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# Microbiological study of fish from the rural market of Katana on the edge of Kahuzi-Biega National Park, South Kivu Province, DRC

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## ABSTRACT

### Introduction

Safeguarding public health requires effective planning and adequate epidemiological surveillance. Microbial foodborne illnesses are a major public health problem.

**Purpose**

This study aims to assess bacterial infestation in fish sold at the rural market of Katana, located on the edge of the Kahuzi-Biega National Park in South Kivu Province, Democratic Republic of the Congo.

**Methods**

Fish samples were collected, and bacterial cultures were performed on culture media. Identification was conducted using Gram staining at the Bacteriology Laboratory of the Fomulac/Katana General Reference Hospital in Katana, located in Kabare Territory, north of Bukavu City. For morphological identification, each sample was analysed in triplicate. Data were statistically analysed using the F test and the $\chi^2$ test with Past version 4.10 software.

**Results**

Rod-shaped bacteria accounted for 52.6% of isolates, of which 93.3% were Gram-negative and 6.6% were Gram-positive. Among the Gram-negative bacteria, 21.4% were identified as *Vibrio cholerae*, while 71.9% were classified as other bacteria. Gram-positive bacteria were observed in species of *Haplochromis sp.* and *Limnotrissa miodon*. Gram-negative diplococci comprised 95.4% of isolates, compared to 4.5% that were Gram-positive. Among cocci, Gram-positive cocci accounted for 60%, while Gram-negative cocci made up 40%. The Hikojima strain of *Vibrio cholerae* was detected in 100% of *Vibrio cholerae* colonies. Biochemical identification revealed the presence of *Vibrio cholerae*, *Citrobacter sp.*, *Enterobacter sp.*, *Salmonella sp.*, *Escherichia coli*, and *Klebsiella sp.* Statistical analysis showed no significant difference between fish species and the bacteria sought or between fish species and fish condition. However, there was a significant difference between colony characteristics and both the culture medium and fish condition, while no significant difference was observed between the culture medium and the bacteria sought.

**Conclusion**

Fish sold at Katana's rural market are contaminated with bacteria regardless of the conditioning methods used. The unsanitary conditions of the market, display cases, and vendors' hygiene practices are the primary sources of contamination.

## INTRODUCTION

Health is an absolute necessity because a morbid or sick person is incapable of performing any productive work. However, the development of a country depends on several factors, the most important of which is undoubtedly human capital (Umba, 2018). Safeguarding public health requires effective planning and adequate epidemiological surveillance (Umba, 2018). Bacterial diseases transmitted through the digestive tract are contracted through the ingestion of contaminated food or water. Poor conditions of production, preservation, and consumption of food products sold in rural and urban markets in the Democratic Republic of Congo (DRC) could lead to the proliferation of microbial germs that cause food poisoning (Zayukua, 2019).

In recent decades, both the livestock and agri-food sectors have been affected by various crises, including cases of food poisoning in the United States caused by Salmonellosis and *Escherichia coli* O157:H7, as well as epidemics in Europe related to bovine spongiform encephalopathy (BSE), avian flu, and influenza A(H1N1) (Hanak et al., 2002). Ensuring the safety of food of animal origin requires implementing measures at every stage of the food chain (Southern African Development Community [SADC], 2011).

A study examining the scope and effects of food waste in some public markets in the DRC revealed that the majority of respondents were women. According to the poll, 40% of vegetables, 30% of fruits, 20% of spices, and 10% of cereals are wasted. Notably, none of this waste is sent to landfills (Mubwele et al., 2024).

Fish is considered a staple food in many cultures and has long been recognized as a significant source of essential nutrients, including protein, omega-3 fatty acids, minerals (iodine, selenium), vitamin D, and amino acids (e.g., taurine, carnitine, melatonin, tryptophan, and polyamines) (Abera & Adimas, 2024). Due to these factors, fish is regarded as a valuable and integral part of a balanced diet. As a result, global fish production and consumption have been on the rise. In 2020, China accounted for approximately 15% of the total global fish catch, which exceeded the combined total of the second and third leading countries (Food and Agriculture Organization [FAO], 2022).

