2026, Scienceline Publication
**World's Veterinary Journal**
***World Vet J,*** 16(2): 402-410.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2026.wvj38
PII: S232245682600038-16

# Biochemical and Morphological Characterization of Bacteria Isolated from Freshwater in Sudan

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ORIGINAL ARTICLE
Received: March 03, 2026
Revised: April 09, 2026
Accepted: April 30, 2026
Published: June 25, 2026

## ABSTRACT

The microbial quality of freshwater fish is a crucial indicator of both aquatic environmental health and food safety. The present study aimed to isolate and characterize bacteria from three common freshwater fish species, including *Oreochromis niloticus*, *Clarias gariepinus*, and *Synodontis alberti*, collected from three sites in Khartoum, Sudan. Six fish of each species were collected from each sampling location where the species were present. Total viable bacterial counts in fish gill and intestinal tissues obtained from Green Belt Sewage, Jebel Aulia fish landing, and Al Mourda fish market ranged from $1.2 \times 10^4$ to $3.9 \times 10^5$ CFU/g, with higher bacterial loads generally observed in intestinal tissues compared to gills. The highest bacterial load ($3.9 \times 10^5$ CFU/g) was recorded in the intestine of *Clarias* species collected from Green Belt Sewage. The current results demonstrated a predominance of Gram-negative, rod-shaped bacteria in gill and intestinal tissue samples across all sampling sites. All isolates were catalase-positive and capable of fermenting glucose, indicating facultative anaerobic metabolism. Oxidase activity of bacterial isolates differed by location; fish samples from Al Murda Fish Market had the highest number of oxidase-positive isolates, suggesting the potential presence of *Aeromonas* and *Pseudomonas* species. Urease activity was predominantly observed in isolates of fish samples collected from Al Murda and Jebel Aulia, suggesting a greater risk of fish spoilage or pathogenicity. Notably, isolates from the Green Belt Sewage fish samples comprised Gram-positive coccus, potentially identified as *Staphylococcus* spp., underscoring the likelihood of anthropogenic contamination. The present results indicated that fish obtained from all sampling locations generally exhibited similar microbial communities. However, the differences in enzyme activity across fish from all sites likely reflected variations in environmental factors, sanitation practices, and potential public health risks.

**Keywords:** Bacterial isolate, Biochemical characterization, Enzyme activity, Fish, Microbial count

## INTRODUCTION

Freshwater and marine fish are vital in the human diet, especially in developing countries, where they provide an accessible, affordable source of high-quality animal protein, essential amino acids, vitamins, and micronutrients. Additionally, freshwater and marine fish support global markets and contribute to the nation's economy (Thilsted et al., 2016). In Sudan, species such as *Oreochromis niloticus* (Nile tilapia), *Clarias gariepinus* (African catfish), and *Synodontis albertis* (squeaker catfish) are widely consumed and constitute a major part of the national inland fisheries, especially in the Nile River and its branches. However, microbial safety and fish quality can be compromised by exposure to contaminated water, unhygienic post-harvest handling, and poor storage conditions (Chichester and Graham, 2013; Elhadi, 2014; Deivansigamani, 2026).

Fish harbor diverse microbiota on their skin, gills, and gastrointestinal tracts, including both commensal and opportunistic bacteria such as *Micrococcus* spp., *Bacillus* spp., *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp., and *Enterobacteriaceae* members (Chichester and Graham, 2013). While many of these microorganisms are harmless or beneficial, others, such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Vibrio cholerae*, and *A. hydrophila* (*A. hydrophila*), can serve as indicators of environmental pollution or as potential pathogens to humans and fish (Austin and Austin, 2012). The composition of bacteria associated with fish can vary depending on the fish species, water quality, and geographic location (Zhang et al., 2022). Commonly reported genera include *Aeromonas*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, and *Staphylococcus*, some of which are linked to spoilage, fish diseases, or infections (Janda and Abbott, 2010). Previous studies have highlighted the microbial risks associated with fish harvested or sold in local markets in Africa and the Middle East (Al-Harbi and Uddin, 2005; Oniong et al., 2018). For instance, Omeji et al. (2022) reported the presence of *A. hydrophila* and *Pseudomonas* spp. in Nile tilapia and catfish from Egyptian and Saudi Arabian waters. InSudan, although limited studies have been conducted, preliminary assessments have suggested microbial contamination in fish sold in Khartoum markets (Elhadi, 2014). Biochemical characterization of bacterial isolates is a crucial approach for identifying and understanding the metabolic functions of fish-associated microbes



