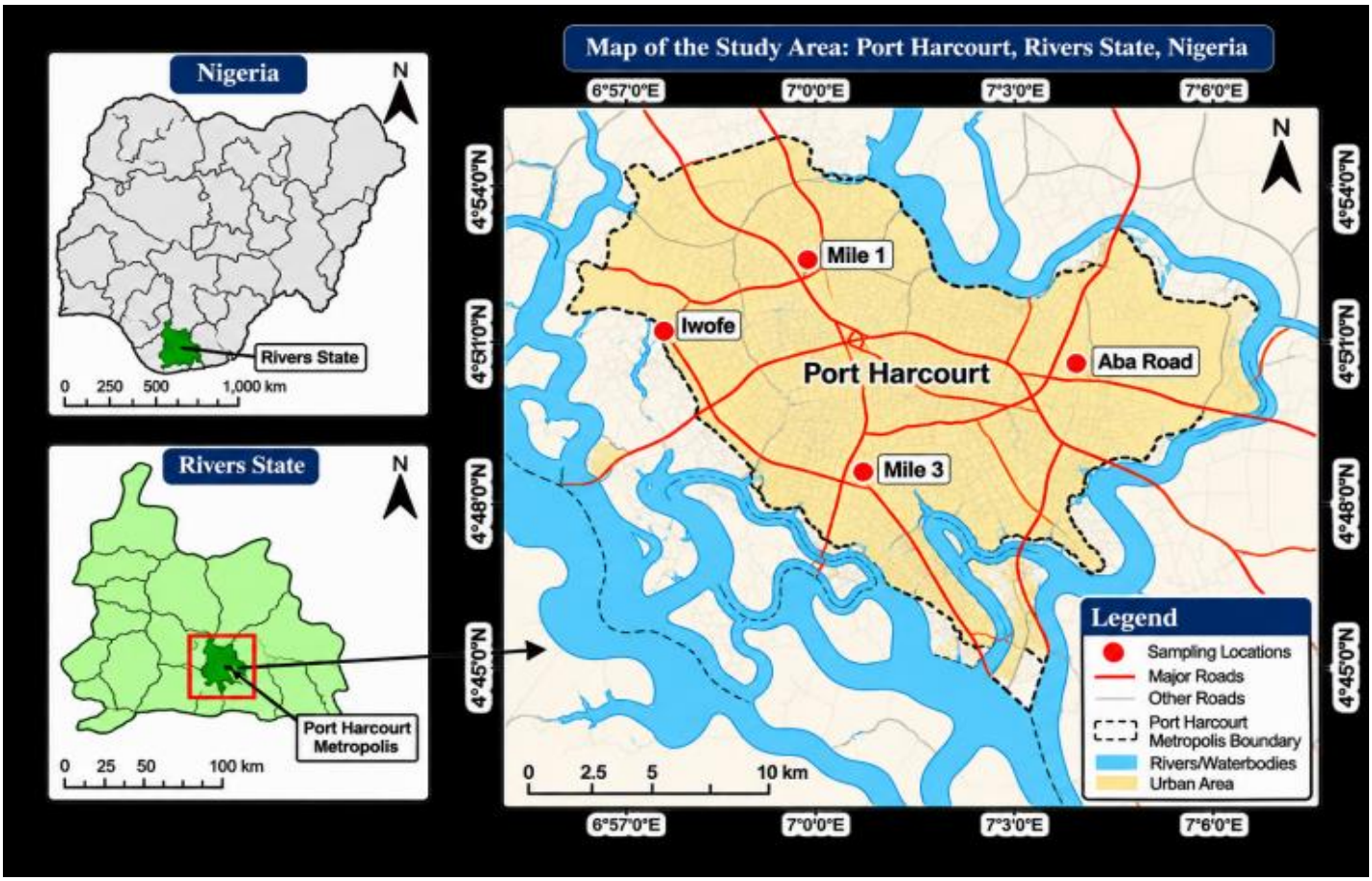


Fig. 1: Map of the Study Area and Sample collection Stations

Bacterial isolation

Bacterial isolation was performed using standard cultural techniques. The culture media employed included nutrient agar for the isolation of total heterotrophic bacteria, MacConkey agar for presumptive coliform bacteria, Salmonella–Shigella agar for presumptive Salmonella and Shigella, mannitol salt agar for presumptive *Staphylococcus* and eosin methylene blue agar for presumptive *Escherichia coli*.

