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Bacterial load and pathogenic isolates in commercially important freshwater fish from River Niger, Onitsha, Nigeria: Food safety implications

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ABSTRACT: Maintaining the microbiological safety of fish is essential for safeguarding public health, particularly in freshwater systems increasingly impacted by anthropogenic activities. This study evaluated the bacterial load and diversity of three commercially important freshwater fish species (*Citharinus citharus*, *Marcusenius kainjii*, and *Synodontis membranaceus*) obtained from two major landing sites (Niger Street and Ose Marine) along the River Niger at Onitsha, Nigeria, with emphasis on food safety implications. Fish samples were collected weekly in May 2024 and analysed for total viable bacterial counts (TVC) and bacterial isolates using standard culture and biochemical methods. Total viable count values ranged from 6.52 to 6.59 $\log_{10}$ CFU g^{-1}, falling within generally accepted limits of 7.0 $\log_{10}$ CFUg^{-1} by the International Commission on Microbiological Specifications for Foods for fresh fish but indicating moderate microbial loads. Gill tissues from Niger Street landing site (6.53 $\log_{10}$ CFU g^{-1}) and Ose Marine landing site (6.59 $\log_{10}$ CFUg^{-1}) showed higher bacterial counts than skin (6.52 $\log_{10}$ CFU g^{-1} and 6.59 $\log_{10}$ CFUg^{-1} respectively), thereby reflecting greater environmental exposure. A diverse bacterial community was identified, dominated by *Vibrio spp.* and *Shigella spp.*, alongside *Escherichia coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Clostridium spp.*, and *Klebsiella spp.* Similar bacterial genera were detected in water samples, demonstrating a strong environmental linkage. Although bacterial loads were within acceptable limits, the presence of potentially pathogenic genera highlights a critical limitation of relying solely on total viable counts as indicators of food safety. The findings indicate that microbial contamination in the study area is driven by a combination of environmental pollution, faecal inputs, and post-harvest handling practices. This study emphasises the need for integrated monitoring approaches utilising quantitative and qualitative microbial assessments, alongside improved wastewater management and hygienic handling practices, to enhance fish food safety and protect public health.

Keywords: Aquatic microbiome, artisanal fisheries, freshwater fish, riverine ecosystem, total viable count.

INTRODUCTION

Fish constitute a major source of high-quality protein, essential fatty acids, and micronutrients, and play a critical role in global food security, particularly in developing countries (Noreen *et al* 2025). However, the microbiological safety of fish and fishery products has become an increasing concern due to their susceptibility to contamination throughout the production and supply chain. Microbial contamination in fish is widely recognised as a major factor affecting both spoilage and foodborne disease risk, with contamination occurring during capture, handling, processing, and storage (Cortés-Sánchez *et al.,* 2025a).

Freshwater ecosystems are particularly vulnerable to anthropogenic pollution, including wastewater discharge, agricultural runoff, and urban activities, which introduce a wide range of microorganisms into aquatic environments (Labbate *et al.,* 2016). Recent studies emphasise that microbial contamination of aquatic systems is largely driven by human activities and represents a significant public health concern due to the persistence and trans-

mission of pathogenic microorganisms in water bodies (Nazmul *et al.,* 2025; Popoola *et al.,* 2025). These contaminated environments serve as reservoirs of bacteria that can colonise aquatic organisms, including fish, thereby linking environmental quality directly to food safety outcomes (Rose*,* 2026).

Fish are highly susceptible to microbial contamination due to their high moisture content, neutral pH, and nutrient-rich tissues, which support bacterial growth (Zhang *et al* 2025). Microorganisms associated with fish include both autochthonous aquatic bacteria and allochthonous contaminants of faecal origin (Cortez-Sanchez et al 2025). The most commonly reported bacterial genera in fish and aquatic environments are *Vibrio spp.*, *Escherichia coli*, *Shigella spp.*, *Staphylococcus spp.*, and *Clostridium spp.*, many of which are associated with foodborne illnesses and public health risks. (Bintsis,2017; Roy *et al.,* 2024; Cortés-Sánchez *et al.,* 2025b).

