Bacteriological Quality of Periwinkles (*Littorina littorea*) and Clams (*Mercenaria mercenaria*) and Antibiotic Sensitivity Pattern of Isolated Bacteria

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Abstract: Periwinkles (*Littorina littorea*) and clams (*Mercenaria mercenaria*) are vital components of aquatic ecosystems, each harbouring diverse bacterial communities including potential pathogens. This study investigated the bacteriological composition and antibiotic sensitivity profiles of periwinkles and clams sampled from different markets in Ondo and Lagos States, Nigeria. The load and array of bacteria in the samples were determined using standard microbiological technique. The disc diffusion method was used to determine the antibiotic sensitivity pattern of the bacterial isolates. The molecular identities of antibiotic resistant bacterial isolates were also determined. Results showed that the bacterial counts in periwinkles ranged from 1.5×10^3 to 5.0×10^3 cfu/g whereas those in clams ranged from 4.0×10^3 to 9.5×10^3 cfu/g. Bacteria belonging to the genera *Escherichia*, *Streptococcus*, and *Proteus* were isolated in both samples of periwinkles and crabs. Some of the bacterial isolates exhibited significant multi-antibiotic resistance and were molecularly identified as *Escherichia coli* and *Enterococcus faecalis.* The findings of this study emphasized the significance of monitoring the bacteriological safety of seafoods such as periwinkles and clams in order to mitigate the spread of antibiotic resistance and protect human health during handling and processing of the sampled seafoods.
Key word: Bacteria, clam, multiple antibiotic resistance, periwinkle, seafoods

INTRODUCTION

Periwinkles (*Littorina littorea*) are, small edible sea snails that play crucial ecological roles in tidal coastal areas worldwide. They are known for their adaptability and nutritional value, particularly in Eastern Nigeria (Eschweiler *et al.,* 2008). Periwinkles may harbour diverse microbial communities, including bacteria, which influence their health and ecological interactions. Understanding the bacterial communities in periwinkles is essential for assessing potential risks of pathogenic or antibiotic-resistant bacteria, thus informing seafood safety measures and environmental management strategies (Eschweiler *et al.*, 2008).

Clams (*Mercenaria mercenaria*), are members of the taxonomic class - bivalvia within the phylum - Mollusca. The Clams are filter-feeders that filter large volumes of water within minutes and may bioaccumulate bacteria in the process. The animals are vital components of marine ecosystems, influencing ecological processes such as nutrient cycling and sediment dynamics. Their adaptability of Clams to various habitats, from intertidal zones to deep-sea sediments, underscores their ecological importance (Clemente and Ingole, 2011).

Beyond their ecological roles, clams hold significant economic value for coastal communities and the seafood industry, serving as a source of protein and essential nutrients. Commercial clam aquaculture operations have been developed to meet market demands, promoting sustainable seafood production and reducing pressure on wild clam populations (Morton *et al.,* 2022). However, concerns regarding the safety of consuming clams, particularly when raw or partially cooked, have emerged due to potential health risks associated with pathogenic bacteria.

The presence of pathogenic bacteria in clams and periwinkles poses significant concerns for food safety and public health. Investigating the presence and prevalence of bacteria in clams and periwinkles is crucial for evaluating their safety and informing regulatory measures. By characterizing bacterial communities and assessing antibiotic sensitivity profiles, this study contributes to safer seafood consumption practices and enhances public health. This study aims to determine bacterial communities in both clams and periwinkles

and examine the antibiotic sensitivity profiles of the isolates through conventional and molecular methods. This is to gain a better understanding on the bacteriological quality and safety of the clams and periwinkles.

MATERIALS AND METHODS

Study area: The periwinkles used were obtained from different markets namely Arakale, Ijomu junction, Oshodi, Ayetoro junction and Nepa markets in Akure. Ondo State, Nigeria, while the clams were obtained from different fish markets namely Otunba fish market, Makoko fish market, Apapa fish market, Ikoyi fish market, and Oyingbo fish market in Lagos State, Nigeria.

Sample collection: Sterile polythene bags were used to collect and transport live periwinkle samples to the laboratory, where they were processed. About 1 g each of fresh clams were procured from the six (6) fish markets in Lagos State. These clams, typically containing five medium-sized specimens, were obtained from the fish markets at two-week intervals. Upon collection, the clams were preserved in sterile buckets filled with water from their respective lagoons and transported to the laboratory for analysis. This process was repeated for each sample.