Despite the significant fishing potential in the South Kivu Province of the DRC, fish availability remains a critical food security issue (ABAKIR, 2020). To address the deficit caused by low local production, the province imports substantial quantities of fish, despite the unsatisfactory health status of some imports (Zayukua, 2019). In the Irhambi-Katana Group, the microbiological characteristics of fish sold at local markets have not been systematically studied. The origin of bacterial diseases such as typhoid fever, diarrhea, cholera, and throat infections recently observed in Irhambi-Katana remains largely unknown. This concern prompted the present microbiological study of fish sold at this market.

## METHODS

### *Description of the Study Environment*

The Irhambi-Katana Group **(Figure 1)** is located in Kabare Territory, approximately 40 km north of Bukavu City, along the Bukavu-Goma road **(Figure 2)**. It is situated between 2°15' and 2°30' South latitude and 28°45' and 28°85' East longitude. The altitude ranges from 1,465 m near Lake Kivu to 2,200 m towards the PNKB. The group is bounded to the north and west by Kalehe Territory, to the south by the Bugorhe Group, to the east by Lake Kivu, and to the west by the PNKB (Ndegeyi, 2009). According to official documents from 2020, the Irhambi-Katana Group (where the Katana market is located) comprises six localities with an average population density of 577.4 inhabitants per km².

### *Fish Sampling*

Fish sampling at the Katana market was conducted with 50 vendors: 13 vendors of *Limnotrissa miodon* fish species, 13 vendors of *Haplochromis sp.* fish species, 4 vendors of *Barbus sp.* fish species, 6 vendors of *Bargus docmak* fish species, 5 vendors of *Lates niloticus* fish species, 4 vendors of *Lates sp.* fish species, 2 vendors of *Oreochromis niloticus* fish species, and 3 vendors of *Bargus sp.* fish species. Sampling was performed by randomly selecting two individual fish from each vendor or shelf for *Limnotrissa miodon* and *Haplochromis sp.*, and one individual fish for the other species. The sample consisted of 76 individual fish: 26 *Limnotrissa miodon*,



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26 *Haplochromis sp.*, 4 *Barbus sp.*, 2 *Oreochromis niloticus*, 4 *Lates sp.*, 5 *Lates niloticus*, 3 *Bargus sp.*, and 6 *Bargus docmak*.

### *Biological Materials*

The biological materials included fish species sold at the Katana market, specifically *Limnotrissa miodon*, *Haplochromis sp.*, *Barbus sp.*, *Bargus docmak*, *Bargus sp.*, *Oreochromis niloticus*, *Lates niloticus*, and *Lates sp.* Biochemical tests and other analyses were conducted following standard methods as described by Quinn et al. (2002), Acharjee et al. (2013), and Cappuccino and Sherman (1996).

### *Culture Media Preparation Techniques*

#### *TCBS Preparation for the Identification of Vibrio cholerae*

A total of 7.816 g of TCBS was dissolved in 200 ml of distilled water. After homogenization, the solution was heated until boiling and then immediately poured into sterilized plates (180°C for 60 minutes).

#### *Hektoen Agar Preparation for the Isolation of Salmonella and Shigella*

A total of 15 g of Hektoen Agar was dissolved in 200 ml of distilled water and heated in a water bath with steam sterilization. The solution was then poured into sterilized plates.

#### *Muller Hinton Solution Preparation*

A total of 7.6 g of Muller Hinton powder was introduced into 200 ml of distilled water. After homogenization and heating, the solution was autoclaved for 15 minutes, then poured into sterile plates.