The presence of enteric bacteria such as *E. coli* and *Shigella spp.* is particularly significant, as these organisms are widely used as indicators of faecal contamination and poor sanitary conditions. Their detection in fish or water suggests contamination from sewage or human and animal waste. In contrast, genera such as *Vibrio* are naturally occurring in aquatic environments but include species capable of causing severe infections in humans. The coexistence of these bacterial groups reflects the combined influence of environmental conditions and anthropogenic inputs on aquatic microbial communities (Chen *et al.,* 2018; Kapetanović *et al.,* 2024; Wang *et al.,* 2024)

Recent research has further highlighted the interconnected relationship between water quality and fish microbiota, demonstrating that microbial communities present in water significantly influence those associated with fish (Li *et al.,* 2026). For example, studies have shown that pathogenic bacteria such as *E. coli* and *Salmonella* can be simultaneously detected in both water and fish, indicating direct environmental transmission pathways (Niu *et al.,* 2025., Wang *et al.,* 2026; Xu *et al.,* 2026). Similarly, microbial source-tracking studies have revealed that environmental and processing conditions contribute substantially to the microbial composition of fish products, reinforcing the role of external contamination sources in shaping fish-associated microbiota (Corral-Jara *et al.,* 2024; Li *et al.,* 2024; Zhang *et al.,* 2025)

In Nigeria, the River Niger represents one of the most important freshwater systems supporting artisanal fisheries and local livelihoods. However, increasing urbanisation and commercial activities in cities such as Onitsha have led to continuous inputs of domestic and industrial waste into the river, potentially compromising water quality and the safety of fish harvested from this environment. Despite the importance of this ecosystem, there remains a paucity of recent, site-specific data on the microbial load and diversity of bacteria in commercially important fish species from this region, particularly in relation to food safety implications (Mgbemena *et al.,* 2011).

Assessment of total viable bacterial counts (TVC) provides an indication of overall microbial load and hygiene status, while identification of bacterial isolates offers insight into contamination sources and potential health risks (Bottini *et al.,* 2011). However, reliance on total counts alone may be insufficient, as the presence of pathogenic bacteria can pose significant risks even when microbial loads fall within acceptable limits. Therefore, integrated approaches combining quantitative microbial assessment with bacterial identification are essential for a comprehensive evaluation of fish safety (Sheng and Wang, 2021).

This study aimed to evaluate the bacterial load and diversity of selected commercially important freshwater fish species (*Citharinus citharus*, *Marcusenius kainjii*, and *Synodontis membranaceus*) obtained from two major landing sites along the River Niger at Onitsha, Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted at two major artisanal fish landing sites (Niger Street Landing and Ose Marine Landing) located along the River Niger in Onitsha North Local Government Area, Anambra State, Nigeria (6º04′ N, 5º04′ E) as shown in the map (Figure 1). These sites are characterized by intense fishing activity and close proximity to densely populated urban settlements that rely heavily on riverine fisheries for food and livelihood. The region lies within a humid tropical climate, with a mean annual temperature of approximately 28ºC and distinct wet (April-October) and dry (November-March) seasons. The River Niger in this area is subject to varying degrees of anthropogenic pollution, including domestic wastewater discharge, market runoff, and other urban inputs, which may influence microbial contamination of aquatic biota.

Fish sampling and experimental design

Three commercially important freshwater fish species; *Citharinus citharus* commonly called moonfish and locally known as ‘Mpete’, *Marcusenius kainjii* commonly called trunkfish and locally known as ‘Obor’ and *Synodontis membranaceus* commonly called Moustache catfish and locally known as ‘Okpor’ were selected based on their economic importance and consumption frequency. Sampling was conducted weekly over month of May 2024 at the two landing sites. A total of 18 fish specimens were collected at each sampling, comprising three individuals per species per site. Freshly caught fish were obtained directly from artisanal fishers using sterile polyethylene bags and transported in insulated ice chests containing crushed ice to the Maeve Academic Research Laboratory,