Culture media were prepared according to the manufacturers' instructions and sterilised by autoclaving at 121°C for 15 minutes, where applicable. A 1-mL aliquot of each water sample was transferred into 9 mL of sterile diluent to obtain a 10^{-1} dilution. Subsequently, 0.1 mL of the diluted sample was inoculated onto freshly prepared agar plates and spread evenly using a sterile bent-glass spreader. Inoculated plates were incubated at 35°C for 24 hours.

Following incubation, colonies displaying distinct morphological characteristics were selected and purified through repeated subculturing on nutrient agar. The purified isolates were subjected to preliminary characterisation and subsequent biochemical and molecular identification. Bacterial identities were reported as presumptive based on their cultural, morphological and biochemical characteristics. Pure isolates were maintained in appropriate culture media for subsequent antibiotic susceptibility testing.

Characterisation and identification of bacterial isolates

Purified bacterial isolates were characterised using cultural, morphological, biochemical and molecular methods. Colony characteristics assessed included colour, shape, elevation, margin and consistency. Gram staining was performed to determine Gram reaction and cellular morphology.

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Biochemical characterisation included motility, catalase activity, methyl red and Voges–Proskauer reactions, carbohydrate fermentation involving glucose, fructose and sucrose, citrate utilisation, indole production and starch hydrolysis. Some selected isolates were identified using molecular methods which involved the DNA extraction using the boiling lysis method and the PCR amplification to determine the organisms to the specie level. The results of the morphological and biochemical tests were compared with standard identification criteria in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) to achieve presumptive identification of the bacterial isolates.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc-diffusion method on Mueller–Hinton agar. Bacterial suspensions were prepared from fresh pure cultures and standardised to a turbidity equivalent of 0.5 McFarland standards. The standardised suspensions were uniformly spread over the surfaces of Mueller–Hinton agar plates using sterile swab sticks. Commercial antibiotic-impregnated discs were aseptically placed on the inoculated agar surfaces using sterile forceps.

The antibiotic panel comprised ciprofloxacin, azithromycin, amoxicillin, levofloxacin, amoxicillin–clavulanic acid, erythromycin, gentamicin, pefloxacin, sparfloxacin, ofloxacin, ampiclox, cefuroxime, cefixime, cefotaxime and ceftriaxone. Roxithromycin was included only where confirmed in the original laboratory records. The concentrations of the antibiotic discs were obtained from the manufacturers' specifications.

Following disc placement, the plates were incubated under the appropriate conditions for the organisms tested. After incubation, the diameters of the inhibition zones were measured in millimetres. Susceptibility interpretations were made using the Clinical and Laboratory Standards Institute (CLSI) guidelines, with susceptible, intermediate and resistant categories assigned only where validated interpretive breakpoints existed for the relevant organism–antibiotic combinations. Where applicable breakpoints were unavailable, inhibition-zone diameters were reported descriptively without assigning clinical susceptibility categories.

DNA extraction

Genomic DNA was extracted from overnight bacterial cultures using the boiling lysis method. Briefly, bacterial colonies were harvested, resuspended in 100 μL of nuclease-free water, and heated at 95°C for 10 minutes in a thermal block to lyse the bacterial cell walls. The suspension was immediately chilled on ice for 5 minutes and then centrifuged at $12{,}000 \times g$ for 5 minutes. The resulting supernatant containing the template DNA was carefully transferred to a sterile tube and stored at -20°C until PCR amplification.

PCR amplification of the *eno* virulence-associated gene

The *eno* gene was investigated in selected bacterial isolates using polymerase chain reaction (PCR). Each reaction was prepared in a final volume of 40 µL containing 2× DreamTaq PCR Master Mix (Inqaba Biotec, South Africa), forward and reverse primers at a final concentration of 0.4 µM each, approximately 50 ng of template DNA and nuclease-free water to make up the final reaction volume. Amplification was performed using an ABI 9700 Applied Biosystems thermal cycler. The cycling programme consisted of initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at the gene-specific temperature for 30 seconds and extension at 72°C for 30 seconds. A final extension was performed at 72°C for 5 minutes.