Enumeration of bacteria in samples: The shells of the clams and periwinkles were aseptically removed, and the tissues were macerated using a sterile scalpel. Serial dilution of 1 g the marcerated and homogenized sample was carried out in tenfold increments. Approximately, 1 ml aliquots from the dilutions were inoculated onto sterile Petri dishes and fresh molten nutrient agar prepared following manufacturer's specification was poured into the plates and swirled gently until solidified. Thereafter, the plated were incubated aerobically at 37°C for 24 h, and colonies that emerged were counted and recorded as colony-forming units per gram (cfu/g). The isolates were subcultured repeatedly to obtain pure cultures, then stored in agar slants at 4°C until further characterization through biochemical tests.

Antibiotic sensitivity test of the bacterial isolates: The Kirby-Bauer antibiotic testing method, also known as disc diffusion antibiotic sensitivity testing, was employed to determine the antibiotic susceptibility pattern of the bacterial isolates. Cultures were standardized using McFarland's standard. Mueller Hinton agar plates were inoculated with isolates from stock cultures using sterile swab sticks. Excess liquid was removed from the swab sticks by gently pressing or rotating them against the inside of the tube. Bacterial lawns were formed on the plates using the streaking method to ensure uniform growth. Plates were streaked in one direction, rotated 90°, and streaked again, repeating this process three times. After allowing the plates to dry for approximately 5 minutes, antibiotic discs containing specific antibiotics were dispensed onto the plates using an antibiotic disc dispenser. Each disc was gently pressed onto the agar with flame-sterilized forceps to ensure attachment. The setup was performed in triplicate for each isolate, with a control plate containing no antibiotic disc. Inoculated plates were then incubated at 37°C for 72 h, following which the diameter of the zone of inhibition around each disc was measured. Interpretation of results was carried out using standard interpretative charts recommended by the Clinical Laboratory Science Institute (2007). Multiple antibiotic resistance was defined as resistance to a minimum of three different antibiotics. For Gram-positive bacteria, antibiotics discs containing standard concentrations of gentamycin (10 µg), erythromycin (30 µg), rocephin (25 µg), amoxicillin (30 µg), streptomycin (30 µg), zinacef (20 µg), pefloxacin (10 µg), septrin (30 µg), ciprofloxacin (10 µg), and ampiclox (30 µg) were used. For Gram negative bacteria, antibiotics discs containing standard concentrations of pefloxacin (10 µg), gentamycin (10 µg), amoxicillin (30 µg), ciprofloxacin (10 µg), streptomycin (30 µg), septrin (30 µg), chloramphenicol (30

μg), sparfloxacin (10 μg), augmentin (10 μg), and tarivid (10 μg) were used.

Molecular identification of multiple antibiotic resistant isolates: The DNA extraction from isolates was performed following the protocol described by Trindade *et al*. (2007). Single colonies were transferred to liquid medium and cultured on a shaker for 48 hours. After centrifugation, the pellets were resuspended in the TE buffer, and SDS and Proteinase K were added before incubation. Following the addition of NaCl and CTAB solution, the suspension was incubated, and chloroform isoamyl alcohol was added. The DNA was precipitated, washed with ethanol, airdried, and dissolved in the TE buffer. For polymerase chain reaction (PCR), a reaction cocktail was prepared, including GoTaq reaction mix, MgCl2, dNTPs mix, primers, Taq DNA polymerase, and DNA template. PCR was carried out with specific temperature cycles. Gel electrophoresis was performed on a 1.5% agarose gel to check the integrity of the amplified gene fragment. After gel integrity confirmation, the amplified fragments were purified by ethanol precipitation. Sodium acetate and ethanol were added to the PCR product, followed by centrifugation, washing with ethanol, and air-drying. The purified fragments were then resuspended in sterile distilled water. Sequencing was conducted using a Genetic Analyzer 3130xl sequencer with the BigDye terminator v3.1 cycle sequencing kit. BioEdit software was used for sequence editing and cluster alignment, while MEGA 6 was utilized for genetic analysis.