#### *Preparation of Seed Media and Identification*

**Kligler Iron Agar (KIA)**

A total of 5.5 g of powder was dissolved in 100 ml of distilled water, heated, and distributed into test tubes (3-5 ml per tube). The tubes were sterilized (15 minutes at 121°C) and tilted for solidification.

**Simmons Citrate Agar**

A total of 3.63 g of powder was dissolved in 150 ml of distilled water, poured into tubes (5 ml each), and autoclaved. The tubes were tilted to form a slope after cooling.

**Tryptophan Broth (TB)**

A total of 1.6 g of powder was dissolved in 100 ml of distilled water, heated, and 3 ml of the solution was introduced into each tube before autoclaving.

**Methyl Red Voges Proskauer (MR-VP)**

A total of 1.7 g of powder was dissolved in 100 ml of distilled water, homogenized, and 5 ml was transferred into tubes, followed by autoclaving.

### *Seeding*

After sterilizing the workspace using a Bunsen burner, the seeding was performed by rubbing sterile swabs on fish samples and streaking on culture media plates. The plates were covered, inverted, and incubated for 24 hours at 37°C.

### *Inoculation and Identification for Biochemical Characteristics*

Culture media were prepared, homogenized, and poured into labeled assay tubes (3-5 ml each). The tubes were sterilized, tilted, and inoculated. After incubation at 37°C for 18-24 hours, colonies were counted. Each sample was analyzed twice to confirm biochemical characteristics.

### *Morphological Identification of Bacteria by Gram Staining*

A sterile slide was labeled and degreased by flaming. A drop of physiological water was added, followed by a bacterial colony from the culture field. After drying, the following stains were applied: gentian violet (1 minute), Lugol (1 minute), alcohol-acetone (1 minute), and Fuchsin (1 minute), with water washes between steps. The slide was dried and examined using a 100x objective lens with immersion oil. Each sample was examined in triplicate.

### *Statistical Analysis*

Statistical analysis was performed using Past version 4.10 software. The F test (Fischer-Snedecor test) was used to compare fish species and states, fish species and bacterial presence, culture medium and fish states, and culture medium and bacteria sought, with a significance level of 0.05. The $\chi^2$ test (chi-square test) was used to compare colony characteristics and the frequency of germs identified based on biochemical characteristics.

### *Ethical Considerations*

Fish samples were collected with the consent of vendors, ensuring ethical compliance.



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## RESULTS

The obtained data are presented in the following tables, which include the characteristics of bacterial colonies on culture media, biochemical characteristics of bacteria, serotyping of *Vibrio cholerae* strains from samples, and the morphology of bacteria isolated from fish.

**Table 1**:
Characteristics of Bacterial Colonies on Selective and Elective Culture Media for Enterobacteria and Cocci from Fish Sold at the Katana Market

