Isolation and identification of selected bacterial pathogens from fish samples in selected markets in Windhoek: An exploratory study

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Abstract

This exploratory study assessed the microbiological quality of fresh fish sold in Windhoek, Namibia, with a focus on detecting selected bacterial pathogens and evaluating their antibiotic resistance profiles. A total of 20 fish samples, comprising *Merluccius* spp. (hake) and *Trachurus* spp. (horse mackerel), were collected from local wholesalers and retailers. Thirteen samples out of 20 (65%) tested positive for at least one of the targeted pathogens: *Escherichia coli* (20%), *Staphylococcus aureus* (20%), *Shigella sonnei* (15%) and *Salmonella* spp. (10%). Contamination levels varied across locations, with Katutura 1 and Katutura 2 showing the highest prevalence. Horse mackerel exhibited a higher contamination rate (57%) compared to hake (43%). Antibiotic susceptibility testing revealed that *E. coli* and *S. aureus* were resistant to ampicillin and cephalothin but remained susceptible to ciprofloxacin and cefotaxime. *Shigella sonnei* showed moderate susceptibility to chloramphenicol and co-trimoxazole, while *Salmonella* spp. displayed resistance to kanamycin and tetracycline. These findings indicate potential public health risks associated with the consumption of contaminated fish and highlight deficiencies in hygienic practices during fish handling, processing and storage. The study underscores the urgent need for strengthened quality control measures, regular microbial assessments and antimicrobial resistance monitoring to ensure consumer safety and maintain food industry standards.

Keywords: Food Safety, Microbial contamination, pathogens, sustainability, Windhoek

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1. Introduction

Food safety in the fish and seafood products remains a significant concern in the food industry due to its direct implications in public health and economic sustainability. Fish is a perishable food product and its contamination is a common source of foodborne illnesses globally, with pathogens such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Shigella* spp. frequently implicated in outbreaks (Galaviz-Silva et al., 2009; Sheng & Wang, 2021). These microorganisms can lead to severe health threats ranging from gastroenteritis to systemic infections, with potential long-term complications such as cancer (Ishaque et al., 2020). In Namibia, fish species such as hake (*Merluccius capensis*) and Cape horse mackerel (*Trachurus trachurus*), which are dietary staples, are highly consumed (Erasmus et al., 2021), making it critical to evaluate their microbiological quality and safety.

The pathways of microbial contamination are multifaceted and can occur at various points along the fish supply chain. Environmental factors, including polluted water sources, and improper handling during processing and storage, are known contributors to contamination (Rahman et al., 2022). For example, the presence of faecal coliforms in fish indicates potential sewage contamination, which is often associated with unhygienic processing practices (Gashe & Mhango, 2010). Additionally, temperature fluctuations during transport or market display facilitates the rapid proliferation of pathogenic bacteria (Sheng & Wang, 2021). Despite global recognition of these risks, the effectiveness of existing decontamination strategies remains a subject of debate, with methods such as chemical disinfection and bio preservation offering varying levels of success (Mir et al., 2022).

The socio-economic implications of contaminated fish further compound the urgency of addressing these challenges. Microbial contamination not only threatens consumer health but also undermines the reputation of the fishing industry, which is a vital economic sector in Namibia. Outbreaks linked to fish products can result in trade restrictions, financial losses from product recalls, and heightened healthcare costs (Cámara & Santero-Sánchez, 2019). Consequently, understanding the prevalence of bacterial pathogens in fish sold at local markets is critical for developing effective interventions and ensuring the safety of fish products.

While studies have documented microbial contamination in fish globally, limited research exists on the microbiological quality of fish in Namibian markets. This gap in knowledge is particularly concerning given the popularity of hake and horse mackerel among consumers

(Erasmus et al., 2021). Addressing this gap requires a systematic assessment of bacterial contamination and the conditions that facilitate pathogen growth during fish production, processing and retail.

The aim of this study was to evaluate the microbiological quality of fresh fish from diverse market sources in Namibia, with a focus on isolating and identifying key bacterial pathogens such as *Salmonella* species*, Shigella* species*, Staphylococcus aureus* and *Escherichia coli*. Additionally, antibiotic susceptibility testing was conducted to assess potential risks associated with resistant strains. This research provides meaningful contributions into the microbiological risks associated with fish consumption and offers practical recommendations for improving food quality standards within the Namibian fishing industry.