Statistical analysis of data obtained: Data obtained from the antibiotic susceptibility testing were subjected to statistical analysis using Microsoft Excel (2013) and SPSS (Statistical Package for the Social Sciences). Other data from the enumeration of colonies and biochemical characterization were analyzed using statistical methods. Descriptive statistics were employed to summarize large datasets using numerical descriptors such as mean, mode, median, or standard deviation, as well as graphical methods (Lorowitz *et al.,* 2019).

RESULTS

The total plate counts of bacteria from fresh periwinkles showed that samples from Arakale market had the highest count of 5.0×10^3 cfu/g, while those obtained from Nepa market had the least count of 1.5×10^3 cfu/g. In addition, the total plate counts of bacteria from fresh clams revealed that samples from Otunba fish market had the highest counts of 9.5×10^4 cfu/g, while those obtained from Apapa fish market had the least counts of 4.0×10^4 cfu/g. In general, the total plate counts of bacteria from fresh clams were higher than those from periwinkles (Table 1).

The cultural, morphological and biochemical characteristics of bacterial isolates from periwinkles revealed the presence of *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, *Proteus vulgaris*, and *Enterococcus feacalis* (Table 2). In clams, the following bacteria were isolated namely *Enterobacter* spp., *Escherichia coli*, *Proteus vulgaris*, *Vibrio cholerae*, *Salmonella* spp., *Streptococcus* spp. and *Staphylococcus aureus* (Table 3).

In periwinkles, *E. coli* and *Bacillus cereus* had the highest percentage frequency of occurrence of 15.6%, while *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Proteus vulgaris* had the least percentage frequency of occurrence of 6.3% each. In clams, *Staphylococcus aureus* had the highest percentage frequency of occurrence of 21.1%, while *Enterobacter* spp., *Escherichia coli*, *Proteus vulgaris* had the least percentage frequency of occurrence of 10.2% (Table 4).

The antibiotic sensitivity testing on bacterial isolates from the periwinkle and clams unveiled diverse patterns across different species. *Staphylococcus aureus* demonstrated sensitivity to antibiotics like ciprofloxacin but, exhibited resistance to others such as ampiclox. Conversely,

Streptococcus spp. showcased susceptibility to erythromycin while demonstrating resistance to rocephin. *Salmonella* spp. and *Enterobacter* spp. displayed varied susceptibility profiles to different antibiotics. Notably, *Enterococcus faecalis, Proteus vulgaris* and *Escherichia coli* exhibited elevated levels of multiple antibiotic resistance compared to other organisms, with multiple resistances observed across different antibiotics (Tables 5, 6, 7, 8).

Two multiple antibiotic resistant bacterial isolates from the periwinkles were identified by 16S rRNA gene sequencing. The two isolates A8 and A10 showed highest sequence identity to *Escherichia coli* (99.76%) and *Enterococcus faecalis* (99.76%), respectively. Similarly, two multidrug resistant bacteria isolates from the clams were also identified as *Escherichia coli* (99.84%) and *Proteus vulgaris* (100%) (Table 9).

Table 1: Total plate counts of bacteria from periwinkles and clams obtained from different markets in Ondo and Lagos State, Nigeria

Week	Source of periwinkles	Total plate count (cfu/g)	Source of clams	Total plate count (cfu/g)
1	ARA	5.0×10^3	TFM	9.5×10^5
2	IJJ	3.6×10^3	MFM	7.2×10^5
3	OSH	2.2×10^3	IFM	6.1×10^5
4	NEP	1.5×10^3	OFM	5.4×10^5
5	AJY	2.7×10^3	AFM	4.0×10^4

Key: ARA-Arakale market; IJJ-Ijomu junction; OSH-Oshodi market; NEP-Nepa market; AYJ-Ayetoro junction; TFM-Otunba fish market; MFM-Makoko fish market; IFM- Ikoyi fish market; OFM-Oyingbo fish market; AFM- Apapa fish market

Table 2: Morphological and biochemical characteristics of bacterial isolates from periwinkles