PCR products were separated by electrophoresis on a 1.5% agarose gel at 120 V for 30–45 minutes. DNA bands were visualised under ultraviolet illumination following staining with an appropriate nucleic acid stain. A 50-bp DNA molecular-weight marker was used to estimate the sizes of the amplified fragments. Positive and negative PCR controls were included in the assays. Isolates producing bands corresponding to the expected amplicon size were recorded as PCR-positive for the target gene.

PCR amplification of antibiotic-resistance genes

The antibiotic-resistance genes investigated were *bla*TEM and *aac(6')-Ib*. The *bla*TEM gene, which encodes a TEM-family β-lactamase, and the *aac(6')-Ib* gene, which encodes an aminoglycoside 6′-N-acetyltransferase, were investigated using gene-specific primers.

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The TEM gene was amplified using the forward primer TEMF (5′-ATGAGTATTCAACATTTCC GTG-3′) and reverse primer TEMR (5′-TTACC AATGCTTAATCAGTGAG-3′). Each reaction was prepared in a final volume of 40 µL containing 2× Dream Taq PCR Master Mix, 0.4 µM of each primer, approximately 50 ng of genomic DNA and nuclease-free water. Amplification was performed using an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds. A final extension was performed at 72°C for 5 minutes. The amplified products were separated on a 1% agarose gel at 120 V for 25 minutes and visualised under ultraviolet illumination. The expected amplicon size was assessed against the appropriate DNA molecular-weight marker.

The *aac(6′)-Ib* target was investigated using the forward primer 5′-TGACCTTGCGATGCTCTATG-3′ and reverse primer 5′-TTAGGCATCACTGCGTG TTC-3′. The reaction mixture and amplification programme were as described for the *bla*TEM assay.

The amplified products were separated by electrophoresis on a 1% agarose gel at 120 V for 25 minutes and visualised under ultraviolet illumination. Amplicon sizes were assessed using a DNA molecular-weight marker. Positive and negative controls were included in the resistance-gene assays.

PCR results were interpreted based on the presence or absence of DNA bands corresponding to the expected product sizes. Detection of the target genes was interpreted as evidence of the presence of the corresponding gene sequences and not as confirmation of gene expression, plasmid localisation or their functional contribution to the observed antibiotic-resistance phenotypes.

Quality assurance and data analysis

Microbiological analyses were performed in duplicate, and culture media were prepared according to the manufacturers' instructions. Aseptic procedures were followed during sample processing, isolation and subculturing to minimise cross-contamination. PCR assays incorporated positive and negative controls, and amplicon sizes were assessed against a DNA molecular-weight marker. Bacterial isolates were summarised using frequencies and percentages. Antibiotic susceptibility results were summarised according to the number and proportion of isolates classified in each interpretive category, where appropriate criteria were available. The occurrence of virulence-associated and antibiotic-resistance genes was expressed as the number and percentage of PCR-positive isolates among the ten isolates examined. Descriptive statistics were used to summarise the findings.

Results

A total of 144 bacterial isolates belonging to ten bacterial groups were recovered from the packaged table-water samples. The identified bacteria included *Staphylococcus* spp., *Lactobacillus* spp., *Bacillus* spp., *Proteus* spp., *Micrococcus leteus*., *Salmonella enterica*., *Escherichia coli, Klebsiella aerogenes*., *Enterobacter* spp. and *Shigella* spp. Figure 2 presents the phylogenetic tree showing the Isolate codes and their percent relatedness to their geneBank relatives. J3 is *Klebsiella aerogenes* with accession number LC571941.1; J4 *Esherichia coli* with accession number CP010213.1; J2 is *Micrococcus luteus* with accession number LN681571.1; and J1 is *Salmonella enterica* with accession number CALNWE01000 0001.1

Antibiotic susceptibility varied among the Gram-positive bacteria groups (Table 1). Among *Staphylococcus* spp., the highest susceptibility was recorded against pefloxacin (40.3%), followed by gentamicin (38.8%) and erythromycin (35.8%). Lower susceptibility was recorded against ampiclox and ceftazidime (20.9% each). Among *Bacillus* spp., gentamicin recorded the highest susceptibility (47.4%), followed by ciprofloxacin and levofloxacin (36.8% each). *Lactobacillus* spp. showed the highest susceptibility to ciprofloxacin (52.2%), followed by roxithromycin (43.5%). *Micrococcus* spp. showed generally low susceptibility, with the highest value recorded against gentamicin (22.2%). Antibiotic susceptibility also varied among the Gram-negative bacteria groups (Table 2). *Salmonella* spp. showed the highest reported susceptibility to amoxicillin (85.7%), followed by pefloxacin (57.1%). Susceptibility to amoxicillin–clavulanic acid, ofloxacin and azithromycin was 42.9% for each agent. *Proteus* spp. demonstrated generally low susceptibility across the tested antibiotics.