| | Fish Sample and Culture medium | Fish state | Bacteria sought | Colonies Characteristics | Observation |
|---|---|---|---|---|---|
| 1 | *L. miodon* on TBCS | Fresh | *V. cholerae* | yellow colonies | Glucose fermented Yellow TBCS |
| | *L. miodon* on Hektoen agar | Fresh | *Salmonella* and *shigella* | Yellow α black colonies | Fermented glucose and of blackish colonies (*Salmonella* sp) |
| | *L. miodon* on Mueller Hinton | Fresh | Any bacteria | abundant colonies | bacterial diversity on fish |
| | *L. miodon* on TBCS | Grilled | *V. cholerae* | Yellow and non-yellow colonies | fermented glucose |
| | *L. miodon* on Hekton agar | Grilled | *Salmonella* and *shigella* | black to red colonies | Salomonella or Shigella |
| | *L. miodon* on Mueller Hinton | Grilled | Any bacteria | isolated chains colonies | Occurrence of diversified bacteria |
| | *L. miodon* on TBCS | Dried | *V. cholerae* | No growth | absence of growth: no germ |
| 2 | *Haplochromis sp* on TBCS | Fresh | *V. cholerae* | yellow colonies +++ | fermented glucose: *V. cholerae* |
| | *Haplochromis sp* on Hektoen agar | Fresh | *Salmonella* and *shigella* | Yellow to red colonies | fermented glucose: S α S |
| | *Haplochromis sp* on Mueller Hinton | Fresh | Any bacteria | abundant colonies | bacteria grown |
| | *Haplochromis sp* on TBCS | Grilled-smoked | *V. cholérae* | Black yellow colonies | Fermentation of glucose |
| | *Haplochromis sp* on Mueller Hinton | Grilled-smoked | Any bacteria | slightly yellow colonies | various bacteria grown |
| | *Haplochromis sp* on Hektoen agar | Grilled-smoked | - | - | - |
| | *Haplochromis sp* on TBCS | Grilled unsmoked | *V. cholerae* | Isolated yellow colonies | fermented glucose |
| | *Haplochromis sp* on Hektoen agar | Grilled-unsmoked | *Salmonella* and *shigella* | yellow-red colonies | fermented glucose |
| | *Haplochromis sp* on Mueller Hinton | Grilled-unsmoked | Any bacteria | abundant chain colonies | growth of various bacteria |
| | *Haplochromis sp* on TBCS | Dried | *V. cholerae* | Black yellow colonies | yellow-black dry colonies |
| | *Haplochromis sp* on Hektoen agar | Dried | *Salmonella* and *shigella* | Isolated black colonies | fermented glucose |
| | *Haplochromis sp* on Mueller Hinton | Dried | Any bacteria | Colonies in chains | growth of various bacteria |
| 3 | *Barbus sp* on TBCS | Dried | *V. cholerae* | blackish yellow colonies | Less fermentation of glucose |
| | *Barbus sp* on Hektoen agar | Dried | *Salmonella* and *shigella* | Yellow-black colonies | fermented glucose |
| | *Barbus sp* on Mueller Hinton | Dried | Any bacteria | Colonies in chains | growth of various bacteria |
| 4 | *O. niloticus* (Tilapia) on TBCS | Salted | *V.cholerae* | small yellow colony | a little fermentation of glucose |
| | *O. niloticus* (Tilapia) on Hektoen agar | Salted | *Salmonella* and *shigella* | growths a little black | *Salmonella* with a black center |
| | *O. niloticus* (Tilapia) on Mueller Hinton | Salted | Any bacteria | abundant colonies | Other *enterobacteria* such as cocci |
| 5 | *Bargus docmak* on TBCS | Smoked | *V.cholerae* | Small yellow growths | There is fermentation of glucose |
| | *Bargus docmak* on Hektoen agar | Smoked | *Salmonella* and *shigella* | Yellow to red colonies | fermented glucose |
| | *Bargus docmak* on Mueller Hinton | Smoked | Any bacteria | abundant colonies | various bacterial strains |
| 6 | *Lates sp* on TBCS | Fresh (ice) | *V.cholerae* | No growth | No bacteria |
| | *Lates sp* on Hektoen agar | Fresh (ice) | *Salmonella* and *shigella* | colonies a little black | less use of medium by germs |

The data in Table 1 show the distribution of bacterial colonies across different seeding media. In the selective TBCS medium used for the detection of *V. cholerae*, six out of eight samples (75%) tested positive, including fresh and grilled *L. miodon*, fresh, smoked, and grilled *Haplochromis sp.*, *O. niloticus*, *Barbus sp.*, *Bargus sp.*, and *L. niloticus*.

Statistical analysis indicated no significant difference between fish species and the bacteria sought ($F = 0.1264$, $p = 0.9437$, $p > 0.05$) or between fish species and fish states ($F = 0.3831$, $p = 0.9258$, $p > 0.05$). However, a significant difference was observed between colony characteristics ($\chi^2 = 24.4$, $p = 0.00000078269$, $p < 0.05$) and the culture medium and fish states ($F = 8.625$, $p = 0.0000825$, $p < 0.05$). Conversely, there was no significant difference between the culture medium and the bacteria sought ($F = 0.005487$, $p = 0.9994$, $p > 0.05$).