Isolates	Form	Elevation	Margin	Colour	Gram reaction	Arrangement	Shape	Coagulase	Catalase	Citrate	H2S	Indole	Motility	Urease	Sugar Triple Iron Test	Mr Test	Vp Test	Probable Organism
1	Irregular	Flat	Lobate	Cream	+	Clusters	Rod	-	+	+	-	-	+	+	Red slant/ yellow butt	+	+	*Bacillus cereus*
2	Filamentous	Flat	Filiform	Cream	-	Scattered	Rod	-	+	+	-	-	+	+	Red slant/ yellow butt	+	+	*Klebisiella pneumonia*
3	Circular	Flat	Entire	White	+	Scattered	Rod	-	+	-	-	-	+	+	Red slant/ yellow butt	_	_	*Micrococcus luteus*
4	Circular	Raised	Entire	Cream	-	Clusters	Rod	-	+	+	-	-	+	+	Yellow slant/ yellow butt	_	_	*Pseudomonas aeroginosa*
5	Irregular	Flat	Entire	White	-	Clusters	Cocci	-	+	+	-	-	+	+	Red slant/ yellow butt	+	+	*Serratia marcescens*
6	Irregular	Flat	Filiform	White	-	Scattered	Cocci	-	+	-	-	+	-	-	Red slant/ yellow butt	+	+	*Shigella dysenteriae*
7	Circular	Flat	Entire	Cream	+	Clusters	Rod	-	+	+	-	-	+	+	Yellow butt/ yellow slant	+	_	*Streptococcus pneumonia*
8	Circular	Flat	Entire	White	-	Scattered	Cocci	-	+	-	-	-	+	+	Red slant/ yellow butt	+	+	*Escherichia coli*
9	Circular	Flat	Entire	Cream	-	Scattered	Rod	-	+	+	+	+	+	+	Red slant/ yellow butt	+	-	*Proteus vulgaris*
10	Irregular	Raised	Entire	Pink	+	Scattered	Rod	-	+	+	-	-	+	+	Red slant/ yellow butt	+	+	*Enterococcus feacalis*

Table 3: Morphological and biochemical characteristics of bacterial isolates from clams

Isolates	Form	Elevation	Arrangement	Colour	Gram reaction	Appearance	Shape	Coagulase	Catalase	Citrate	H_2S	Indole	Motility	Urease	Lactose	Sucrose	Glucose	Mannitol	Probable Organism
1	Irregular	Convex	Cluster	Cream	-	Small, round	Rod	+	+	+	-	-	+	+	+	+	+	-	*Enterobacter* spp.
2	Filamentous	Convex	Singly	Cream	-	Opaque. round	Rod	+		-	-	+	+	-	+	+	+	+	*Escherichia coli*
3	Circular	Convex	Singly	Cream	-	Translucent, round	Rod	+	+	+	+	-	+	+	-	+	+	-	*Proteus vulgaris*
4	Circular	Convex	Singly	Yellow	-	Circular, smooth	Curved rod	+	+	+	-	+	+	-	+	+	+	+	*Vibrio cholerae*
5	Irregular	Convex	Singly	White	-	Translucent, round	Rod	-	+		+	+	+	+	-	+	+	+	*Salmonella* spp.
6	Irregular	Convex	Chain	Cream	+	Small, mucoid	Cocci	-	-	+	+	-	-	-	+	+	+	+	*Streptococcus* spp.
7	Irregular	Convex	Cluster	Cream	+	Round, rough	Cocci	+	+	+	+	-	-	+	+	+	+	+	*Staphylococcus aureus*

Table 4: Percentage frequency of occurrence of bacterial isolates in periwinkles and clams

Samples	Isolates	Week 1	Week 2	Week 3	Week 4	Week 5	Frequency of occurrence	Percentage frequency of occurrence (%)
Periwinkles	*Bacillus cereus*	+	+	+	+	+	5	15.6
	Klebisiella pneumonia	+	-	+	+		3	9.4
	Micrococcus luteus	+	-	-	+	+	3	9.4
	Pseudomonas aeroginosa	-	+	-	-	+	2	6.3
	Serratia marcescens	+	-	+	-	+	3	9.4
	Shigella dysenteriae	+	+	-	+	+	4	12.5
	Streptococcus pneumonia	+	-	-	+	-	2	6.3
	Escherichia coli	+	+	+	+	+	5	15.6
	Proteus vulgaris	-	-	+	+	-	2	6.3
	Enterococcus feacalis	+	+	-	-	+	3	9.4
Clams	*Enterobacter* spp.	-	-	-	+	+	2	10.2
	Escherichia coli	-	+	+	-	-	2	10.2
	Proteus vulgaris	-	+	-	-	+	2	10.2
	Vibrio cholerae	+	-	+	-	+	3	15.8
	Salmonella spp.	-	+	-	+	+	3	15.8
	Streptococcus spp.	+	+	-	-	+	3	15.8
	Staphylococcus aureus	+	+	+	-	+	4	21.1