To cite this paper: Sulieman HMA and Bakhet HGAB (2026). Biochemical and Morphological Characterization of Bacteria Isolated from Freshwater in Sudan. *World Vet. J.*, 16(2): 402-410. DOI: https://dx.doi.org/10.54203/scil.2026.wvj38

(Newaj-Fyzul et al., 2008). Evaluation methods such as Gram staining, catalase, oxidase, glucose fermentation, urease activity, and nitrate reduction offer important insights into the taxonomy and possible pathogenicity of bacterial isolates (Newaj-Fyzul et al., 2008). Furthermore, the biochemical profiles assist in distinguishing between environmental and clinically relevant strains. The current study aimed to isolate and biochemically characterize bacteria isolated from the gills and intestines of three common freshwater fish species, including *Oreochromis niloticus* (Nile tilapia), *Clarias gariepinus* (African catfish), and *Synodontis albertis*, collected from three different locations in South and West Khartoum, Sudan.

## MATERIALS AND METHODS

### Ethics approval

The present study did not involve any live experimental manipulation of fish. All samples were purchased from local fish markets after harvest for commercial purposes, and all procedures adhered to national and institutional guidelines for ethical handling and sample processing.

### Study location and design

A cross-sectional study was conducted using fish samples collected from three sites in South and West Khartoum, Sudan, including Green Belt Sewage, Jebel Aulia Fish Landing, and Al Murda Fish Market. These sites were selected due to their importance in local fish marketing and human consumption. A total of 36 fish samples representing three species (*Clarias gariepinus*, *Oreochromis niloticus*, and *Synodontis alberti)* were collected from three different locations. A total of six fish per species were collected at each sampling location where that species was present. However, *Oreochromis niloticus* was not available in the Green Belt Pond at the time of sampling; therefore, this species was collected only from the remaining two locations. Consequently, the total number of fish samples collected for the study was 36. Each fish sample was considered an independent biological replicate. Tissue samples from the gills and intestine were analyzed from each specimen to assess microbial contamination associated with external exposure and colonization of the digestive tract. The experimental design consisted of two primary factors, including the sampling location and fish species. The present study involved a comparative analysis of microbial load across different species and collection sites.

### Sample collection

Immediately after purchase, fish samples were aseptically placed into sterile polyethylene bags and transported in insulated containers filled with ice to maintain a temperature of approximately 4°C, thereby reducing microbial growth and ensuring sample integrity. Standard cold chain procedures were maintained during transportation. All samples were transported to the Central Veterinary Laboratories and Research Center in Soba, Khartoum, and processed within four hours of collection to ensure microbial stability. In the laboratory, fish samples were aseptically dissected with sterile tools. Gill tissues and intestinal contents were removed separately and homogenized in sterile phosphate-buffered saline (PBS, pH 7.2) at a ratio of 1:10 (w/v). Serial tenfold dilutions were then prepared from each homogenate using sterile diluent by transferring one mL of homogenate into nine mL of sterile normal saline, yielding successive decimal dilutions ($10^{-1}$ to $10^{-6}$) for subsequent microbiological analysis.

### Microbiological procedures

Total viable bacterial counts were determined using the drop plate technique following standard microbiological methods (Newaj-Fyzul et al., 2008). Nutrient agar plates were dried in an incubator and inverted for two hours prior to use. Each plate was divided into eight equal segments using a glass marking pencil and labeled according to the corresponding dilution. One gram of the sample was homogenized in ten mL of sterile normal saline to create the initial inoculum. From this solution, one mL was transferred into nine mL of sterile normal saline, and the process was repeated to produce a series of decimal dilutions up to $10^{-9}$. The tubes were thoroughly mixed to ensure proper homogenization. From each dilution, 0.02 mL aliquots were inoculated onto the labeled segments of nutrient agar plates using a sterile micropipette. The inoculated plates were allowed to absorb the inoculum and then incubated at 37°C for 24 hours under aerobic conditions. Following incubation, colonies appearing in each segment were counted. The average colony count from three plates for each dilution was calculated, and the results were expressed as colony-forming units per gram (CFU/g) of sample.

