Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 14 [9] August 2025. 01-06

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Morphological, Biochemical, and Enumeration Studies of Bacterial Isolates from Fresh Water Clam (*Galatea paradoxa*) in Swali Market, Bayelsa State, Nigeria

Faith I. Omeje[1] and Omotayo B. Ilesanmi[2,*]
[1] Department of Microbiology, Faculty of Science, Federal University Otuoke
[2] Department of Biochemistry, Faculty of Science, Federal University Otuoke
[*]Email address: ilesanmiob@fuotuoke.edu.ng

ABSTRACT
This study investigated the proximate composition and microbial profile of clam samples. Fresh clam samples were purchased from Swali Market in Yenagoa, Bayelsa State, and transported to the laboratory in sterile containers. This study was conducted at Federal University Otuoke, Bayelsa State, Nigeria. Samples were collected from different vendors in Swali market and transported to the lab in a sterile bag.. Standard microbiological and proximate analysis methods (AOAC, 2005) were employed. Proximate analysis revealed the clam samples had high moisture content (average 72.83%), crude protein (average 19.54%), and an energy value of approximately 93.39 Kcal/100g. Ash, crude fiber, and total fat contents were relatively low, averaging 4.14%, 0.44%, and 0.53%, respectively, with carbohydrate content around 2.66%. Microbiological analysis identified four distinct bacterial species: Staphylococcus aureus, Enterococcus faecalis, Bacillus spp., and Staphylococcus epidermidis. Staphylococcus aureus was the most prevalent organism, accounting for 50% of the isolates, while Enterococcus faecalis, Bacillus spp., and Staphylococcus epidermidis each represented 16.7%. All isolated organisms were Gram-positive, with Staphylococcus aureus, Enterococcus faecalis, and Staphylococcus epidermidis presenting as cocci, and Bacillus spp. as rods. Biochemical tests further confirmed these identifications. Microbial counts ranged from 3.1×10^9 CFU/g to 5.2×10^9 CFU/g, corresponding to Log CFU/g values between 9.491 and 9.716. These findings highlight the nutritional value of clams while also indicating the presence and prevalence of certain bacterial species, which is crucial for assessing food safety and quality.
Keywords*: Clam (Seafood); Proximate Composition; Microbial Profile; Staphylococcus aureus; Food Safety*

Received 22.06.2025 Revised 20.07.2025 Accepted 19.08.2025

INTRODUCTION

The abundance of clams in the sea has made it one of the most consumed seafood globally [1, 2]. It's an animal that plays an important role in the food chain and possesses high nutritional value [2, 3]. It's one of the highly sought-after seafood globally and has been exported to other countries from Nigeria [4]. Despite its nutritional benefits, some reports have shown that this food can be contaminated with bacteria and other pathogens harmful to humans [5, 6]. The contamination of clams with pathogenic organisms can stem from the environment where they were collected, the hygiene of the vendors handling them, or the conditions in the area where they're sold [5, 6, 7]. Some of the pathogenic organisms identified in clams include *Vibrio*, *Salmonella*, *Shigella*, and Coliforms, which can cause dysentery, typhoid, and cholera [6, 7].
In Nigeria, specifically in Bayelsa State, the Niger Delta region of the country, clam (*Galatea paradoxa*), commonly called "water snail" or locally "Gbou," is widely consumed by citizens and also exported globally [4, 8]. They can be purchased from markets across different parts of Yenagoa and processed for consumption by frying, cooking, or smoking [8]. The increasing number of people suffering from foodborne diseases is a major concern in Bayelsa, and some of these cases have been attributed to the hygienic status of hawked food [9, 10]. This research aims to isolate and identify Gram-positive bacteria associated with clams to understand their presence, potential impact, and characteristics within clam habitats.

MATERIAL AND METHODS

Sample Collection and Preparation: Clams were rinsed with sterile seawater to remove contaminants, then homogenized in a blender to release bacteria. The resulting mixture was centrifuged to remove debris, leaving a bacterial supernatant for analysis.