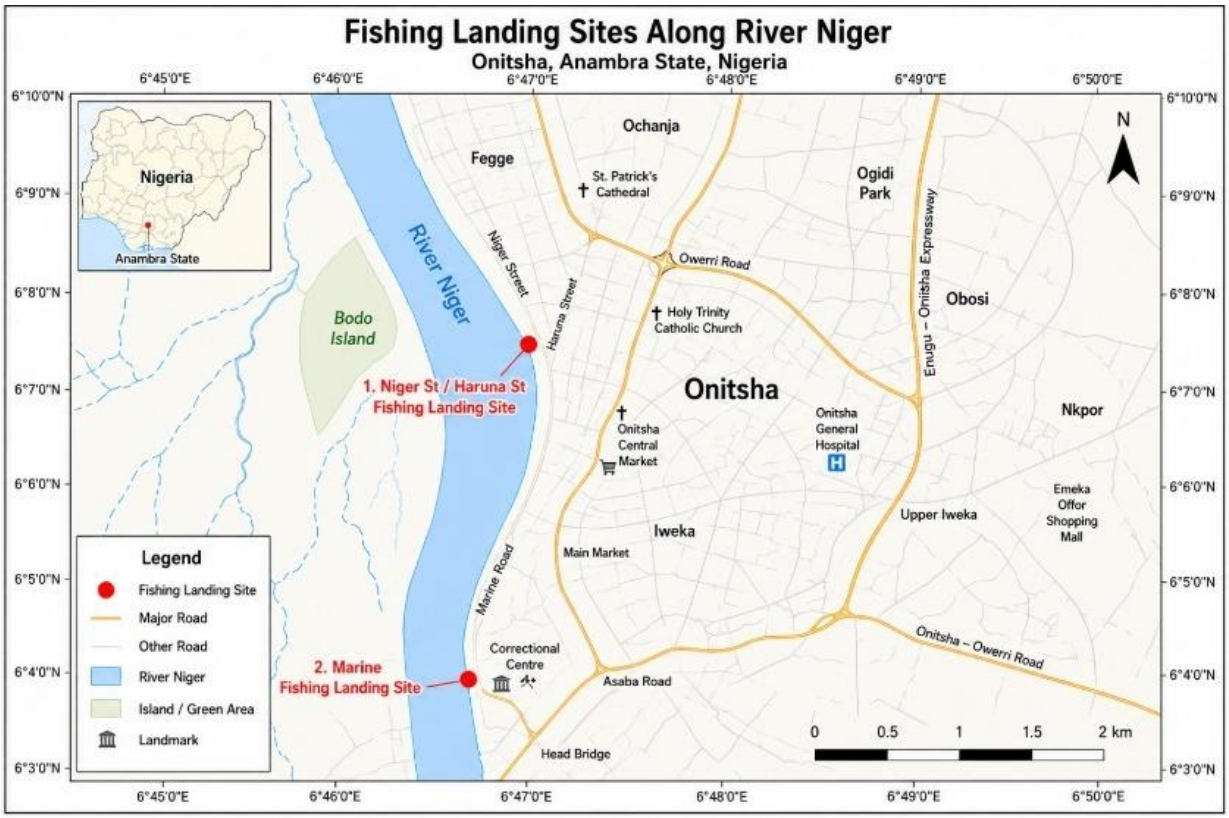

Figure 1. Niger Street Landing and Ose Marine Landing.

Onitsha, within 30 minutes of collection. The study followed a factorial design considering:

- Site (Niger Street vs Ose Marine)
- Species (3 species)
- Tissue type (skin and gills) (Chittaranjan 2024)

All samples were processed immediately upon arrival to minimise post-harvest microbial changes.

Sample preparation

Fish samples were handled aseptically on sterilised laboratory surfaces. Each specimen was rinsed with sterile distilled water, blotted dry with sterile tissue, and dissected using sterile instruments. Approximately 5 g of skin and gill tissues were excised, weighed (±0.01 g), and transferred into sterile containers. Samples were either processed immediately or stored at 4 ºC for a maximum of 12 hours prior to analysis (Alikunhi *et al.,* 2017).

Microbiological analysis

Serial dilution and plating

Each 5 g tissue sample was homogenized in 45 mL of sterile physiological saline (0.85% NaCl) using a stomacher to obtain a 10^{-1} dilution. Serial tenfold dilutions were prepared up to 10^{-5}. Aliquots (1 mL) of appropriate dilutions were plated in duplicate using the pour plate method (Terrones-Fernandez *et al* 2023) on:

- Nutrient Agar (NA) for total viable counts (TVC),
- MacConkey Agar (MAC) for enteric and coliform bacteria, and
- Eosin Methylene Blue (EMB) agar for confirmation of faecal coliforms.

Plates were incubated at 37 ± 2ºC for 24 - 48 hours. Only plates containing 30 - 300 colonies were considered for enumeration (APHA, 2017; Thomas *et al.,* 2015).

Enumeration and Identification of Isolates

Bacterial isolates were presumptively identified based on colony morphology, growth on selective media, and standard biochemical tests. Colonies were enumerated using a digital colony counter and expressed as colony-forming units per gram (CFU g^{-1}). Representative colonies were selected based on distinct morphological characteristics and purified by repeated sub-culturing. Identification of isolates was carried out using a combination of colony morphology, Gram staining, and biochemical characterisa-

tion, including catalase, oxidase, indole, coagulase, citrate utilisation, and carbohydrate fermentation tests. All tests were performed following standard microbiological procedures. The identification scheme was guided by standard taxonomic keys and biochemical identification protocols, with reference to *Bergey's Manual of Systematic Bacteriology* (Whitman, 2015) and standard microbiological methods (APHA, 2017). Quality assurance was ensured using reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (Al Qahtani et al., 2025).