### Biochemical characterization

Pure bacterial isolates were chosen for biochemical characterization according to distinct colony morphology observed after primary cultivation. From each sample, 3-6 representative colonies exhibiting differences in size, shape,

color, and texture were carefully selected and purified by subculturing to obtain single, well-isolated colonies. The purified isolates were then subjected to a series of standard biochemical tests for preliminary identification, following the protocols described by Newaj-Fyzul et al. (2008). Catalase activity was determined by adding 3% hydrogen peroxide to bacterial smears, where bubble formation indicated a positive reaction. Oxidase activity was assessed using oxidase strips; a purple color indicated a positive result. Glucose fermentation was evaluated using phenol red glucose broth to detect acid and/or gas production. Oxidative and fermentative metabolic activities were distinguished utilizing Hugh and Leifson's oxidation-fermentation (O/F) test. Nitrate reduction was assessed by adding sulfanilic acid and α-naphthylamine to nitrate broth cultures; a color change indicated a positive result. Urease activity was determined using Christensen's urea agar, where a color change indicated ammonia production. Motility was examined by stab inoculation into motility test medium and observing the growth pattern. Arginine dihydrolase activity was tested using Moeller's decarboxylase medium supplemented with arginine. Indole production was determined by incubating isolates in tryptone broth, then adding Kovac's reagent; a red layer indicated a positive reaction. The Voges-Proskauer (VP) test was performed using Barritt's reagents to detect acetoin production. Quality control for biochemical tests was ensured by using established positive and negative control bacterial strains. Control organisms with established biochemical reactions were tested alongside the isolates to confirm the accuracy of the test procedures and reagents. All biochemical tests, including catalase, oxidase, indole production, citrate utilization, urease activity, nitrate reduction, motility, and O/F tests, were performed according to established microbiological procedures, and test results were interpreted only when control reactions exhibited expected results (Newaj-Fyzul et al., 2008).

**Data analysis**

All results were analyzed in SPSS version 20 using descriptive statistics to determine the prevalence and percentage of pathogens.

## RESULTS

The highest viable microbial count was recorded in the intestine of *Clarias gariepinus* from the Green Belt Sewage site ($3.9 \times 10^5$ CFU/g; Tables 4, Figure 2), suggesting significant microbial contamination likely associated with sewage influence. In all sampling locations and across all fish species, intestinal samples consistently demonstrated higher bacterial loads than gill samples. *Oreochromis niloticus* and *Synodontis alberti* generally exhibited lower viable microbial counts than *Clarias gariepinus* across the study locations (Table 4, Figure 5). At Al Murda Fish Market, the predominant bacteria isolated from *Oreochromis niloticus* were *Pseudomonas* spp., while *Proteus* spp. were commonly found in *Clarias gariepinus* and *Synodontis alberti* (Table 4, Figure 2). The isolates obtained from fish collected in the Green Belt Sewage site were *Aerococcus* spp., *Staphylococcus* spp., and *Bacillus mycoides* (Tables 1,4). In samples from the Jebel Aulia fish landing, *E. coli* was the dominant species found in *Oreochromis* spp. and *Clarias* spp., whereas *Synodontis* spp. was associated with *Staphylococcus* spp. Fish samples collected from Green Belt Sewage exhibited the most diverse and abundant bacterial populations, indicating contamination from untreated domestic or industrial waste (Tables 1 and 4, Figures 2 and 4). Fish samples collected from Al Murda fish market demonstrated post-harvest bacterial contamination, whereas samples collected from Jebel Aulia fish landing indicated the presence of *E. coli*, suggesting fecal contamination (Tables 2, 3, and 4, Figures 2 and 4).