Key: + = Positive to the test; - = Negative to the test

Table 5: Antibiotic sensitivity patterns of Gram negative bacterial isolates from periwinkles

Isolates	S	OFX	PEF	CN	AU	AM	CPX	SP	CH	SXT
Klebsiella pneumoniae	16.00±1.00^{de}	13.66±1.52^{bcd}	0.00±0.00^{a}	0.00±0.00^{a}	19.33±1.15^{e}	0.00±0.00^{a}	14.66±2.51^{cd}	10.67±1.15^{bc}	12.66±2.52^{bcd}	10.33±1.52^{b}
Proteus vulgaris	19.33±2.51^{cd}	0.00±0.00^{a}	20.00±0.00^{cd}	15.67±3.21^{bc}	0.00±0.00^{a}	22.00±3.00^{d}	0.00±0.00^{a}	16.67±0.57$_{c}$	11.66±1.53^{b}	0.00±0.00^{a}
Serratia marcescens	16.67±0.58^{bc}	14.67±3.78^{b}	19.33±1.52^{bcd}	17.66±0.57^{bcd}	0.00±0.00^{a}	20.67±1.15^{cd}	0.00±0.00^{a}	15.66±1.15^{b}	21.00±2.64^{cd}	22.33±0.58^{d}
Escherichia coli	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.00±2.65^{b}	0.00±0.00^{a}	0.00±0.00^{a}	13.67±2.08^{bc}	11.33±1.15^{b}	0.00±0.00^{a}	16.00±1.00^{c}
Shigella dysenteriae	18.67±3.51^{bc}	13.67±2.89^{b}	0.00±0.00^{a}	18.33±1.15^{bc}	14.67±2.52^{b}	0.00±0.00^{a}	0.00±0.00^{a}	23.33±1.53^{c}	21.67±3.21^{c}	0.00±0.00^{a}
Pseudomonas aeroginosa	16.00±3.00^{c}	16.67±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	9.33±1.53^{b}	15.67±1.53^{c}	14.67±1.53^{c}	15.00±3.00^{c}	0.00±0.00^{a}	17.00±2.00^{c}

Key: Values are mean ±standard deviation of three determinations. Values not followed by the same superscripts in the same column are significantly different ($p<0.05$). PEF- Pefloxacin (10Ug), CN-Gentamycin (10Ug), AM- Amoxacillin (30Ug), CPX-Ciprofloxacin (10Ug), S-Streptomycin (30Ug), SXT- Septrin (30Ug), CH- Chloramphenicol (30Ug), SP- Sparfloxacin (10Ug), AU- Augmentin (10Ug), OFX- Tarivid (10Ug)

Table 6: Antibiotic sensitivity patterns of Gram positive bacterial isolates from periwinkles

Isolates	E	SXT	S	CPX	R	AM	Z	APX	CN
Bacillus cereus	0.00±0.00^{a}	14.33±2.52^{bc}	0.00±0.00^{a}	24.33±2.52^{d}	19.33±1.15^{cd}	19.67±2.08^{cd}	14.67±2.52^{bc}	0.00±0.00^{a}	9.67±3.51^{b}
Micrococcus luteus	0.00±0.00^{a}	0.00±0.00^{a}	20.00±0.00^{d}	0.00±0.00^{a}	19.67±2.08^{cd}	22.33±2.52^{d}	0.00±0.00^{a}	17.33±1.53^{bc}	13.33±3.51^{b}
Streptococcus pneumoniae	16.67±0.57^{bc}	17.33±1.53^{bcd}	20.00±1.00^{de}	18.00±1.00^{bcd}	0.00±0.00^{a}	0.00±0.00^{a}	20.67±1.15^{e}	16.00±1.00^{b}	20.33±2.52^{de}
Enterococcus feacalis	18.67±1.53^{bc}	16.67±1.53^{b}	17.67±1.53^{c}	16.33±1.53^{b}	21.33±1.15^{c}	15.00±2.65^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Key: Values are mean ±standard deviation of three determinations. Values not followed by the same superscripts in the same column are significantly different ($p<0.05$). PEF- Pefloxacin (10Ug), CN-Gentamycin (10Ug), AM- Amoxacillin (30Ug), CPX-Ciprofloxacin (10Ug), S-Streptomycin (30Ug), SXT- Septrin (30Ug), APX-Ampiclox (30Ug), Z- Zinnacef(20Ug), R- Rocephin (25Ug), E- Erythromycin (10Ug).