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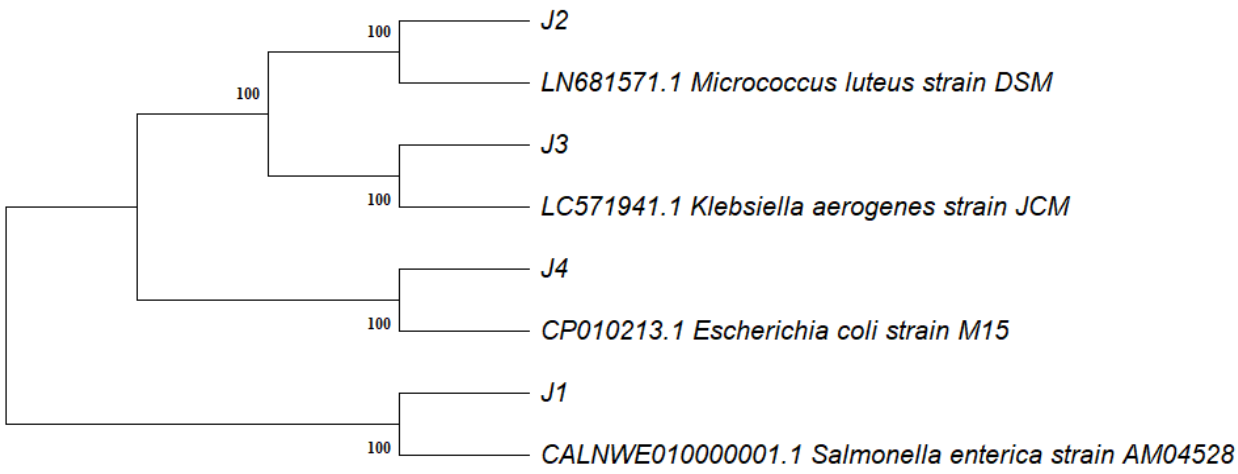


Fig. 2: Phylogenetic Tree of the isolates and their geneBank relatives

Table 1: Antibiotic susceptibility of Gram-positive bacteria isolates recovered from packaged table water

Antibiotic	*Staphylococcus* spp.	*Bacillus* spp.	*Lactobacillus* spp.	*Micrococcus* spp.
Ciprofloxacin	18 (26.9%)	7 (36.8%)	12 (52.2%)	1 (11.1%)
Azithromycin	19 (28.4%)	4 (21.1%)	4 (17.4%)	0 (0.0%)
Levofloxacin	20 (29.9%)	7 (36.8%)	1 (4.3%)	1 (11.1%)
Erythromycin	24 (35.8%)	5 (26.3%)	5 (21.7%)	0 (0.0%)
Pefloxacin	27 (40.3%)	5 (26.3%)	6 (26.1%)	1 (11.1%)
Gentamicin	26 (38.8%)	9 (47.4%)	6 (26.1%)	2 (22.2%)
Ampiclox	14 (20.9%)	4 (21.1%)	3 (13.0%)	0 (0.0%)
Ceftazidime	14 (20.9%)	5 (26.3%)	7 (30.4%)	1 (11.1%)
Amoxicillin	16 (23.9%)	6 (31.6%)	3 (13.0%)	0 (0.0%)
Roxithromycin	15 (22.4%)	6 (31.6%)	10 (43.5%)	0 (0.0%)

The highest susceptibility was recorded against amoxicillin (30.8%), followed by gentamicin (23.1%). No susceptibility was recorded against amoxicillin–clavulanic acid, ofloxacin or cefixime. Among the three *E. coli* isolates, susceptibility was recorded against amoxicillin and ciprofloxacin (33.3% each), while no susceptibility was recorded against the other tested agents. The single *Klebsiella* isolate was susceptible to amoxicillin, gentamicin and azithromycin, whereas the single *Enterobacter* isolate showed susceptibility to several of the tested agents. Results for *Shigella*, *Klebsiella* and *Enterobacter* were based on individual isolates and were therefore descriptive.