**Table 1.** Biochemical characteristics of bacteria isolated from fish in Green Belt Sewage, South Khartoum, Sudan

| Sample | Shape and gram reaction | Catalase | Oxidase | Glucose | Oxidation fermentation | Nitrate reduction | Arginine | Urea | Motility | Indole | V.P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Clarias gariepinus* (intestine) | G +ve Cocci | + | - | + | F | + | - | - | - | - | - |
| *Clarias gariepinus* (gill) | G –ve Rod | + | - | + | F | + | - | - | + | - | - |
| *Syuodontis alberti* (intestine) | G –ve Rod | + | - | + | F | + | - | - | + | - | - |
| *Syuodontis alberti* (gill) | G –ve Rod | + | - | + | F | + | - | - | + | - | - |

G –ve: Gram-negative, + / –: Positive/Negative result, F: Fermentative metabolism, V.P: Voges-Proskauer test

**Table 2.** Biochemical characteristics of bacteria isolated from fish species in Al Mourda Fish Market, West of Khartoum, Sudan

| Test Sample | Shape and gram reaction | Catalase | Oxidase | Glucose | Oxidation fermentation | Nitrate reduction | Arginine | Urea | Motility | Indole | V.P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Orechromis niloticus* (intestine) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Orechromis niloticus* (gill) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Orechromis niloticus* (intestine) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Orechromis niloticus* (gill) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Clarias gariepinus* (intestine) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Clarias gariepinus* (Gill) | G –ve Rod | + | + | + | F | + | + | - | + | - | - |
| *Syuodontis alberti* (intestine) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |
| *Syuodontis alberti* (gill) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |

G –ve: Gram-negative, + / –: Positive/Negative result, F: Fermentative metabolism, V.P: Voges-Proskauer test

**Table 3.** Biochemical characteristics of bacteria isolated from different fish species in Jebel Aulia fish Landing, South of Khartoum, Sudan

| Test sample | Shape and gram reaction | Catalase | Oxidase | Glucose | Oxidation fermentation | Nitrate reduction | Arginine | Urea | Motility | Indole | V.P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Orechromis niloticus* (intestine) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |
| *Orechromis niloticus* (gill) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |
| *Clarias gariepinus* (intestine) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |
| *Clarias gariepinus* (Gill) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |
| *Syuodontis alberti* (intestine) | G –ve Rod | + | - | + | F | - | - | + | + | - | - |
| *Syuodontis alberti* (gill) | G –ve Rod | + | - | + | F | + | - | + | + | - | - |

G –ve: Gram-negative, + / –: Positive/Negative result, F: Fermentative metabolism, V.P: Voges-Proskauer test

**Table 4.** Viable microbial counts and bacterial isolates from different organs of the studied fish species and locations in Khartoum, Sudan

| Location | Fish species | Organ | Viable count (CFU/g) | Isolated bacteria |
|---|---|---|---|---|
| **Al Mourda fish market** | *Oreochromis niloticus* | Intestine | $3.35 \times 10^4$ | *Pseudomonas* |
| | *Oreochromis niloticus* | Gill | $9.65 \times 10^3$ | *Pseudomonas* |
| | *Syuodontis alberti* | Intestine | $3.2 \times 10^4$ | *Proteus* |
| | *Syuodontis alberti* | Gill | $1.5 \times 10^4$ | *Proteus* |
| | *Clarias gariepinus* | Intestine | $1.5 \times 10^4$ | *Proteus* |
| | *Clarias gariepinus* | Gill | $1.3 \times 10^4$ | *Proteus* |
| **Green Belt Sewage** | *Syuodontis alberti* | Intestine | $3.7 \times 10^4$ | *Bacillus mycoides* |
| | *Syuodontis alberti* | Gill | $1.8 \times 10^4$ | *Bacillus mycoides* |
| | *Clarias gariepinus* | Intestine | $3.9 \times 10^5$ | *Aerococcus* |
| | *Clarias gariepinus* | Gill | $1.5 \times 10^4$ | *Staphylococcus* |
| **Jebel Aulia fish landing** | *Oreochromis niloticus* | Intestine | $1.9 \times 10^4$ | *Escherichia coli* |
| | *Oreochromis niloticus* | Gill | $1.3 \times 10^4$ | *Escherichia coli* |
| | *Syuodontis alberti* | Intestine | $3.2 \times 10^4$ | *Staphylococcus* |
| | *Syuodontis alberti* | Gill | $1.4 \times 10^4$ | *Staphylococcus* |
| | *Clarias gariepinus* | Intestine | $3.9 \times 10^4$ | *Escherichia coli* |
| | *Clarias gariepinus* | Gill | $1.2 \times 10^4$ | *Escherichia coli* |

Viable microbial counts are expressed as colony-forming units per milliliter (CFU/g).