Ethical considerations

All procedures involving fish handling were conducted in accordance with the guidelines of the Nnamdi Azikiwe University Animal Research Ethics Committee (Approval No. NAU/AREC/2024/0094). Fish were transported on ice to minimise stress and handled aseptically in accordance with institutional protocols for aquatic research.

Statistical analysis

Bacterial counts were $\log_{10}$-transformed prior to analysis to achieve normality. Data were expressed as mean ± standard deviation. Differences in bacterial loads were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS version 26.0 (IBM Corp, USA).

RESULTS

Total viable bacterial counts (TVC) in fish tissues

Total viable bacterial counts (TVC) in fish samples, expressed as $\log_{10}$ CFU g^{-1}, are presented in Table 1. Across both sampling sites, TVC values ranged from 6.52 ± 0.02 to 6.59 ± 0.01 $\log_{10}$ CFU g^{-1}. At the Niger Street landing site, mean bacterial loads were 6.52 ± 0.02 $\log_{10}$ CFU g^{-1} in skin samples and 6.53 ± 0.01 $\log_{10}$ CFU g^{-1} in gill samples. Similarly, at the Ose Marine site, TVC values were 6.53 ± 0.03 $\log_{10}$ CFU g^{-1} for skin and 6.59 ± 0.01 $\log_{10}$ CFU g^{-1} for gill tissues. Across both locations, gill tissues exhibited slightly higher bacterial loads than corresponding skin samples. However, these differences were not statistically significant (one-way ANOVA, $p > 0.05$). Likewise, no significant variation in microbial load was observed between the two sampling sites. All recorded bacterial loads were below the maximum permissible limit of 7 $\log_{10}$ CFU g^{-1} recommended by the International Commission on Microbiological Specifications for Foods (ICMSF), indicating that the fish samples were within acceptable microbiological limits.

Table 1. Total viable bacterial counts (TVC) in skin and gill tissues of fish from River Niger, Onitsha.

Sample Site	Tissue	TVC ($\log_{10}$ CFU g^{-1})
Niger Street	Skin	6.52 ± 0.02
Niger Street	Gills	6.53 ± 0.01
Ose Marine	Skin	6.53 ± 0.03
Ose Marine	Gills	6.59 ± 0.01

Values are expressed as mean ± standard deviation (n = 3). No significant differences were observed between tissues or sampling sites (one-way ANOVA, $p > 0.05$). The maximum acceptable limit for fresh fish is ≤ 7 $\log_{10}$ CFU g^{-1} (ICMSF, 2022).

Distribution of bacterial isolates in fish tissues

The mean counts of bacterial isolates recovered from the skin and gill tissues of the three fish species across both landing sites are presented in Table 2. Across all samples, *Vibrio spp*. and *Shigella spp.* consistently exhibited the highest counts, with values ranging from 5.50 ± 0.58 to 7.25 ± 0.50 ×10^5 CFU g^{-1}, indicating their dominance in both sampling locations. These organisms were detected in both skin and gill tissues of all fish species, with generally higher counts observed in gill samples. *Staphylococcus spp.* and *Streptococcus spp.* showed moderate abundance, with counts ranging from 2.00 ± 0.00 to 3.75 ± 0.50 ×10^5 CFU g^{-1}, and were widely distributed across species and sampling sites. In contrast, *Escherichia coli* and *Klebsiella spp.* were detected at relatively lower levels, typically ranging between 1.25 ± 0.50 and 2.00 ± 0.82 ×10^5 CFU g^{-1}. Notably, *Klebsiella spp.* was not detected in samples from the Ose Marine site but was present at low levels in samples from Niger Street. *Clostridium spp*. were also identified at low to moderate levels across all species, with counts ranging from 1.75 ± 0.50 to 2.75 ± 0.50 ×10^5 CFU g^{-1}, showing slightly higher values in gill tissues compared to skin. Across fish species, *Synodontis membranaceus* and *Marcusenius kainjii* generally exhibited higher bacterial loads compared to *Citharinus citharus* although variations among species were not pronounced. Similarly, gill tissues consistently showed higher bacterial counts than skin tissues across most bacterial groups and sampling sites. The distribution pattern of bacterial isolates was consistent between the two landing sites, with only minor variations in abundance, indicating similar microbial contamination profiles in fish from both locations.