**Table 2**:
Serotyping of *Vibrio cholerae* Strains from Fish Sold at the Katana Market

| N° | Sample | Antiserum Polyvalent | Antiserum Ogawa | Antiserum Inaba | Interpretation |
|---|---|---|---|---|---|
| 1 | *L. miodon* fresh | + | + | + | HIKOJIMA |
| | *L. miodon* roasted | + | + | + | HIKOJIMA |
| 2 | *Haplochromis* sp fresh | + | + | + | HIKOJIMA |
| | *Haplochromis* sp roasted-smoked | + | + | + | HIKOJIMA |
| | *Haplochromis* sp roasted no smoked | + | + | + | HIKOJIMA |
| 3 | *Barbus* sp | + | + | + | HIKOJIMA |
| 4 | *O. niloticus* | + | + | + | HIKOJIMA |
| 5 | *Bargus* sp | + | + | + | HIKOJIMA |
| 6 | *L. niloticus* | + | + | + | HIKOJIMA |

Table 2 indicates that all fish species from the research area contain the HIKOJIMA strain of *V. cholerae*, except for *Bargus docmak* and *Lates sp.*

**Table 3**:
Morphology of Bacteria Isolated from Fish Sold at the Katana Market

| N° | SAMPLE | Morphology of the bacteria on TBCS medium | Morphology of the bacteria on HEKTOEN | Morphology of the bacteria on MULLER HINTON |
|---|---|---|---|---|
| 1 | *L. miodon* Fresh | - Curved Gram-negative rods<br>- Gram-negative diplococci | - Gram-negative bacteria<br>- Gram-negative diplococci | - Gram-negative<br>- Gram-negative diplococci |
| | *L. miodon* roasted | - Gram-negative rods curved | - Gram-negative rods<br>- Gram-negative diplococci | - Gram-negative rods<br>- Gram-negative diplococci |
| | *L. miodon* Dried | - | -Gram-negative diplococci<br>- Gram-negative rods | - Gram-negative cocci (Diplococci)<br>- Gram-negative |
| 2 | *Haplochromis sp* Fresh | -Gram-negative diplococcic<br>- Gram-negative rods curved | - Gram-positive rods large<br>- Gram-negative diplococci | - Gram-negative rods<br>- Gram-negative diplococci |
| | *haplochromis* sp roasted-smoked | - Gram-negative rods<br>-Gram-negative diplococci | - Gram-negative rods short<br>- Gram-negative diplococci | - Gram-negative rods short<br>- Gram-negative rods<br>- Gram-negative diplococci |
| | *Haplochromis* sp Grilled | - Gram-negative rods curved | - Gram-negative rods<br>-Gram-negative diplococci | - Gram-negative rods<br>- Gram-negative diplococci |
| 3 | *Barbus sp* | - Gram-negative rods curved | - Gram-negative rods | - Gram-negative diplococci |
| 4 | *Haplochromis* sp dried | - Gram-negative rods | - Gram-negative rods | - Gram-negative rods<br>- Gram-negative diplococci |
| 5 | *Lates sp* | - | -Gram-negative diplococci | - Gram negative cocci |
| 6 | *Bagrus sp* | - Gram-negative rods | - Gram-negative rods | - Gram-negative rods<br>- Gram negative cocci<br>- Gram-negative diplococci |
| 7 | *Oréochromis niloticus* | - Cocci Positive<br>- Gram-negative rods | -Gram-negative rods | - Gram-negative rods<br>- Gram positive cocci |
| 8 | *Bagrus docmac* | - Gram negative cocci | - Gram-negative rods<br>-Gram-negative diplococci | - Gram-negative diplococci |
| 9 | *L. niloticus* | - Gram-negative rods curved | - Gram-negative rods<br>-Gram-negative diplococci | - Gram-negative rods<br>- Gram-negative diplococci |



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**Table 3** presents the diverse morphologies of bacteria, including rods, curved rods, cocci, and diplococci, which can be either Gram-negative or Gram-positive.