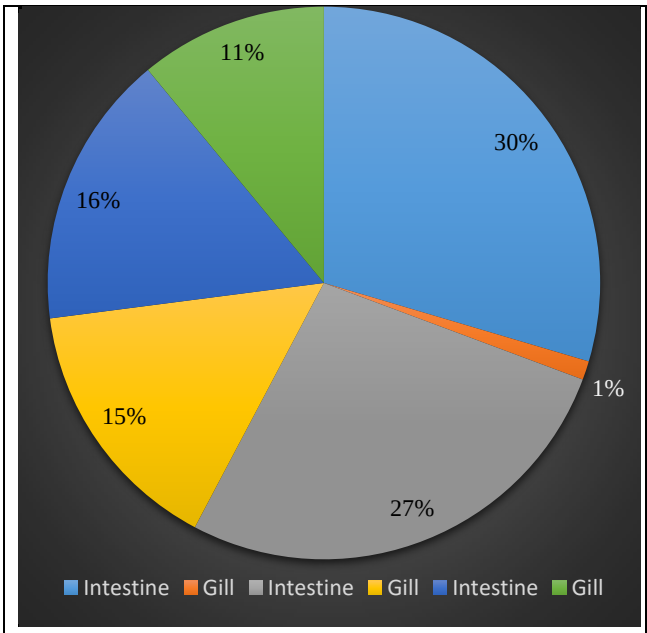


**Figure 1.** Viable bacterial counts in the gills and intestines of *Oreochromis niloticus* collected from West and South Khartoum, Sudan. Results were expressed as percentage colony-forming units per gram (CFU/g) of the original sample.

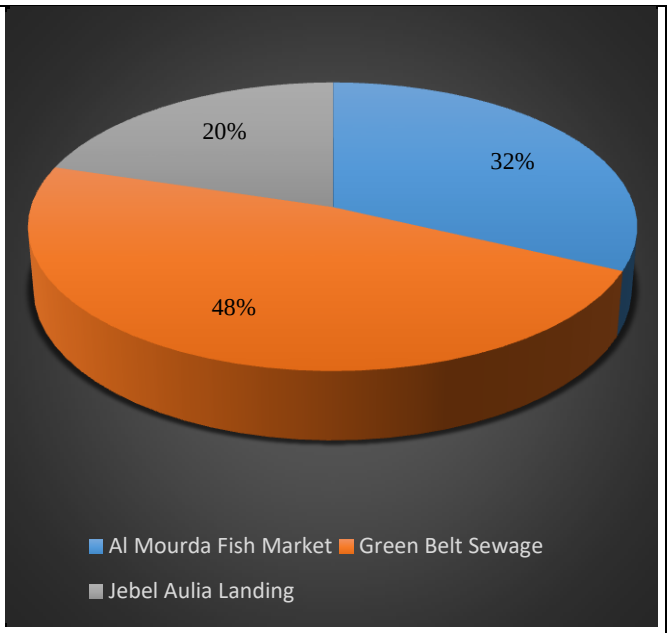


**Figure 2.** The viable bacterial counts in West and South Khartoum, Sudan. Results were expressed as percentage colony-forming units per gram (CFU/g) of the original sample.