Biochemical characterization of bacterial isolates

The biochemical characteristics of bacterial isolates recovered from fish samples are summarised in Table 3. Identification of isolates was presumptive, based on standard morphological and biochemical tests. The isolates comprised both Gram-positive and Gram-negative bacteria, indicating a diverse microbial community

Table 2. Mean counts of bacterial isolates (×10⁵ CFU g⁻¹) in skin and gill tissues of selected fish species from River Niger, Onitsha.

Bacterial Species	Site	Tissue	*C. Citharus*	*M. Kainjii*	*S.membranaceus*
Staphylococcus spp.	Ose	Gill	2.75 ± 0.50	1.75 ± 1.26	2.50 ± 0.58
	Ose	Skin	2.05 ± 0.58	1.75 ± 1.26	2.25 ± 0.50
	Niger	Gill	2.75 ± 0.50	2.00 ± 0.82	2.00 ± 0.00
	Niger	Skin	2.50 ± 0.58	1.75 ± 1.26	2.00 ± 0.82
Escherichia coli	Ose	Gill	1.50 ± 0.58	1.75 ± 0.50	1.50 ± 0.58
	Ose	Skin	1.25 ± 0.50	1.50 ± 0.58	1.25 ± 0.50
	Niger	Gill	1.75 ± 0.50	2.00 ± 0.82	1.75 ± 0.50
	Niger	Skin	1.50 ± 0.58	1.75 ± 0.50	1.50 ± 0.58
Vibrio spp.	Ose	Gill	6.75 ± 0.50	7.00 ± 0.00	7.25 ± 0.50
	Ose	Skin	6.25 ± 0.50	6.50 ± 0.58	6.75 ± 0.50
	Niger	Gill	6.25 ± 0.50	6.50 ± 0.58	6.50 ± 0.58
	Niger	Skin	5.75 ± 0.50	6.00 ± 0.00	6.25 ± 0.50
Shigella spp.	Ose	Gill	6.25 ± 0.50	6.50 ± 0.58	6.75 ± 0.50
	Ose	Skin	5.75 ± 0.50	6.00 ± 0.00	6.25 ± 0.50
	Niger	Gill	6.00 ± 0.00	6.25 ± 0.50	6.25 ± 0.50
	Niger	Skin	5.50 ± 0.58	5.75 ± 0.50	6.00 ± 0.00
Streptococcus spp.	Ose	Gill	3.25 ± 0.50	3.50 ± 0.58	3.75 ± 0.50
	Ose	Skin	3.00 ± 0.00	3.25 ± 0.50	3.50 ± 0.58
	Niger	Gill	3.00 ± 0.00	3.25 ± 0.50	3.25 ± 0.50
	Niger	Skin	2.75 ± 0.50	3.00 ± 0.00	3.00 ± 0.00
Clostridium spp.	Ose	Gill	2.25 ± 0.50	2.50 ± 0.58	2.75 ± 0.50
	Ose	Skin	2.00 ± 0.00	2.25 ± 0.50	2.50 ± 0.58
	Niger	Gill	2.00 ± 0.00	2.25 ± 0.50	2.25 ± 0.50
	Niger	Skin	1.75 ± 0.50	2.00 ± 0.00	2.00 ± 0.00
Klebsiella spp.	Ose	Gill	ND	ND	ND
	Ose	Skin	ND	ND	ND
	Niger	Gill	1.50 ± 0.58	1.75 ± 0.50	1.50 ± 0.58
	Niger	Skin	1.25 ± 0.50	1.50 ± 0.58	1.25 ± 0.50

Values are expressed as mean ± standard deviation (×10⁵ CFU g⁻¹; n = 3). ND = Not detected.

associated with the fish samples. Gram-negative isolates were characterised by variable indole reactions, motility, and predominantly oxidase-negative profiles, consistent with enteric bacteria. In particular, isolates presumptively identified as *Escherichia coli* showed positive indole and motility reactions with oxidase negativity, while *Klebsiella spp.* and *Shigella spp.* were non-motile and oxidase-negative, supporting their classification as enteric organisms.

In contrast, isolates presumptively identified as *Vibrio spp.* exhibited motility and oxidase-positive reactions, distinguishing them from other Gram-negative bacteria and supporting their identification as typical aquatic microorganisms. This biochemical profile is consistent with organisms adapted to aquatic environments and aligns with their widespread occurrence across all samples in the present study.