PCR analysis of ten selected bacterial isolates for the Molecular detection of virulence-associated and antibiotic-resistance genes revealed the presence of the three investigated genetic targets (Table 3). The *eno* virulence-associated gene was detected in seven isolates (70.0%), with amplification products of approximately 300 bp as shown in the gel image of plate 1. The *bla*TEM gene was detected in four isolates (40.0%), with amplicons of approximately 450 bp, while *aac(6')-Ib* was detected in five isolates (50.0%), with amplicons of approximately 500 bp as shown in the gel image in plate 2. Three isolates (30.0%) carried all three genetic targets, whereas one isolate (10.0%) was negative for all three genes.

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Table 2: Antibiotic susceptibility of Gram-negative bacteria isolates recovered from packaged table water

Antibiotic	*Proteus* spp.	*Salmonella* spp.	*E. coli*	*Shigella* spp.	*Klebsiella* spp.	*Enterobacter* spp.
Amoxicillin	4 (30.8%)	6 (85.7%)	1 (33.3%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Amoxicillin–clavulanic acid	0 (0.0%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gentamicin	3 (23.1%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Pefloxacin	2 (15.4%)	4 (57.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
Ofloxacin	0 (0.0%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	0 [0.0%]	1 (100.0%)
Azithromycin	1 (7.7%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1 (100.0%)
Levofloxacin	2 (15.4%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	0 [0.0%]	1 (100.0%)
Ciprofloxacin	1 (7.7%)	3 (42.9%)	1 (33.3%)	0 (0.0%)	0 [0.0%]	1 (100.0%)
Sparfloxacin	2 (15.4%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	0 [0.0%]	1 (100.0%)
Cefixime	0 (0.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)

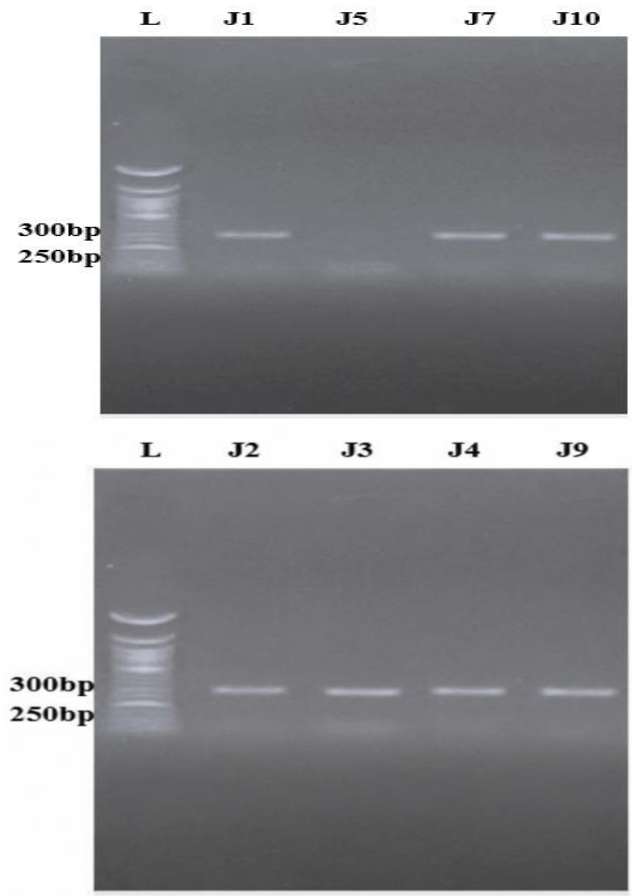


Plate 1: Agarose gel electrophoresis of PCR amplicons for the virulence-associated *eno* gene. (Lanes J1, J2, J3, J4, J7, J9 and J10 show positive amplification at approximately 300 bp. Lane L represents the 50-bp DNA molecular-weight ladder).