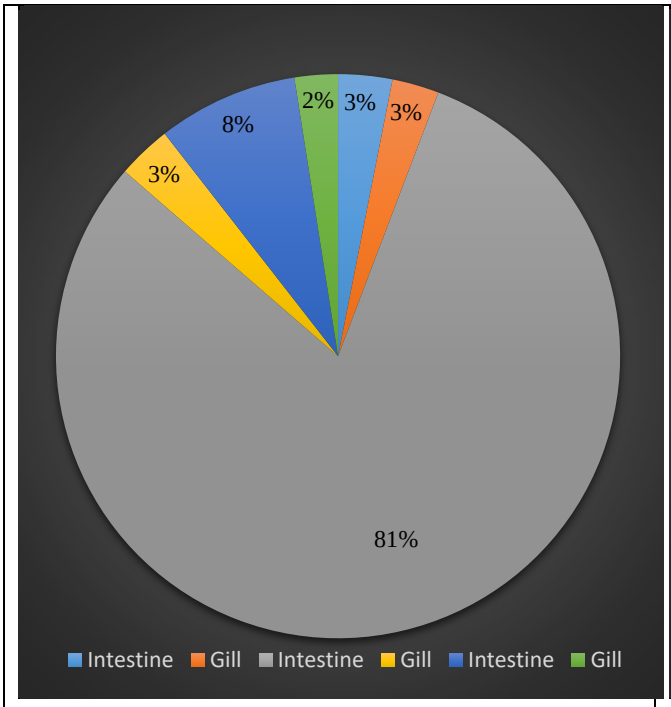


**Figure 3.** Viable bacterial counts in the gills and intestines of *Clarias* spp. collected from West and South Khartoum, Sudan. Results were expressed as percentage colony-forming units per gram (CFU/g) of the original sample.

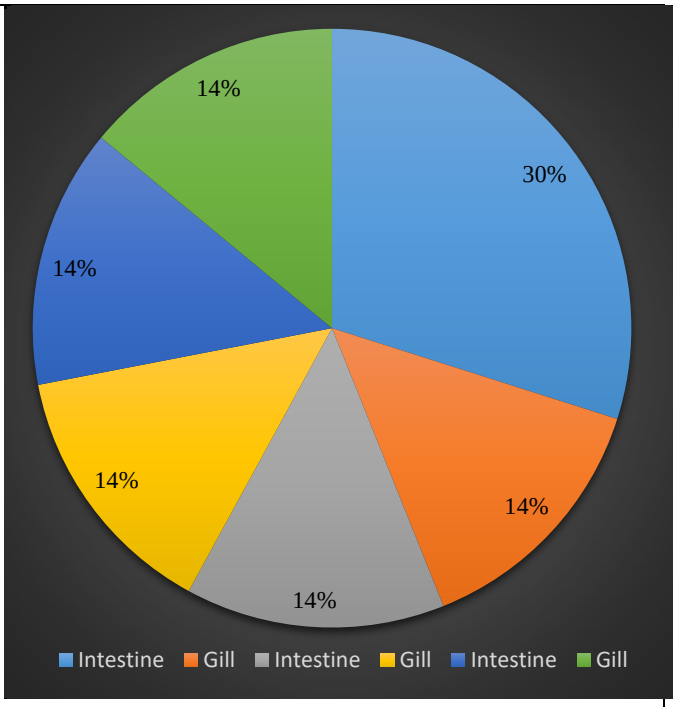


**Figure 4.** Viable bacterial counts in the gills and intestines of *Synodontis* spp. collected from West and South Khartoum, Sudan. Results were expressed as percentage colony-forming units per gram (CFU/g) of the original sample.

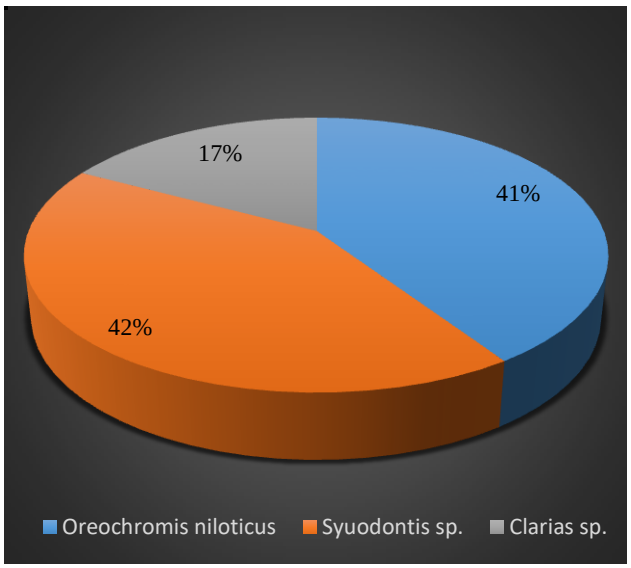


**Figure 5.** The viable bacterial counts of the gill and intestine fish species collected from West and South Khartoum, Sudan. Results were expressed as percentage colony-forming units per gram (CFU/g) of the original sample.