**Table 4**:
Biochemical Characteristics of Bacteria from Fish Sold at the Katana Market

| № | SAMPLE ↓ | Test → Sample fish state | Glucose | Lactose Fermentation | $CO_2$ Production | $H_2S$ | Indole | Voges Proskauer | Oxidase | Citrate | Germ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *L. miodon* on TCBS | Fresh | + | - | + | + | + | + | + | + | *V. cholerae* |
| | | | + | + | + | + | + | - | - | - | *E.coli* |
| | *L. miodon* on Hektoen agar | Fresh | + | - | + | + | + | + | - | ± | *Salmonella paratyphie* B |
| | *L. miodon* on Mueller Hiton | Fresh | + | + | + | + | + | ± | - | ± | *Enterobacter* freu |
| 2 | *L. miodon* on TCBS | Grilled | + | - | - | - | + | - | + | ± | *V. cholerae* |
| | *L. miodon* on hektoen agar | Grilled | - | - | ± | - | + | + | - | ± | *Proteus Vulgaris* |
| | *L. miodon* on Mueller H | Grilled | - | - | ± | + | + | + | - | ± | *P. vulgaris* |
| 3 | *Limnitrissa miodon* on Hektoen | Dried | + | - | - | + | + | + | - | + | *Salmonella sp* |
| | *L. miodon* on Mueller H | Dried | - | - | - | + | + | - | - | + | *P. vulgaris* |
| | *Haplochromis* sp on TBCS | Fresh | + | - | ± | + | + | + | + | + | *V. cholerae* |
| 4 | *Haplochromis* sp on Hektoen agar | Fresh | + | - | + | + | + | + | - | + | *Salmonella sp* |
| | *Haplochromis* sp on Mueller H | Fresh | - | - | - | + | + | + | - | + | *Proteus* sp |
| | *Haplochromis* sp on TCBS | Smoked | - | - | - | - | + | + | + | + | *V. cholerae* |
| | *Haplochromis* sp on Hektoen agar | Smoked | - | - | + | + | + | ± | - | + | *P. mirabilis* |
| | *Haplochromis* sp on Mueller Hinton | Smoked | - | - | - | + | + | ± | - | + | *Proteus* sp |
| | *Haplochromis* sp on TCBC | Grilled | + | - | - | - | + | ± | + | + | *V. cholerae* |
| | *Haplochromis* sp on Hektoen agar | Grilled | + | - | + | - | + | + | - | + | *Enterobacter irmedium* |
| | *Haplochromis* sp on Mueller Hinton | Grilled | + | + | + | - | + | ± | - | ± | *Klebsiella* sp |
| | *Haplochromis* on TCBS | Dried | + | - | - | + | + | ± | - | ± | *V. cholerae* |
| | *Haplochromis* sp on CLED | Dried | + | + | + | - | - | - | - | - | *E. coli* |
| | *Barbus* sp on TBCS (1°) (2°) | Dried | - | - | - | - | - | + | - | ± | *Pseudomonas* |
| | | | + | - | - | - | + | + | + | ± | *V. cholerae* |
| | *Barbus sp* on CLED | Dried | - | + | + | - | - | + | - | - | *E. coli* |
| | *Lates sp* (Tomson) sur Hektoen agar | | - | - | - | - | + | - | - | + | *Pseudomonas* sp |
| | *Lates* sp (Tomson) on Hektoen agar | | + | + | - | - | - | - | - | - | *Citrobacter* sp |
| | *O. niloticus* on TCBC | Salted | - | - | - | - | - | - | + | + | *Pseudomonas* sp |
| | | | + | - | - | - | + | ± | | + | *V. cholerae* |
| | *O. niloticus* on Hekton agar | Salted | - | - | - | - | - | - | - | + | *Pseudomonas* sp |
| | *O. niloticus* on Mueller Hinton | Salted | - | - | - | - | - | - | - | + | *Pseudomonas sp* |
| | *Bagrus docmak* on TCBS | Salted | + | + | - | - | - | + | - | - | *Shigella sp* |
| | *Bagrus docmak* on Hektoen | Smoked | + | + | - | - | - | ± | - | - | *Shigella mirabilis* |