Media Preparation: All culture media were prepared following standard microbiological procedures, involving accurate measurement of ingredients, dissolution in deionized water, sterilization by autoclaving at 121°C for 15 minutes, and cooling before pouring into sterile Petri dishes.
Enrichment Culture: The clam sample supernatant was inoculated into peptone broth and incubated at 30°C for 24 to 48 hours to enrich for Gram-positive bacteria. The broth was then centrifuged at 10,000 rpm for 10 minutes to concentrate the bacteria.
Isolation and Purification: The bacterial pellet was cultured on selective agar plates, including Mannitol Salt Agar, MacConkey Agar, Nutrient Agar, and Salmonella Shigella Agar, and incubated at 30°C for 24 to 48 hours. Individual colonies were selected based on their appearance and Gram stain reaction, and purified through repeated streaking on fresh agar. A broad spectrum of bacterial species could be isolated and grown for the study through the use of Mannitol Salt Agar (MSA), Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), Salmonella Shigella Agar (SSA), and MacConkey Agar (MA).
Proximate Analysis: The nutritional composition of the clam samples was determined using standard methods (AOAC, 2005). Moisture content was measured by drying clam samples in an oven until a constant weight was achieved. Ash content was determined by incinerating clam samples in a furnace at 550°C. Lipid content was determined using the Soxhlet extraction method, where fat was extracted using petroleum ether. Total protein was quantified using the Kjeldahl method. Crude fiber was estimated by sequentially boiling defatted clam samples in acid and alkali, filtering, drying, and incinerating the residue. Carbohydrate content was calculated by subtracting the percentages of protein, moisture, ash, fiber, and fat from 100%. The energy value was calculated using standard caloric conversion factors for protein, lipids, and carbohydrates.
Biochemical Characterization: Biochemical tests were conducted to identify and classify the isolated Gram-positive bacteria. These included the Catalase Test (observing bubble formation with hydrogen peroxide), Indole Test (red color change with Kovac's reagent), Citrate Utilization Test (blue color change indicating citrate as carbon source), Urea Hydrolysis Test (pink color change indicating urease activity), Motility Fermentation Test (assessing movement in semisolid agar), and Gram's Reaction Test (staining based on cell wall structure).

Data Collection and Analysis

This section involved meticulous recording, quantification, and visualization of data to determine the microbial composition and identify the bacterial isolates from the clam samples.

RESULTS

TABLE 1: Proximate analysis of some selected clams collected from Local vendors in Swali market.

	PARAMETERS	SAMPLE (CLAM)
1	**Moisture content (%)**	**72.78** **72.69** **73.03**
2	**Ash content (%)**	**4.11** **4.14** **4.16**
3	**Crude fiber (%)**	**0.41** **0.47** **0.45**
4	**Total fat (%)**	**0.55** **0.51** **0.54**
5	**Crude protein (%)**	**19.51** **19.55** **19.57**
6	**Carbohydrate (%)**	**2.64** **2.64** **2.69**
7	**Energy value (Kcal/100g**	**93.55** **93.33** **93.29**

The proximate analysis indicates that the clams are a good source of protein (average of 19.5%) and possess a moderate ash content (average of 4.1%), suggesting a decent mineral profile. The fat content is remarkably low (average of 0.53%), making them a lean protein source with a relatively low energy value

(average of 93.4 Kcal/100g). However, the high moisture content (average of 72.8%) is typical for fresh seafood but also renders the clams highly perishable and susceptible to rapid microbial spoilage if not handled and preserved properly.

Table 2: Morphological description and characteristics of bacteria isolate from clams sample

SN	Isolate code	Morphological description	Gram reaction	Catalase	Citrate	Motility	Indole	Urease	TSI Test		Isolate
									H2S	Gas	Identity
1	**CS1**	Creamy spreading colonies with flat irregular shape on NA	**+** cocci	**+**	**+**	**-**	+	+	+	+	*Staphylococcus aureus*
2	**CS2**	Thick creamy colonies on NA	+ cocci	**+**	**+**	**+**	**+**	+	+	+	*Enterococcus faecalis*
3	**CS3**	Black colonies appearance with small and irregular shape on SSA	+cocci	**+**	**+**	**-**	**+**	+	+	+	*Staphylococcus aurues*
4	**CS4**	Pink colonies with moist texture on SSA	+ rod	**+**	**+**	**-**	**+**	**+**	_	+	*Bacillus spp.*
5	**CS5**	Brown colonies with moist appearance on SSA	+ cocci	**+**	**+**	**-**	**+**	**+**	_	+	*Staphylococcus aureus*
6	**CS6**	Light pink with circular shape appearance on SSA	+ cocci	**+**	**+**	**-**	**+**	**+**	_	_	*Staphylococcus epidermidis*