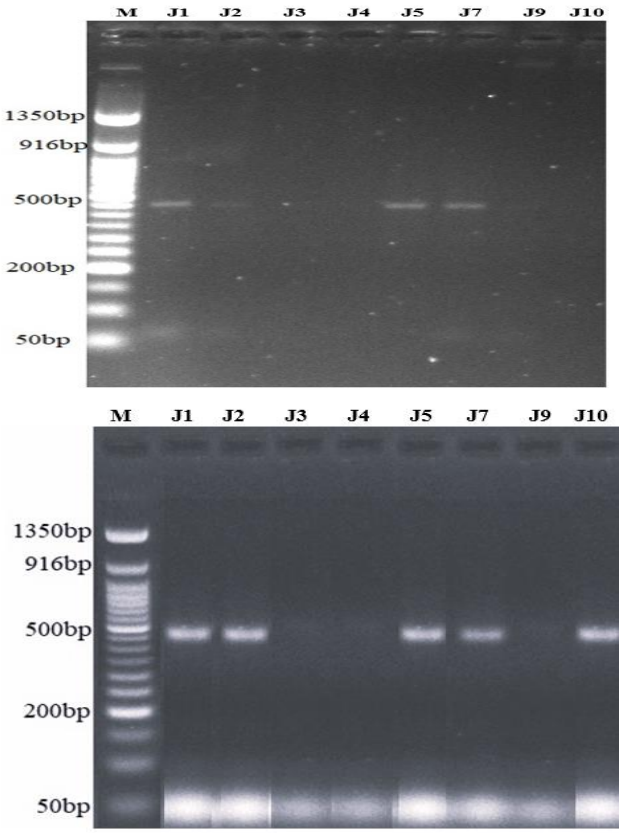


Plate 2 (A and B): Agarose gel electrophoresis of PCR amplicons for antibiotic-resistance genes. (A) *bla*TEM amplification at approximately 450 bp in isolates J1, J2, J5 and J7. (B) *aac(6')-Ib* amplification at approximately 500 bp in isolates J1, J2, J5, J6 and J7. Lane L represents the DNA molecular-weight ladder.

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Table 3: Detection of selected virulence-associated and antibiotic-resistance genes among ten bacterial isolates

Isolate	*Eno*	*Bla*TEM	*aac(6')-Ib*
J1	+	+	+
J2	+	+	+
J3	+	−	−
J4	+	−	−
J5	−	+	+
J6	−	−	+
J7	+	+	+
J8	−	−	−
J9	+	−	−
J10	+	−	−
Total positive	**7**	**4**	**5**
Occurrence (%)	**70.0**	**40.0**	**50.0**

Note: + = PCR-positive; − = PCR-negative. Isolate identities and individual results must be verified against the original PCR records and gel images before submission.

Discussion

The recovery of diverse bacterial groups from commercially packaged table water highlights the importance of maintaining microbiological quality throughout treatment, packaging, transportation, storage and handling. Contamination may arise from inadequate source-water treatment, ineffective disinfection, poor production hygiene, contaminated processing equipment or packaging materials, and improper handling during distribution and retail storage (Berry et al., 2006; LeChevallier & Au, 2019). The occurrence of *Staphylococcus* and *Micrococcus luteus* is noteworthy because these organisms are commonly associated with human skin and environmental surfaces, respectively. Their recovery may indicate possible contamination during production or handling, although their presence alone cannot establish the specific route of entry. Similar concerns regarding variations in the bacteriological quality of packaged water have been reported by Igbeneghu and Lamikanra (2014) in Ile-Ife, Nigeria. The recovery of *Bacillus* sp. is also relevant because its ability to produce endospores may enhance survival under adverse environmental conditions, including exposure to some routine disinfection procedures.

Lactobacillus species, which occur in diverse biological and environmental habitats, may also be encountered in water samples, although their significance depends on the species and strain. These findings demonstrate the range of bacterial groups that may occur in commercially packaged drinking water (Madigan et al., 2021; Brooks et al., 2023). More importantly, the presumptive recovery of *Salmonella enterica, Shigella, Escherichia coli, Proteus, Klebsiella aerogenes* and *Enterobacter* raises concerns because some members of these groups are associated with gastrointestinal infections or opportunistic infections. However, the cultural, morphological, biochemical and molecular methods used in this study provide presumptive identification and some definitive species confirmation. Furthermore, identification at genus level does not establish the pathogenicity of individual isolates, as virulence characteristics vary among species and strains (Brooks et al., 2023; Winn et al., 2017).