Table 7: Antibiotic sensitivity patterns of Gram negative bacterial isolates from clams

Isolates	CH	SXT	CPX	SP	AM	AU	OFX	CN	PEF	S
Salmonella spp	0.00±0.00^{a}	14.33±2.52^{bc}	24.33±2.52^{d}	19.33±1.15^{cd}	19.67±2.08^{cd}	14.67±2.52^{bc}	00.00±00.00^{a}	9.67±3.51^{b}	10.33±1.53^{b}	0.00±0.00^{a}
Proteus vulgaris	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	19.67±2.08^{cd}	22.33±2.52^{d}	0.00±0.00^{a}	17.33±1.52^{bc}	13.33±3.51^{b}	16.0±1.0^{bc}	20.00±0.00^{cd}
Esherichia coli	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.33±1.15^{b}	0.00±0.00^{a}	0.00±0.00^{a}	13.66±2.08^{bc}	11.33±1.15^{b}	0.00±0.00^{a}	16.00±1.00^{d}
Enterobacter spp	16.67±0.57^{d}	0.00±0.00^{a}	13.00±1.00^{b}	23.33±1.52	0.00±0.00^{a}	15.67±3.21^{bc}	23.33±2.08^{d}	14.33±1.15^{bc}	24.33±0.57^{d}	14.33±1.15^{bc}
Vibrio cholerae	16.67±0.57^{bc}	14.67±3.78^{b}	19.33±1.53^{abc}	17.67±0.57^{bcd}	0.00±0.00^{a}	20.67±1.15^{cd}	0.00±0.00^{a}	15.67±1.15^{b}	21.00±2.65^{cd}	22.33±0.57^{d}

Key: PEF –Pefloxacin 10ug, CN – Gentamycin 10ug, AM – Amoxicillin 30ug, CPX-Ciprofloxacin 10ug, S –Streptomycin 30ug, SXT –Septrin 30ug, CH –Chloramphenicol 30ug, SP –Sparfloxacin 10ug, AU – Augmentin 10ug, OFX –Tarivid 10ug.

Table 8: Antibiotic sensitivity patterns of Gram positive bacterial isolates from clams

Isolates	E	SXT	S	CPX	R	AM	Z	APX	CN
Staphylococcus aureus	16.00±3.00^{c}	16.67±0.57^{c}	0.00±0.00^{a}	9.33±1.53^{b}	15.67±1.53^{c}	14.67±1.53^{c}	15.00±3.00^{c}	0.00±0.00^{a}	17.00±2.00^{c}
Streptococcus pneumoniae	19.33±2.52^{cd}	0.00±0.00^{a}	20.00±0.00^{cd}	0.00±0.00^{a}	0.00±0.00^{a}	22.00±3.00^{d}	0.00±0.00^{a}	16.7±0.57^{c}	0.00±0.00^{a}

Key: PEF –Pefloxacin 10ug, CN – Gentamycin 10ug, APX –Ampiclox 30ug, Z –Zinnacef 20ug, AM – Amoxacillin 30ug, R –Rocephin 25ug, CPX-Ciprofloxacin 10ug, S –Streptomycin 30ug, SXT –Septrin 30ug, E –Erythromycin 10ug.