2. Materials and Methods

2.1 Research Design

Laboratory analysis was conducted in duplicates to evaluate microbial contaminants in hake and horse mackerel samples, focusing on prevalent pathogens such as *Salmonella* spp., *S. aureus*, *Shigella* spp. and *E. coli*. Bacterial isolation was done on the same day of purchase and code names were assigned to the samples based on their location and species. Thus, code "A" refers to hake, "B" refers to horse mackerel and "S" denotes the sample number. Key bacterial pathogens were isolated using differential and/or selective media, followed by various biochemical tests for confirmatory identification. Antibiotic sensitivity testing using the Kirby-Bauer disc diffusion method was performed to observe resistance patterns. Final identification of the isolates was carried out using VITEK® machine. Samples were collected from six markets in Windhoek, Namibia.

2.2 Data Collection Methods and Procedures

Sample Collection Fresh fish were sourced from various markets in Windhoek, Namibia, including wholesalers and retailers within the Windhoek city (Figure 1). Retailers were selected randomly,and the sampling sites were distributed across a range of approximately 10 km. The collection consisted of two frozen fish samples from each market, representing two distinct species: *Trachurus* species (horse mackerel) and *Merluccius* species (hake).

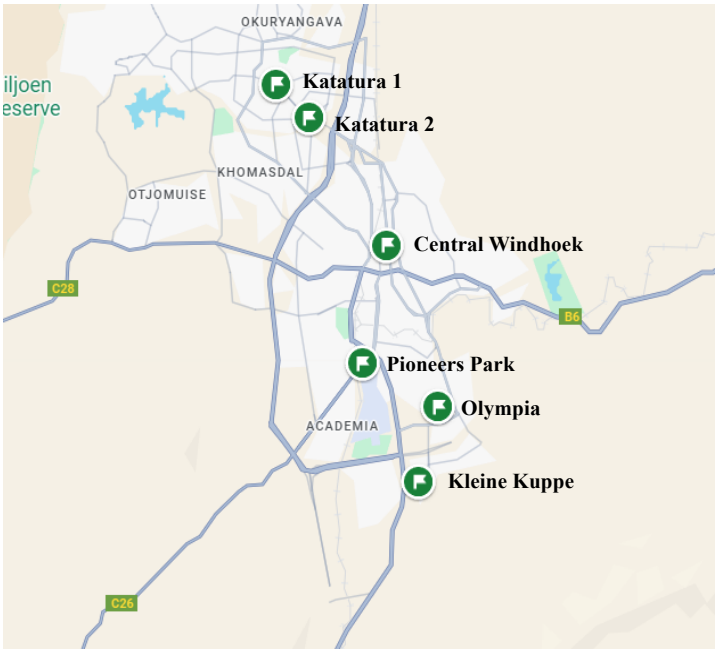

Figure 1. Geographical location of fish sample collection sites.

2.2.2 Microbiological Analysis

2.2.2.1 Isolation and Identification of Salmonella spp.

Salmonella spp. were isolated and identified using the method described by Mitiku et al. (2023). Approximately 25 g of fish muscle tissue was placed in 225 mL of buffered peptone water and homogenised using a blender. The homogenised sample was incubated for 24 hours at 37°C for pre-enrichment. Subsequently, 1 mL of the aliquot (pre-enrichment broth) was inoculated into a tube containing 10 mL of Rappaport-Vassiliadis Soya Peptone (RVS) broth, followed by incubation at 37°C for 24 hours. A loopful of the RVS broth inoculum was transferred and streaked separately onto the surface of Xylose Lysine Deoxycholate (XLD) agar plates, which were incubated at 37°C for 24 hours. The plates were examined for the presence of suspected *Salmonella* colonies displaying brown, grey, or pink colour with a black centre. Suspected colonies of *Salmonella* spp. were further biochemically confirmed by inoculation into triple sugar iron agar, methyl-red-Voges-Proskauer broth, indole test, Simmons' citrate agar, urea agar, lysine iron agar, Kligler's iron agar and Sulphide Indole Motility (SIM) medium and incubated at 37°C for 24 hours.