## DISCUSSION

The bacterial loads observed in the present study were within the range commonly reported for freshwater fish in both natural and polluted environments. Previous studies indicated that bacterial counts in fish intestines typically ranged from $10^3$ to $10^7$ CFU/g, reflecting the naturally dense microbial community in the intestinal tract (Olugbojo and Ayoola, 2015; Austin and Austin, 2016). The guidelines of the International Commission on Microbiological Specifications for Foods stated that microbial counts below $10^6$ CFU/g in fresh fish are generally considered acceptable, depending on fish species and conditions (Oliva-Teles, 2012). Similarly, reports by the Food and Agriculture Organization (FAO, 2018) indicated that bacterial loads on the skin and gills of freshly caught fish commonly ranged from $10^2$ to $10^5$ CFU/g, whereas intestinal microbial counts were higher due to the normal microbiota.

Austin and Austin (2016) and Omeji et al. (2022) reported comparable bacterial loads in freshwater fish from polluted waters, with total viable microbial counts ranging from $10^4$ to $10^6$ CFU/g, particularly in bottom-feeding species such as *Clarias gariepinus*. In contrast, *Oreochromis niloticus* and *Synodontis alberti* exhibited comparatively lower bacterial loads, potentially attributable to differences in feeding habits, habitat preferences, and environmental exposure. The present findings were consistent with those of Austin and Austin (2012) and Mohanta and Goel (2014), who reported *Enterobacteriaceae*, *Aeromonadaceae*, and *Pseudomonadaceae* as the predominant bacterial families associated with freshwater fish across all sampling sites and fish species. The predominant morphology of bacteria associated with fish was Gram-negative rods. The present results were consistent with those of Omeji et al. (2022), who reported a high prevalence of Gram-negative bacteria in Nile tilapia and catfish within aquatic environments in sub-Saharan Africa. The predominance of these bacteria indicated persistent exposure to water contaminated with fecal material or organic waste, thereby promoting the proliferation of facultative anaerobic bacteria, including *Aeromonas* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Klebsiella* spp. The intestine of *Clarias gariepinus* collected from the Green Belt sewage was the only sample that yielded Gram-positive cocci, possibly *Staphylococcus* spp., suggesting contamination from external sources, such as human handling or contaminated sediments (Olugbojo et al., 2015). All isolates were catalase positive, reflecting their aerobic or facultative anaerobic characteristics. However, the oxidase test was used to differentiate the isolates. Fish samples collected from the Al Murda fish market exhibited multiple oxidase-positive isolates, particularly from *Oreochromis niloticus* and *Clarias gariepinus*, indicating the possible presence of *Aeromonas hydrophila* or *Pseudomonas* spp. These microorganisms are known to colonize the gill and intestinal mucosa of fish and act as opportunistic pathogens (Janda and Abbott, 2010). Fish samples from Jebel Aulia fish landing and Green Belt Sewage demonstrated primarily oxidase-negative isolates, suggesting dominance of *Enterobacteriaceae* spp. such as *Enterobacter*, *Proteus*, or *Citrobacter* spp. These patterns reflected differences in microbial ecology across different locations, potentially attributable to water quality, market sanitation, and post-harvest handling, as reported by Janda and Abbott (2010). Glucose fermentation by the isolates was detected in the O/F test, confirming that the bacteria were facultative anaerobic bacteria typically found in aquatic environments and in fish intestines. These bacteria proliferate in environments rich in organic matter, indicating that the nutrient-enriched waters are likely contaminated by agricultural activities (Olugbojo et al., 2015).

Urease-positive bacteria were prevalent, especially in fish samples obtained from the Al Murda Fish Market and Jebel Aulia fish landing. Urease activity is often correlated with pathogenicity, facilitating bacterial survival in acidic environments such as the fish gut or infected tissues (Al-Harbi and Uddin, 2005). Bacteria such as *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Aeromonas* spp. are known for their urease production and have been associated with fish spoilage and disease (Ludwig et al., 2015). Nitrate reduction was observed in the majority of isolates, thereby confirming their capacity for anaerobic respiration, a characteristic frequently associated with enteric and environmental bacteria, as documented by Mahmoud et al. (2022). Nitrate-negative isolates were observed in intestinal samples from *Synodontis alberti* collected at Jebel Aulia, potentially indicating the presence of bacterial strains with reduced metabolic diversity or experiencing environmental stress, consistent with findings reported by Ali (2022). Most isolates were negative for arginine utilization, except those of *Synodontis alberti* obtained from Al Murda fish market, indicating bacterial diversity within this species. Additionally, all isolates were negative for indole and VP tests, indicating that Enterobacter cloacae and other typical enteric bacteria were not predominant, or that the isolates were non-pathogenic environmental strains (Veljović et al., 2015). All isolates were motile, consistent with facultative or free-living aquatic bacteria, capable of colonizing different fish tissues. Motility, a pathogenicity trait of *Aeromonas hydrophila*, enhances its ability to invade host tissues and colonize mucosal surfaces (Semwal et al., 2023).