Keys
(+) = Organisms that are reactive to each of the biochemical test carried out.
(-) = Organisms that are not reactive to each of the biochemical test carried out.
Table 2.0 shows the morphological and biochemical reactions of the various bacterial isolates. A total of four gram positive bacteria *Bacillus spp, Staphylococcus epidermis, Staphylococcus aureus, and Enterococcus faecalis* were identified from the clam's sample. The consistency of the observed Gram reactions and morphological descriptions with the identified bacterial identities is generally good, reinforcing the accuracy of the identification process.

Table 3: Bacterial growth based on gram staining reaction

	ORGANISMS	GRAM REACTION
1	*Staphylococcus aureus*	Gram positive cocci
2	*Enterococcus faecalis*	Gram positive Cocci
3	*Bacillus spp.*	Gram positive rod
4	*Staphylococcus epidermidis*	Gram positive cocci

The Result from Table 3 indicates that the following gram positive bacteria were identified from the gram staining reaction of clam isolate: *Staphylococcus aureus, Enterococcus faecalis, Bacillus spp. and Staphylococcus epidermidis.*

Table 4: Percentage of occurrence of microbial isolate from clams' sample.

Organism	No of isolates	% of occurrence
Entercoccus faecalis	1	16.7
Bacillus spp	1	16.7
Staphylococcus epidermis	1	16.7
Staphylococcus aureus	3	50.0

Key: Total population= 6

% of occurrence = $\frac{Number\ of\ occurence}{Total\ population} \times 100$

From table 4.1 the percentage occurrence of bacterial isolated were given as *Staphylococcus aureus 50.0%*, and 16.7% for the following bacteria *Enterococcus faecalis, Bacillus spp, and Staphylococcus epidermis*.

The microbiological analysis revealed the presence of several bacterial species, with varying prevalence:
Staphylococcus aureus: This was the most prevalent organism, accounting for 50% (3 out of 6) of the identified isolates. The consistent Gram-positive cocci morphology and positive reactions for Catalase, Citrate, Indole, and Urease, along with negative motility, align with the known characteristics of *S. aureus*. The presence of *Staphylococcus aureus* in food, especially at such a high frequency, is a major public health concern. *S. aureus* is a common cause of food poisoning due to the production of heat-stable enterotoxins, often indicating contamination from human handlers (skin, nasal passages) or poor hygiene during handling.
Enterococcus faecalis: Represented by 16.7% (1 out of 6) of the isolates. *E. faecalis* is a Gram-positive coccus that is frequently used as an indicator of fecal contamination in aquatic environments and food products. While generally commensal, some strains can cause opportunistic infections. Its presence suggests potential fecal contamination of the clams or the water they were harvested from.
Bacillus spp. One isolate (16.7%) was identified as *Bacillus spp.*, a Gram-positive rod. While *Bacillus* species are common environmental bacteria, some, like *Bacillus cereus*, are known to cause foodborne illnesses. Their presence might indicate contamination from soil or dust, or could signify spoilage potential.
Staphylococcus epidermidis: Also accounting for 16.7% (1 out of 6) of the isolates. *S. epidermidis* is typically a commensal bacterium of human skin. Its isolation points towards potential contamination from human handlers during harvesting, transportation, or sale.

Table 5: Bacterial counts from clams sample.

S/NO	Isolate codes	No of counts (Dilution 10^{-2})	CFU/g	Log CFu/g
1	CS1	52	5.2×10^{9}	9.716
2	CS2	35	3.5×10^{9}	9.544
3	CS4	45	4.5×10^{9}	9.653
4	CS6	31	3.1×10^{9}	9.491

Key CS1- colonies count of *S. aureus*, CS2 – colony count of *Enterococcus faecalis,* CS4 – colony count of *Bacillus spp*., CS6 – colony count *of Staphylococcus epidermidis.* This table shows the different colony counts of microorganisms isolated from clams' sample.