2.2.2.2 Isolation and Identification of Shigella spp.

The *Shigella* spp. were isolated and identified using the methods described by Mitiku et al. (2023). A mass of 25 g of the homogenised sample was added to 225 mL of Gram-negative

enrichment medium and incubated overnight at 37°C. A loopful of the enriched culture was then directly streaked onto Hektoen Enteric Agar and incubated aerobically at 37°C for 24 hours. The culture plates were examined for the presence of *Shigella* spp., indicated by small greenish colonies. Colonies suspected of being *Shigella* spp. were further characterised by standard biochemical tests, including triple sugar iron (TSI) agar, urea agar base and the sulphide indole motility (SIM) test. The culture results for each biochemical test were read after incubation for 24 to 48 hours at 37°C. The results were registered.

2.2.2.3 Isolation and Identification of Staphylococcus aureus

The isolation and identification of *Staphylococcus aureus* from fish samples were conducted as adapted from Mitiku et al. (2023). A 25 g portion of fish muscle was transferred aseptically into a sterile blender containing 225 mL of buffered peptone water and homogenised for 1–3 minutes. A 10-fold serial dilution was prepared and from appropriate serial dilutions, 0.1 mL aliquots were spread on mannitol salt agar and incubated for 24–48 hours at 37°C. Presumptive colonies were inoculated on blood agar plates, which were incubated aerobically at 37°C and examined after 24 hours for growth and the haemolytic pattern of *S. aureus*. Further identification was based on staining reactions with Gram's stain, pigment production, colony morphology, catalase test and coagulase test. On blood agar, *S. aureus* displayed a light to golden yellow pigment and colonies surrounded by zones of clear alpha haemolysis. Non-inoculated media were incubated as a negative control to check for sterility. Observations and findings were systematically included in the results section.

2.2.2.4 Isolation and Identification of Escherichia coli

Total and faecal coliforms were determined using methods previously described by Gashe and Mhango (2010). The fish samples (25 g) were homogenised with 225 mL of 0.1% sterile peptone water, followed by blending at 12,000 rpm for 2 minutes. Serial dilutions were prepared using 0.1% sterile peptone water. A presumptive test for total coliforms was conducted using a 3-tube multiple fermentation most probable number (MPN) technique. Aliquots (1 mL from each dilution) for three consecutive dilutions were added to Lauryl Tryptose Broth (LTB) containing test tubes. These test tubes were incubated at 35°C for 24–48 hours and examined for growth and gas production.

For total coliforms confirmation, a loopful of broth from gas-positive tubes was transferred into 2% brilliant green broth and incubated at 35°C for 48 hours. Another loopful from Lauryl Tryptose Broth was transferred to *E. coli* broth and incubated at 44.5°C for 48 hours. The MPN

for faecal coliforms was calculated from confirmed gas producing isolates. Positive *E. coli* samples were streaked onto eosin methylene blue agar (EMBA) and incubated at 35°C for 24–48 hours. *E. coli* presented as small colonies with a green metallic sheen. All experimental outcomes were documented in the results section.

2.2.4. Identification of Bacterial Isolates using the VITEK® System

The identification procedure using VITEK® analysis was adapted from US Environmental Protection Agency et al. (2023) manual. The isolates that were presumed positive for *Salmonella* (2 isolates), *Shigella* (3 isolates), *Staphylococcus aureus* (4 isolates) and *Escherichia coli* (4 isolates) were analysed. These isolates were subcultured on nutrient agar for 4 hours. Subsequently, 3 mL of saline were placed in vials and 3-5 colonies were inoculated into each saline vial. The VITEK® card inoculation was performed, followed by loading the inoculated cards into the VITEK® machine. The machine took 6-18 hours to analyse and read the samples, after which the results were recorded.

2.2.3 Antibiotic sensitivity testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar, as adapted by Bantawa et al. (2019). The bacterial inoculum was prepared and adjusted to 0.5 McFarland equivalents using a spectrophotometer. The inoculum was then spread onto the agar surface. Antibiotic discs were placed on the agar and the plates were incubated at 37°C for 18-24 hours. Zones of inhibition around each disc were measured and categorised as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). The antibiotics tested included ampicillin, azithromycin, cefotaxime, cephalothin, chloramphenicol, ciprofloxacin, co-trimazole, gentamicin, kanamycin and tetracycline. For quality control, cultures of *E. coli, S. aureus, Salmonella* spp. and *Shigella* spp. were used. Methanol and double-distilled water were employed as controls.