Among the Gram-positive isolates, *Staphylococcus spp.* were characterised by oxidase positivity and lack of motility, while *Streptococcus spp.* were non-motile and oxidase-negative. Isolates classified as *Clostridium spp.* showed positive reactions for indole, motility, and glucose fermentation, consistent with characteristics of spore-forming bacteria.

All isolates demonstrated the ability to utilise and ferment glucose, indicating active metabolic capacity and adaptability to nutrient-rich environments. The combination of biochemical reactions observed across isolates

Table 3. Biochemical characteristics of presumptively identified bacterial isolates from fish samples.

Bacterial Isolate	Gram Reaction	Indole	Motility	Oxidase	Glucose Fermentation	Interpretation
Staphylococcus spp.	+	–	–	+	+	Consistent with Gram-positive cocci associated with handling contamination
Escherichia coli	–	+	+	–	+	Consistent with enteric, fecal indicator bacteria
Vibrio spp.	–	+	+	+	+	Consistent with aquatic, oxidase-positive bacteria
Shigella spp.	–	–	–	–	+	Consistent with non-motile enteric pathogens
Streptococcus spp.	+	–	–	–	+	Consistent with Gram-positive cocci of environmental/host origin
Clostridium spp.	+	+	+	–	+	Consistent with anaerobic or facultative spore-forming bacteria
Klebsiella spp.	–	–	–	–	+	Consistent with non-motile enteric bacteria

(+): positive reaction; (–): negative reaction. Identification of isolates was presumptive, based on standard morphological and biochemical characteristics.

Table 4. Bacterial Isolates in water samples.

Bacterial Isolate	Niger Street	Ose Marine
Staphylococcus spp.	3.00 ± 0.00	2.50 ± 0.58
Escherichia coli	0.50 ± 1.00	0.25 ± 0.50
Vibrio spp.	2.50 ± 0.58	2.25 ± 0.50
Shigella spp.	1.50 ± 1.00	2.67 ± 0.58
Streptococcus spp.	2.00 ± 1.83	1.25 ± 1.50
Clostridium spp.	1.00 ± 1.15	1.00 ± 1.15
Klebsiella spp.	0.50 ± 1.00	ND

Values are expressed as mean ± standard deviation ($\times 10^5$ CFU mL^{-1}; n = 3). ND = Not detected.

provided a consistent basis for differentiating bacterial groups and supported the identification of the major genera reported in this study.

Generally, the biochemical profiles obtained corroborate the distribution of bacterial genera observed in Table 2 and confirm the presence of a mixed population of environmental, enteric, and handling-associated bacteria in fish samples from the study area.

Bacterial Isolates in water samples

The mean counts of bacterial isolates recovered from water samples at the Niger Street and Ose Marine landing sites are presented in Table 4. A diverse range of bacterial genera was detected in water samples from both locations, including *Staphylococcus spp.*, *Escherichia coli*, *Vibrio spp.*, *Shigella spp.*, *Streptococcus spp.*, *Clostridium spp.*, and *Klebsiella spp*. Overall, the composition of bacterial isolates in water samples closely mirrored those identified in fish tissues.

At the Niger Street landing site, *Staphylococcus spp.* exhibited the highest mean count (3.00 ± 0.00 $\times 10^5$ CFU mL^{-1}), followed by *Vibrio spp.* (2.50 ± 0.58 $\times 10^5$ CFU mL^{-1}) and *Streptococcus spp.* (2.00 ± 1.83 $\times 10^5$ CFU mL^{-1}). Lower counts were recorded for *Shigella spp.* (1.50 ± 1.00 $\times 10^5$ CFU mL^{-1}), *Clostridium spp.* (1.00 ± 1.15 $\times 10^5$ CFU mL^{-1}), and both *Escherichia coli* and *Klebsiella spp.* (each 0.50 ± 1.00 $\times 10^5$ CFU mL^{-1}).

At the Ose Marine site, *Shigella spp.* showed the highest abundance (2.67 ± 0.58 $\times 10^5$ CFU mL^{-1}), followed by *Staphylococcus spp.* (2.50 ± 0.58 $\times 10^5$ CFU mL^{-1}) and *Vibrio spp.* (2.25 ± 0.50 $\times 10^5$ CFU mL^{-1}). Moderate levels of *Streptococcus spp.* (1.25 ± 1.50 $\times 10^5$ CFU mL^{-1}) and *Clostridium spp.* (1.00 ± 1.15 $\times 10^5$ CFU mL^{-1}) were also observed, while *Escherichia coli* was detected at low levels (0.25 ± 0.50 $\times 10^5$ CFU mL^{-1}). *Klebsiella spp.* was not detected in water samples from this site.