**Table 4** reveals the diversity of biochemical traits among germs isolated from fish in the Katana market. These biochemical characteristics aid in distinguishing the bacterial species.

**Table 5**:
Summary of Germs Identified According to Biochemical Characteristics

| Species | frequency | Percentage |
|---|---|---|
| *Proteus* sp. | 9 | 25.8 |
| *V. cholerae* | 8 | 22.8 |
| *Citrobacter* sp | 2 | 5.7 |
| *Enterobacter* sp | 2 | 5.7 |
| *Pseudomonas* sp | 5 | 14.3 |
| *Salmonella* sp | 3 | 8.6 |
| *Shigela* sp | 2 | 5.7 |
| *E. coli* | 3 | 8.6 |
| *Klebsiella* sp | 1 | 2.8 |
| **Total** | **35** | **100%** |

The summary in **Table 5** shows that nine bacterial species were identified from fish samples sold at the Katana market. The most frequently identified species was *Proteus vulgaris* (25.8%), while *Klebsiella sp.* had the lowest frequency (2.8%). Statistical analysis revealed a significant difference between the frequencies of germs identified based on biochemical characteristics ($\chi^2 = 4.7778$, $p = 0.028829$, $p < 0.05$).

## DISCUSSION

Out of 422 non-compliant samples in our study, 44.68% of these drugs were sourced from India. This differs from Koumaré's (2018) study, which recorded that 72.22% of non-compliant samples came from China. Furthermore, in

### *Characteristics of bacterial colonies*

From the data in **Table 1**, 2 out of 8 samples (25%) did not show the presence of glucose fermentation, which suggests the absence of *Vibrio cholerae*. Of the 8 samples inoculated on Hektoen Agar media, a selective medium for the detection of *Salmonella* and *Shigella*, 3 samples (37.5%) exhibited yellow-blackish bacterial colonies, indicating the presence of *Salmonella*. On Mueller Hinton Agar, a general-purpose medium, bacterial colonies were abundant. These findings confirm our hypothesis that bacteria would be distributed diversely on fish sold in the Katana market. Our results are consistent with Adingra et al.'s (2010) study, which evaluated bacterial loads in *Tilapia* (*O. niloticus*) from markets in Abidjan. Their study found *V. cholerae* to be the second most abundant bacterium, following *E. coli* and preceding *Salmonella*.

### *Serotyping of bacterial strains of Vibrio cholerae*

According to **Table 2**, the polyvalent antisera, OGAWA and INABA, demonstrated agglutination after contact with a colony of *V. cholerae*. Indeed, 6 samples containing *V. cholerae* all revealed the presence of the HIKOJIMA strain, as indicated by agglutination with OGAWA and INABA sera (polyvalent antiserum). Zayukua et al. (2019) also reported the presence of *V. cholerae* in fish sold in the Kinshasa market, noting three cases of infection.