3. Results

A total of 20 fish samples were analysed, of which 13 tested positive for bacterial contamination based on isolation on selective media and biochemical tests (Table 1). Among these, *Escherichia coli* was detected in 4 samples (20%), *Shigella sonnei* in 3 samples (15%), *Staphylococcus aureus* in 4 samples (20%) and *Salmonella* spp. in 2 samples (10%).

Contamination varied across locations, with Katutura 1 and Katutura 2 showing the highest contamination rates, where four bacterial isolates were identified from each location. Central Windhoek and Kleine Kuppe had two contaminated samples each, while Olympia had one contaminated sample. No bacterial contamination was detected in samples from Pioneers Park (Figure 2). Regarding bacterial species distribution, *Escherichia coli* and *Staphylococcus aureus* were the most prevalent, each accounting for 31% of the isolates, followed by *Shigella sonnei* (23%) and *Salmonella* spp. (15%) (Figure 3). Comparison of bacterial contamination in different fish species revealed that 57% of the contaminated samples were from horse mackerel, while 43% were from hake (Figure 4).

Antibiotic susceptibility testing (Table 7) showed varying resistance patterns among isolates. *Escherichia coli* and *Staphylococcus aureus* exhibited susceptibility to Ciprofloxacin and Cefotaxime but were resistant to Ampicillin and Cephalothin. *Shigella sonnei* displayed moderate susceptibility to Chloramphenicol and Co-Trimoxazole, while *Salmonella* spp. exhibited resistance to multiple antibiotics, including Kanamycin and Tetracycline (Figure 5).

Table 1. Identified bacterial pathogens isolated from the fish samples using VITEK® analysis.

Sample No.	Location	Isolated Bacteria Identified	Probability (%)
1	Central Windhoek	*Escherichia coli*	99
2	Katatura 1	*Escherichia coli*	99
3		*Shigella sonnei*	98
4	Katatura 2	*Escherichia coli*	99
5		*Salmonella* spp.	99
6		*Shigella sonnei*	98
7		*Staphylococcus aureus*	99
8		*Staphylococcus aureus*	99
9		*Salmonella s*pp.	99
10	Kleine Kuppe	*Escherichia coli*	99
11		*Shigella sonnei*	98
12		*Staphylococcus aureus*	99
13	Olympia	*Staphylococcus aureus*	99