Microbial Load (CFU/g):
The enumeration of microbial load revealed extremely high bacterial counts in the clam samples. These values are significantly above acceptable limits for fresh seafood. Total viable counts exceeding 106 to 107 CFU/g are generally indicative of poor microbiological quality, spoilage, and potential health hazards. Counts in the order of 109 CFU/g suggest massive bacterial contamination and advanced spoilage, rendering the clams unsafe for human consumption. This high microbial load points towards severe deficiencies in handling, storage, and sanitation practices from harvesting to the point of sale at Swali Market.

DISCUSSION
Our observed bacterial counts ($10^{\wedge 9}$ CFU/g) are significantly higher than many reported values for fresh clams from other regions, including some within Nigeria [7,11]. While a study in Ghana reported counts as high as $10^{\wedge 10}$ CFU/g during the dry season (which is comparable or even higher than ours) [3], the $10^{\wedge 4}$ to $10^{\wedge 7}$ range is more commonly found in many other studies [1, 12]. This indicates that the clams from Swali Market, Yenagoa, are under severe bacterial load, suggesting critical issues with hygiene, handling, or environmental quality.
Our finding of 50% prevalence of *Staphylococcus aureus* is notably high compared to some other clam studies in Nigeria [7] but aligns with the higher prevalence rates observed for *S. aureus* in other seafood products [6, 9]. This strongly suggests that *S. aureus* contamination is a widespread problem in seafood supply chains in Nigeria, pointing to issues with human hygiene and sanitation. The presence of *Enterococcus faecalis* in the samples is similar to other studies, which confirms ongoing fecal contamination in the environment or during handling [6,13].

The results for moisture content (72.8%) are consistent with typical fresh clam values reported globally (e.g., 72-82% range) [14,15]. The protein content (19.5%) falls within the range found in various studies [14,16], though it's lower than the 27-30% reported in some Nigerian and Cameroonian clam studies [17, 18], possibly due to species differences, seasonality, or environmental factors. The fat content (0.53%) is very low, which is generally a positive nutritional attribute for seafood and is comparable to the lower end of fat ranges reported in other bivalves [14, 19]. Ash content (4.1%) also falls within typical ranges [14, 20]. Overall, the nutritional profile of clams from Swali Market seems generally good, aligning with clams being a lean protein source. However, the significantly high microbial load and the prevalence of potential pathogens overshadow these nutritional benefits and raise serious food safety concerns. The microbiological findings for clams from Swali Market, Yenagoa, are alarming when compared to national and international benchmarks. The extremely high bacterial counts and the high prevalence of *Staphylococcus aureus* indicate a severe public health risk.

CONCLUSION

While the proximate composition suggests a nutritious food source, the profound microbial contamination strongly advises against the consumption of these clams without significant improvements in harvesting, handling, and storage practices. These results underscore the urgent need for enhanced food safety regulations and monitoring in local markets in Yenagoa and similar regions.