The recovery of *E. coli* warrants particular attention because it is widely used as an indicator of faecal contamination. The World Health Organization recommends that *E. coli* or thermotolerant coliform bacteria should not be detectable in any 100-mL sample of drinking water (WHO, 2022). Its detection therefore raises concerns about the microbiological quality of the affected products. Nevertheless, the isolation procedure in this study involved plating 0.1 mL of a diluted water sample; consequently, the findings do not constitute a direct assessment of compliance with the WHO 100-mL requirement unless a separate examination of that volume was performed. Similarly, although *Klebsiella* and *Enterobacter* may occur in faecally influenced environments, their recovery alone does not confirm faecal contamination. The antibiotic susceptibility profiles provide additional insight into the antimicrobial response characteristics of the recovered bacteria. Differences in susceptibility may reflect intrinsic resistance, acquired resistance determinants and species-specific physiological characteristics. Bacterial resistance can arise through mechanisms such as enzymatic antibiotic inactivation, modification of target sites, reduced membrane permeability and active efflux (Davies & Davies, 2010). The occurrence of resistant bacteria in packaged drinking water is relevant because aquatic environments may serve as reservoirs of antimicrobial-resistant organisms and associated genetic determinants.

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However, their recovery does not establish that resistance originated within the water products or resulted from antibiotic exposure during production. Interpretation of the susceptibility results depends on the standardisation of testing procedures and the suitability of the interpretive criteria. Although the Kirby–Bauer disc-diffusion method is widely used to assess antimicrobial susceptibility, reliable interpretation requires appropriate inoculum density, culture medium, incubation conditions and validated breakpoints (Bauer et al., 1966; CLSI, 2017). The use of a 0.5 McFarland standard in this study helped standardise the bacterial inocula. However, the absence of reference quality-control organisms limits independent verification of test performance against established quality-control ranges. In addition, clinical breakpoints should not automatically be applied to environmental isolates or organism–antibiotic combinations for which validated interpretive criteria are unavailable. Accordingly, susceptible, intermediate and resistant classifications should be restricted to combinations with appropriate established criteria, while inhibition-zone diameters should be reported descriptively where such criteria are unavailable.

The molecular detection of selected virulence-associated and antibiotic-resistance genes provides complementary information on the genetic characteristics of the recovered isolates. Detecting the *eno* gene is important because bacterial enolase does more than just help with metabolism; when expressed on the cell surface, it can bind to host proteins like plasminogen to help bacteria attach to host tissues. Pancholi (2001) described the multifunctional properties of bacterial α-enolase, while Bergmann et al. (2001) demonstrated its plasminogen-binding capacity in *Streptococcus pneumoniae*. Nevertheless, enolase is a widely distributed metabolic enzyme, and its detection does not independently establish virulence. Conventional PCR confirms the presence of the targeted DNA sequence but does not demonstrate gene transcription, protein production or surface localisation. Therefore, *eno* detection should be interpreted as evidence of the target sequence rather than confirmation of an expressed virulence function (Pancholi, 2001; Bergmann et al., 2001).

The detection of *blaTEM* is significant because TEM-type beta-lactamases confer resistance to susceptible beta-lactam antibiotics through hydrolysis of the beta-lactam ring. However, TEM variants differ in their substrate profiles, and detection of a generic *blaTEM* sequence does not establish the presence of an extended-spectrum beta-lactamase. Sequence characterisation and appropriate phenotypic confirmation are required to determine the specific variant and its functional significance (Partridge et al., 2018).

Similarly, *aac(6')-Ib* encodes an aminoglycoside 6′-N-acetyltransferase, which reduces the activity of susceptible aminoglycosides through enzymatic modification. The *aac(6')-Ib-cr* variant has an additional capacity to modify selected fluoroquinolones, including ciprofloxacin, as demonstrated by Robicsek et al. (2006). This additional activity, however, cannot be attributed to the generic *aac(6')-Ib* gene without sequence confirmation of the -cr variant.

The detection of multiple resistance-associated or virulence-associated genes within individual isolates may indicate the presence of diverse genetic determinants with different biological functions. Mobile genetic elements, including plasmids, transposons and integrons, are important in the mobilisation and dissemination of antimicrobial-resistance genes among bacterial populations. Plasmids may carry multiple resistance determinants and facilitate their movement between compatible bacterial hosts through horizontal gene transfer (Carattoli, 2018; Partridge et al., 2018).