Comparatively, bacterial profiles were broadly similar between the two landing sites, with only minor differences in the relative abundance of individual genera. Notably, enteric bacteria such as *Escherichia coli*, *Shigella spp.*, and *Klebsiella spp.* were present in water samples, indicating fecal contamination of the aquatic environment. Overall, the occurrence of similar bacterial genera in both water and fish samples suggests that the surrounding aquatic environment serves as a primary source of microbial contamination in fish from the study area.

DISCUSSION

The total viable bacterial counts (TVC) observed in this study (6.52–6.59 $\log_{10}$ CFU g^{-1}) indicate moderate microbial loads in fish obtained from both landing sites. Although these values fall below the generally accepted maximum limit for fresh fish (≤7 $\log_{10}$ CFU g^{-1}), their proximity to this threshold suggests that the fish were not of optimal microbiological quality and were likely exposed to environmental contamination and post-harvest handling influences. Total viable counts in this range have been reported in fish from urbanised freshwater systems, where microbial loads are strongly influenced by organic pollution and human activities (Bintsis, 2017, Marijani, E. 2022; Mitiku *et al.,* 2022).

The relatively narrow range of TVC values across both sites suggests comparatively homogeneous environmental conditions influencing microbial colonisation. In river systems impacted by urbanisation, continuous inputs from wastewater discharge, surface runoff, and anthropogenic activities elevate baseline microbial loads, which are subsequently reflected in fish microbiota (Parwin *et al.,* 2025; Bharti *et al.,* 2023). This pattern indicates that contamination in the present study is likely driven by diffuse pollution sources rather than localised point inputs.

Furthermore, recent research in aquatic microbiology highlights that microbial load alone is an incomplete indicator of fish health and safety (Abdallah-Ruiz *et al.,* 2022). Shifts in microbial communities (dysbiosis), even at moderate bacterial loads, can increase disease susceptibility and facilitate the proliferation of opportunistic pathogens (Ling *et al.,* 2025; Shen *et al.,* 2025). This suggests that the observed TVC levels, although acceptable, may still reflect underlying environmental stressors capable of influencing microbial dynamics in fish.

The consistently higher bacterial loads observed in gill tissues compared to skin are in agreement with recent microbiome-based studies, which demonstrate that fish gills serve as primary interfaces with the aquatic environment and hotspots for microbial accumulation (Wang *et al.,* 2025; Sehnal *et al.,* 2021). Gill and skin microbiomes are highly dynamic and responsive to environmental conditions, with gill surfaces particularly prone to colonisation due to continuous water filtration and exposure to suspended microorganisms (Debnath *et al.,* 2025). This anatomical and functional role explains the slightly elevated bacterial loads recorded in gill tissues in the present study.

The distribution of bacterial isolates provides further insight into contamination pathways. The predominance of *Vibrio spp.* across all samples reflects their status as indigenous aquatic bacteria that thrive in nutrient-rich environments. Their widespread occurrence is consistent with their ecological adaptability and association with organic enrichment (Sampaio *et al.,* 2022; EFSA Panel on Biological Hazards *et al.,* 2024). In contrast, the detection of *Shigella spp., Escherichia coli*, and *Klebsiella spp*. indicates faecal contamination, most likely arising from untreated wastewater, surface runoff, and human activities around the landing sites. These enteric bacteria are widely recognised indicators of compromised sanitary conditions and reinforce the influence of anthropogenic inputs on water quality (Pereira *et al*., 2024).

The presence of Staphylococcus spp. across fish and water samples suggests an additional contribution from post-harvest handling and human contact, as these organisms are commonly associated with skin and handling surfaces (Haifaa 2014). Similarly, the occurrence of *Streptococcus spp. and Clostridium spp*. reflects contributions from environmental and sediment-associated microbial communities, highlighting the complexity of microbial assemblages in freshwater ecosystems (Li *et al.,* 2022).

Interspecific variation in bacterial load among fish species, although not statistically significant, may be attributed to differences in ecological behaviour, habitat preference, and feeding strategies. Species associated with benthic environments are likely to encounter higher microbial densities due to interaction with sediments rich in organic matter, which are known reservoirs of diverse microbial populations (Hou *et al.,* 2022).