### *Morphology of bacteria isolated from fish sold in Katana market*

The bacterial morphology found on fish sold at Katana includes three types: rods, diplococci, and cocci **(Table 3)**. Gram-negative rods appeared 28 times out of 30 observations (93.3%), with 21.4% of these being curved rods, likely *V. cholerae*, while the remaining 71.9% were other Gram-negative rods. Gram-positive rods appeared 2 times (6.6%), with Haplochromis sp. on Hektoen Agar and *L. miodon* in grilled form on Mueller Hinton Agar. Diplococci were observed 22 times, with Gram-negative diplococci representing 95.45%, while Gram-positive cocci, including *Staphylococcus* and *Streptococcus*, appeared 3 times (60%), compared to 2 times (40%) for Gram-negative

cocci. Among the 57 bacterial observations, 52.6% were rod-shaped, 38.6% were diplococci, and 8.8% were cocci forming clusters. The prevalence of rod-shaped bacteria aligns with the abundance of Enterobacteriaceae family members on fish sold at Katana. Enterobacteriaceae are Gram-negative bacilli and known to be parasitic. The significant proportion of diplococci reflects the presence of *Enterococcus*, while Gram-positive cocci are likely from *Staphylococcus* or *Streptococcus*, which are common in normal human flora. These results support those of Zhou et al. (2018), who observed bacterial colonization in sick catfish (*Clarias batrachus* and *C. macrocephalus*). Poor conservation and the marketing process are often responsible for increased contamination and bacterial proliferation. Adingra et al. (2010) similarly found that *Tilapia* specimens were contaminated by multiple bacterial species simultaneously, suggesting that fish act as reservoirs and vectors of pathogenic germs. According to Senderovich et al. (2010), a single fish can transmit several infections to humans.

### *Biochemical characteristics of isolated enterobacteria*

The bacteria isolated from fish sold at Katana Market exhibited a range of biochemical characteristics **(Table 4)**, including glucose or lactose fermentation, CO2 production, hydrogen sulfide (H2S) production, indole production, Voges-Proskauer test, citrate fermentation, and oxidase activity. **Table 5** shows that Proteus was the most frequent genus, appearing 9 times (25.8%), followed by *Vibrio* (8 times, 22.8%), *Pseudomonas* (5 times, 14.3%), *E. coli* and *Salmonella* (3 times, 8.6%), *Enterobacter*, *Citrobacter*, and *Shigella* (2 times, 5.8%), and *Klebsiella* (1 time, 2.8%). Statistical analysis indicated no significant difference in the frequency of bacterial species found on fish from Katana market ($\chi^2 = 4.7778$, $p = 0.28829$, $p > 0.05$). These bacteria belong to the Enterobacteriaceae family, which is part of the normal human microbiota. The presence of these bacteria on fish sold at Katana is likely due to the unsanitary conditions under which the fish are handled. Sellers frequently touch money, scratch, and engage in various unsanitary practices without proper hygiene measures, transferring bacteria to the fish. These findings are in agreement with Horman (2006), who reported that over 80% of food sold in the informal sector in Kinshasa was contaminated with pathogenic bacteria.

## CONCLUSION AND RECOMMENDATIONS

Gram-negative rod-shaped bacteria, derived from human feces, predominated in all 13 of the samples examined in this study. Regarding the biochemical characteristics of these bacteria, the current investigation demonstrated that some of them can ferment lactose and glucose, as well as produce carbon dioxide, hydrogen sulphide, and various other biochemical markers. The high contamination rate suggests that inadequate handling, storage, and unsanitary market conditions significantly contribute to bacterial proliferation in fish.

Therefore, we recommend conducting further studies to compare the bacterial pathogens found in fish obtained in situ and ex situ by the Katana population. Additionally, we recommend performing genetic analyses of the HIKOJIMA strain of *Vibrio cholerae* identified in this study and comparing it with the human strain of *V. cholerae* to determine the source of human contamination. Further research on the molecular characterization of pathogens is also needed.

Customers are advised to avoid consuming fish that is immediately on display and to properly cook the fish they purchase from Katana Market and other rural markets at a temperature that effectively eliminates these germs. To decision-makers, we recommend providing sanitary equipment to fish vendors in remote marketplaces to ensure safe business practices.

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