The fish microbiota across all sites generally had a similar structure, primarily consisting of Gram-negative, catalase-positive, fermentative bacteria. Fish samples collected from Al Murda fish market exhibited the most biochemically active bacteria (oxidase-positive, urease-positive), indicating that market conditions might promote the growth of opportunistic fish pathogens. Jebel Aulia Landing indicated more uniform oxidase-negative profiles, suggesting possible dominance of *Enterobacteriaceae* and lower environmental diversity. The detection of urease and nitrate-reducing bacteria in all sites posed potential public health risks through zoonotic transmission, particularly due to poor hygiene, inadequate fish storage, or consumption of undercooked fish, as reported by Omeji et al. (2022).

Despite geographic variation among sampling locations, bacterial isolates from all sites exhibited similar phenotypic characteristics, including Gram-negative rod morphology, catalase positivity, and fermentative metabolism. This similarity might be attributed to comparable environmental conditions across the aquatic systems in Khartoum, Sudan, particularly water temperature, organic matter content, and nutrient availability, which are known to influence microbial community structure. Aquatic environments receiving organic inputs and sewage contamination often support the proliferation of Gram-negative bacteria such as *Aeromonas*, *Pseudomonas*, *Enterobacter*, and *E. coli*, which are commonly associated with fish and aquatic habitats. Therefore, the similarity in bacterial characteristics across locations

indicated that environmental factors, rather than geographical distance alone, played a major role in shaping microbial populations in these aquatic ecosystems. Similar findings were reported by Zhang et al. (2022), who observed that environmental conditions and water quality parameters notably influenced the microbial communities associated with fish and aquatic environments. Fish samples collected from the Green Belt sewage exhibited the highest biochemical diversity, specifically Gram-positive cocci. Bacterial isolates obtained from fish samples at Al Murda Fish Market exhibited the highest biochemical activity, as evidenced by positive oxidase and urease tests. This indicated a greater potential for pathogenicity or spoilage, potentially due to inadequate hygiene practices or suboptimal fish-handling conditions. Jebel Aulia fish landing isolates were more uniform and oxidase-negative, suggesting the dominance of enteric-type bacteria (Austin and Austin, 2016).

## CONCLUSION

According to the present findings, the microbial communities associated with fish were broadly similar across all sites; however, site-specific differences, particularly in enzyme activity, indicated distinct environmental conditions, hygiene practices, and associated public health risks. Continuous monitoring of bacterial communities in commercially well-known fish species is essential to ensure food safety and aquatic health. Nevertheless, the present study is limited by its reliance on culture-dependent techniques and a relatively restricted sample size, which may underestimate the full diversity of microbial communities and overlook non-culturable taxa.

## DECLARATIONS

### Acknowledgements

The authors sincerely appreciate the support of the Department of Fisheries and Wildlife at Bahri University, Sudan, for providing laboratory resources and assistance for the present study.

### Authors' contributions

Data analysis was conducted by Hassan Mohammed Adam Sulieman and Hnadi Gism Alla Bilal Bakhet, who assisted with the study design and scheduling the experiments. Additionally, samples were collected from different regions by Hnadi Gim, Alla Bilal, and Bakhet following the review of the data analysis. All authors have reviewed and approved the final edition of the manuscript prior to its publication in this journal.

### Availability of the data and materials

The paper contains the original data used in this investigation, which may be obtained from the corresponding author upon reasonable request.

### Competing interests

The authors declare that there are no conflicts of interest.

### Ethical considerations

The authors stated that this is an original study, authored collectively by all contributors, and submitted to the journal for the first time. The authors confirmed that no AI tools were utilized in the preparation and writing the present study.

### Funding

The present study received no external funding and was entirely self-funded.

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