Biochemical characterisation of isolates revealed a metabolically diverse bacterial community comprising both Gram-positive and Gram-negative organisms. This diversity is consistent with current understanding that fish microbiota represents a combination of autochthonous aquatic bacteria and allochthonous contaminants introduced through environmental and anthropogenic inputs (Talwar *et al.,* 2018).

The observed variation in indole production, motility, and oxidase activity provided a consistent basis for differentiating bacterial groups and supported the presumptive identification of isolates reported in this study. The coexistence of oxidase-positive aquatic bacteria (e.g., *Vibrio spp.*) and oxidase-negative enteric bacteria (e.g., *E. coli* and *Shigella spp.*) further highlights the dual influence of environmental and fecal contamination sources. Importantly, the biochemical profiles obtained in this study provide supportive but not definitive evidence for bacterial identification, as conventional biochemical methods have inherent limitations in resolving closely related taxa. Recent studies emphasise the importance of molecular approaches, such as 16S rRNA gene sequencing, for accurate taxonomic identification and characterisation of microbial communities (Alikunhi *et al.,* 2017). Therefore, the identities of isolates reported in this study should be interpreted as presumptive, and future studies incorporating molecular techniques would provide greater taxonomic resolution.

The microbial composition of water samples closely mirrored that of fish tissues, providing strong evidence of a direct environmental transmission pathway. The detection of similar bacterial genera in both matrices confirms that the surrounding aquatic environment serves as a

Primary source of microbial contamination in fish. This relationship is well established, with fish microbiota strongly influenced by the microbial composition of the surrounding water (Bruno *et al.,* 2023; Niu *et al.,* 2025).

From a food safety perspective, the detection of potentially pathogenic genera, including *Vibrio spp.*, *Shigella spp.*, and *Escherichia coli*, is of significant concern. While total bacterial loads were within acceptable limits, the presence of these organisms highlights a critical limitation of relying solely on TVC as an indicator of safety. Total viable counts reflect overall microbial burden but do not distinguish between harmless and pathogenic bacteria. Consequently, fish may meet general microbiological standards while still posing potential health risks, particularly under conditions of inadequate handling, storage, or cooking (Mitiku *et al.,* 2022; Cortés-Sánchez *et al.,* 2025a).

Generally, the findings of this study demonstrate that microbial contamination of fish in the River Niger at Onitsha is driven by a combination of environmental exposure, anthropogenic pollution, and post-harvest handling practices. The integration of fish and water microbiological data provides a comprehensive understanding of contamination pathways and highlights the need for improved wastewater management, routine environmental monitoring, and strict adherence to hygienic handling practices. Addressing these factors is essential for safeguarding fish quality, reducing public health risks, and ensuring the sustainability of artisanal fisheries in the region.

Conclusion

This study demonstrates that freshwater fish from the River Niger at Onitsha harbour a moderate but ecologically driven microbial burden, with total viable bacterial counts (6.52–6.59 $\log_{10}$ CFU g^{-1}) remaining within acceptable limits yet approaching thresholds indicative of compromised microbiological quality. These findings reveal that compliance with conventional microbial standards does not necessarily equate to true food safety, particularly in environments subjected to sustained anthropogenic pressure.

The concurrent detection of indigenous aquatic bacteria (*Vibrio spp.*) alongside enteric and handling-associated bacteria (*Shigella spp.*, *Escherichia coli*, *Staphylococcus spp.*, *Klebsiella spp.*) highlights a converging contamination pathway driven by environmental pollution, fecal inputs, and post-harvest handling practices. The strong concordance between bacterial profiles in water and fish tissues provides compelling evidence that the aquatic environment functions as a primary reservoir and transmission route for microbial contamination.

Critically, the presence of potentially pathogenic genera in fish that meet acceptable total bacterial limits reveals a fundamental limitation in current food safety assessment approaches, whereby quantitative microbial thresholds alone are insufficient to capture risk. This finding reinforces the need to transition from reliance on total viable counts toward integrated, pathogen-informed monitoring frameworks that better reflect real world exposure risks.

The results in this study position the River Niger at Onitsha as a system under persistent microbial pressure, shaped by cumulative environmental and anthropogenic influences. Addressing this requires a shift from isolated interventions to coordinated, multi-level management strategies, including effective wastewater control, routine microbiological surveillance of both water and fish, and enforcement of hygienic handling practices across the fish supply chain.

Ultimately, ensuring the safety of fish in such environments demands a One Health-informed approach, recognising the interconnectedness of environmental quality, food safety, and human health. Without such integrated action, the risk of microbial contamination and associated public health implications will remain an enduring challenge in artisanal fisheries systems.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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