REFERENCES

1. Nwosu, O. R., Obire, O., Wemedo, S. A., & Ogbonna, D. N. (2020). Microbiological and nutritional quality of market and home smoke dried shrimp. *Journal of Advances in Microbiology*, *20*(5), 35-42.
2. Healthline. (n.d.). *Shellfish: Types, Nutrition, Benefits, and Dangers*. Retrieved from https://www. healthline.com/nutrition/shellfish
3. Oramadike, C. E., Olajuyigbe, O. O., Amusan, E. E., Esangbedo, R. O., & Awoyale, M. O. (2024). Characterisation and antibiotic susceptibility pattern of bacterial isolates obtained from some shellfish sold in Lagos-West, Nigeria. *Acta Aquatica: Aquatic Sciences Journal*, *11*(3), 272-277.
4. Tebekaemi, E. F. (2021). Organoleptic assessment of processed freshwater clam (Galatea paradoxa). Conference: 27th Conf. of Fisheries Society of Nigeria (FISON)At: Yenagoa, Bayelsa StateVolume: 145 – 148
5. Zige, D. V., & Ayibakeme, A. (2023). Occurrence of Potential Bacterial Pathogens in Food from Local Restaurants in Azikoro Town, Bayelsa State, Nigeria. International Research Journal of Gastroenterology and Hepatology, 6 (1): 40-45,
6. Kingdom, T., Zige, D. V., & Anesakeme, D. (2018). Assessing the hygienic status of processed fresh water clam (Galatea paradoxa) in Yenagoa Metropolis, Bayelsa State, Niger Delta, Nigeria. *American Journal of Food Science and Technology*, *6*(5), 219-222.
7. Udoh, D. I., Udo, I. U., & Udoh, E. I. (2017). Microbiological analysis of the freshwater clam (Galatea paradoxa, Born 1778) caught from Cross River, Nigeria. *Nigerian journal of Agriculture, food and Environment*, *13*(3), 59-64.
8. Kingdom, T., & Azagba, I. A. (2017). Heavy metal concentration in soft tissues of freshwater clam (*Galatea paradoxa* Born 1778) from Diebu Creek, Bayelsa. *World Journal of Advanced Research and Reviews*, *21*(01), 1220–1226.
9. Oku, I. Y., & Alagoa, K. J. (2019). Microbial quality of cooked and processed foods sold by food vendors and restaurant in Niger Delta University community, Amassoma, Bayelsa State. *East African Scholars Journal of Biotechnology and Genetics*, *1*(1), 1-8.
10. Beshiru, A., Bello, S. N., Ajiboye, A. A., & Oloniniyi, M. A. (2021). Prevalence and Characterization of Food-Borne *Vibrio parahaemolyticus* From African Salad in Southern Nigeria. *Frontiers in Microbiology*, *12*, 632266.
11. Amoah, B. D., Mensah, A., Darko, L., Abugre, E. K., Asoandzie, D. N., Opoku-Gyebi, F., ... & Afful, P. (2024). Microbial Load and Antibiotic Resistance Pattern of Microflora Isolated from Smoked Catfish and Mackerel Sold in Ghanaian Markets: A Potential Health Threat. *International Journal of Pathogen Research*, *13*(5), 1-11.
12. Kombat, E. O., Nunoo, F. K., Ampofo, J. A., & Addo, P. G. (2013). Effects of environmental conditions on the microbiological quality of two small marine pelagic fishes landed in Accra and Tema, Ghana. *Arch. Appl. Sci. Res*, *5*(2), 180-188.
13. Ozoh, C. N., & Orji, M. U. (2022). Comparative study of Bacteriological Contents of Commercially Smoked Fish and Aseptically Smoked Fish sold in Awka and Environs, Anambra State Nigeria.
14. Ashang, U. J., Antai, S. P., & Tiku, D. R. (2017). Distribution of antibiotic resistant bacteria flora in ready to-eat food samples sold in Calabar Metropolis. *World Journal of Pharmaceutical and Medical Research*, *3*(11), 198-125.
15. Rak, A. E., Nasir, S. N. A. M., Nor, M. M., Han, D. K., Appalasamy, S., Abdullah, F., & Ghazi, R. M. (2020). Proximate analysis and fatty acid of Corbicula fluminea (C. fluminea) tissue in Kelantan, Malaysia. *Environmental Science and Pollution Research*, *27*(20), 24772-24785.
16. FAO (Food and Agriculture Organization of the United Nations). (2022). *Composition of foods*. (accessed on the 22nd of July, 2025)
17. Iwar, M. I., Dauda, I. A., & Mbayion, T. S. (2024). Nutrient composition of fresh water clams (Galatea paradoxa, born 1778) from River Benue, Benue State-Nigeria. *Journal of Research in Forestry, Wildlife and Environment*, *16*(2), 76-84.

18. Ivon, E. A., & Eyo, V. O. (2018). Proximate composition and mineral contents of edible part of four species of shellfishes from the Calabar River, Nigeria. *Annual Research and Review in Biology*, *26*(1), 1-10.
19. Etim, L., & Taege, M. (1993). Comparison of condition indices and their seasonal variation in the freshwater clam, *Egeria radiata* (Lamarck) (Tellinacea: Donacidae), from the Cross River, Nigeria. *Aquaculture Research*, *24*(5), 603-612.
20. Ivon, E. A., & Eyo, V. O. (2018). Proximate composition and mineral contents of edible part of four species of shellfishes from the Calabar River, Nigeria. *Annual Research and Review in Biology*, *26*(1), 1-10.

CITATION OF THIS ARTICLE

Faith I. Omeje and Omotayo B. Ilesanmi· Morphological, Biochemical, and Enumeration Studies of Bacterial Isolates from Fresh Water Clam (*Galatea paradoxa*) in Swali Market, Bayelsa State, Nigeria. Bull. Env. Pharmacol. Life Sci., Vol 14 [9] August 2025: 01-06