Table 9: Molecular identity of multiple antibiotic resistant bacterial isolates from periwinkles and clams

Sample	Isolate ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Percentage Identity	Accession
Periwinkles	A8	*Escherichia coli*	232900%	2329	99.00%	0	99.76%	PP268155
	A10	*Enterococcus faecalis*	233500%	2335	99.00%	0	99.76%	PP268156
Clams	L1	*Escherichia coli*	232900%	2329	99.00%	0	99.84%	PP269453
	L2	*Proteus vulgaris*	234600%	2346	99.00%	0	100.00%	PP269453

DISCUSSION

The isolation and identification of bacteria in clams and periwinkles are essential for assessing potential public health risks associated with these seafood specimens (Ekanem and Adegoke, 1995; Udoh *et al.*, 2017). Employing a combination of microbiological culture techniques, biochemical tests, and antibiotic sensitivity testing allows for a thorough analysis of the microbial communities within these shellfish.

In the case of clams, samples were systematically collected from various markets in Lagos, including Otunba, Makoko, Ikoyi, and Oyingbo, while periwinkles were obtained from markets in different locations, such as Arakale, Ijomu junction, Oshodi, Ayetoro junction in Akure, Nigeria. The sampling methodology prioritized proper handling and transportation to maintain sample integrity and prevent contamination, considering insights from related clam species studies to ensure effective and non-destructive collection methods. The microbiological culture technique adopted in this study provided valuable insights into the metabolic capabilities and physiological characteristics of the bacteria present (Madigan *et al.*, 2009).

Total plate count revealed significant insights into the abundance and distribution of bacteria in both clams and periwinkles sourced from different markets. Specific markets showed higher levels of bacterial contamination, with the prevalence of certain bacterial families varying accordingly. For example, Staphylococcaceae were predominant in clams, while *Bacillus cereus* was notable in periwinkles, highlighting market-specific variations in microbial contamination levels (Adeyemo, 2023). Further characterization of bacterial isolates enriched the understanding of the microbial landscape within both clams and periwinkles. Distinctive features such as colonial morphology, colour, shape, elevation, and biochemical traits provided a nuanced view of the diverse bacterial species present. Notable isolates included *Staphylococcus aureus*, *Streptococcus* spp, *Salmonella* spp, and *Enterobacter* spp, among others, identified in both shellfish species.

Antibiotic sensitivity testing provided critical insights into the susceptibility of bacterial isolates from both clams and periwinkles to various antibiotics. The findings revealed diverse responses to different antibiotics, emphasizing the importance of a targeted approach to

antibiotic therapy (Shah *et al.*, 2014). *Staphylococcus aureus*, for instance, exhibited sensitivity to certain antibiotics but, demonstrated resistance to others, underscoring the necessity of precise antibiotic selection in its treatments. The observed sensitivity and resistance patterns, particularly in *Enterococcus faecalis, Escherichia coli* and *Proteus vulgaris*, underscored the complexity of antibiotic interactions and emphasized the need for precision in treatment approaches. The prevalence of antibiotic resistance raised concerns about the potential development of antibiotic-resistant strains, necessitating ongoing monitoring and surveillance efforts. Comparison with other studies, such as those by Zhou *et al.* (2013), highlights the consistency of molecular identification methods in environmental and clinical samples. These studies reinforce the importance of strain-level identification in understanding the clinical and environmental significance of bacterial isolates. Additionally, insights from Lee *et al*. (2022), emphasized the prevalence of *Bacillus cereus* and *Escherichia coli* in seafoods, suggesting potential environmental exposures and faecal contamination during production or processing. The comprehensive analysis of bacterial isolates from both clams and periwinkles contributes significantly to the understanding of microbial ecology in these seafood specimens. These findings have important implications for public health interventions, highlighting the importance of safe handling, thorough cooking, and vigilant monitoring of seafood quality to mitigate potential health risks.

CONCLUSION

This study has provided valuable insights into the presence and composition of bacterial communities in periwinkles and clams obtained from fish markets in Akure and Lagos respectively. A diverse array of bacterial species, including potentially pathogenic strains were identified. It is noteworthy that multiple antibiotic resistant bacteria, such as *Enterococcus faecalis, Staphylococcus aureus, Proteus vulgaris* and *Escherichia coli* were detected in the samples. The presence of antibiotic-resistant bacteria in clams and periwinkles poses significant challenges to public health, highlighting the need for proactive measures to mitigate the spread of these pathogens.

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