However, the simultaneous detection of multiple genes does not establish their physical linkage, co-location on the same plasmid or capacity for joint transfer. Likewise, gene detection does not necessarily demonstrate expression or fully explain the observed susceptibility profile. Phenotypic susceptibility testing and molecular detection therefore provide complementary but distinct evidence: the former describes the observed antimicrobial response under laboratory conditions, whereas the latter identifies selected genetic sequences. Further sequencing, plasmid analysis and functional studies would be required to establish the precise genetic and biological significance of the detected determinants. The recovery of diverse bacterial groups, including 5 organisms of potential public-health significance, indicates the need for effective treatment, hygienic production practices and routine microbiological quality monitoring of commercially packaged drinking water.

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The accompanying susceptibility and molecular findings also provide information on selected antimicrobial-resistance and virulence-associated characteristics of the recovered isolates. Continued surveillance, supported by appropriate microbiological and molecular methods, is important for evaluating product quality and monitoring antimicrobial resistance in packaged drinking water.

Conclusion

This study recovered diverse bacterial groups from commercially packaged table water sold in Port Harcourt, with varying antibiotic susceptibility patterns. Molecular analysis of selected isolates detected the eno, blaTEM and aac(6′)-Ib genes markers. These findings indicate the presence of selected virulence-associated and antibiotic-resistance genetic markers among bacteria recovered from packaged water, although gene detection alone does not confirm pathogenicity or functional resistance. Routine bacteriological monitoring, reliable susceptibility testing and improved quality control during packaged-water production are therefore important. Further studies should incorporate confirmatory bacterial identification and expanded molecular characterisation.

References

American Public Health Association, American Water Works Association, & Water Environment Federation. (2023). *Standard methods for the examination of water and wastewater* (24th ed.). APHA Press.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493–496.

Berry, D., Xi, C., & Raskin, L. (2006). Microbial ecology of drinking water distribution systems. *Current Opinion in Biotechnology, 17*(3), 297–302.

Bergmann, S., Rohde, M., Chhatwal, G. S., & Hammerschmidt, S. (2001). α-Enolase of *Streptococcus pneumoniae* is a plasmin(ogen)-binding protein displayed on the bacterial cell surface. *Molecular Microbiology, 40*(6), 1273–1287.

Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A., & Mietzner, T. A. (2023). *Jawetz, Melnick & Adelberg's medical microbiology*. McGraw Hill.

Cabral, J. P. S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health, 7*(10), 3657–3703.

Carattoli, A. (2009). Resistance plasmid families in Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy, 53*(6), 2227–2238.

Clinical and Laboratory Standards Institute. (2017). *Performance standards for antimicrobial susceptibility testing* (27th ed.; CLSI supplement M100). CLSI.

Clinical and Laboratory Standards Institute. (2024). *Performance standards for antimicrobial susceptibility testing* (34th ed.; CLSI supplement M100). CLSI.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins.

Igbeneghu, O. A., & Lamikanra, A. (2014). The bacteriological quality of different brands of bottled water available to consumers in Ile-Ife, south-western Nigeria. *BMC Research Notes, 7*, Article 859.

LeChevallier, M. W., & Au, K.-K. (2019). *Water treatment and pathogen control: Process efficiency in achieving safe drinking-water*. IWA Publishing.

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2021). *Brock biology of microorganisms* (16th ed.). Pearson.

Odeyemi, O. A. (2015). Public health implications of microbial contamination of packaged water in Nigeria. *Journal of Microbiology and Antimicrobials, 7*(2), 53–59.

Pancholi, V. (2001). Multifunctional α-enolase: Its role in diseases. *Cellular and Molecular Life Sciences, 58*, 902–920.

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Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews, 31*(4), e00088-17.

Robicsek, A., Strahilevitz, J., Jacoby, G. A., Macielag, M., Abbanat, D., Park, C. H., Bush, K., & Hooper, D. C. (2006). Fluoroquinolone-modifying enzyme: A new adaptation of a common aminoglycoside acetyltransferase. *Nature Medicine, 12*, 83–88.

Winn, W. C., Allen, S. D., Janda, W. M., Koneman, E. W., Procop, G., Schreckenberger, P., & Woods, G. (2017). *Koneman's color atlas and textbook of diagnostic microbiology*. Wolters Kluwer.

World Health Organization. (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda*. World Health Organization.

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