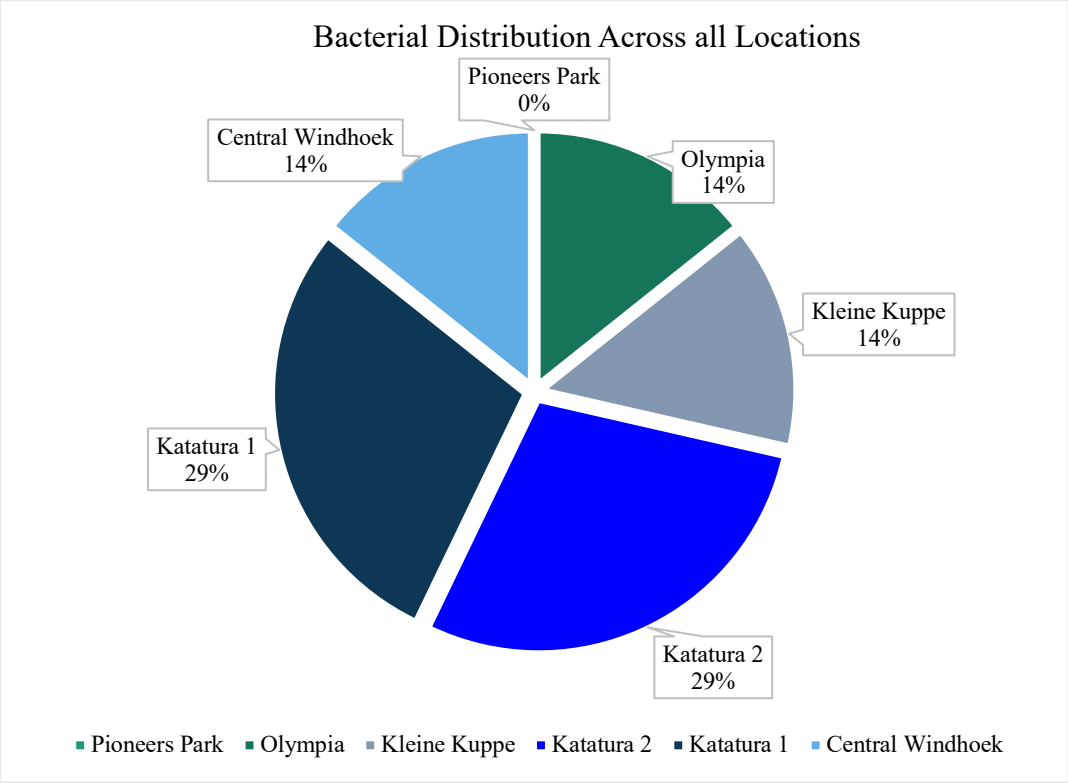

Figure 2. Overall prevalence of bacterial species across different locations, highlighting the most contaminated.

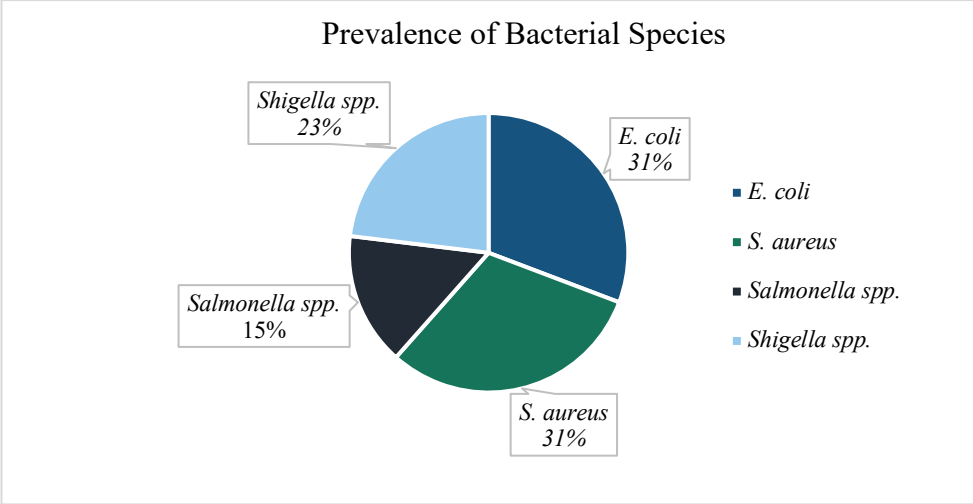

Figure 3. Prevalence of identified Isolates.

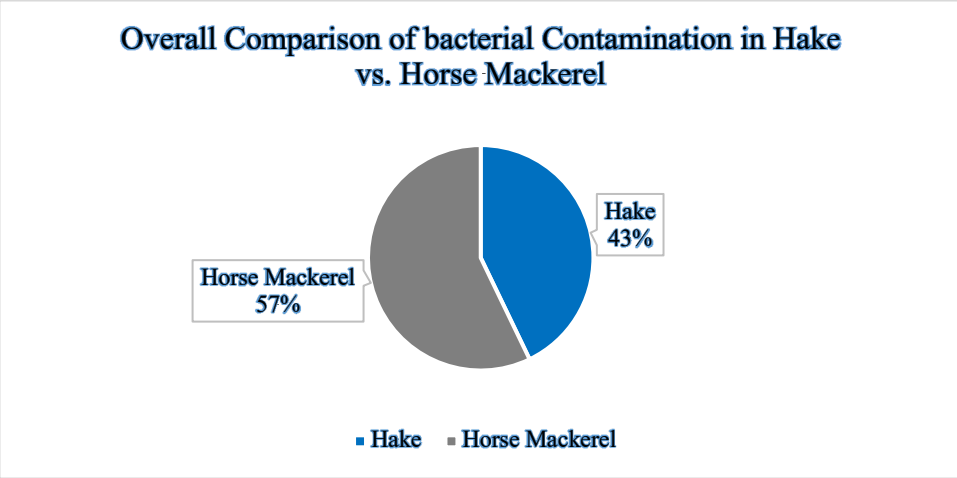

Figure 4. Bacterial contamination of hake and horse mackerel.

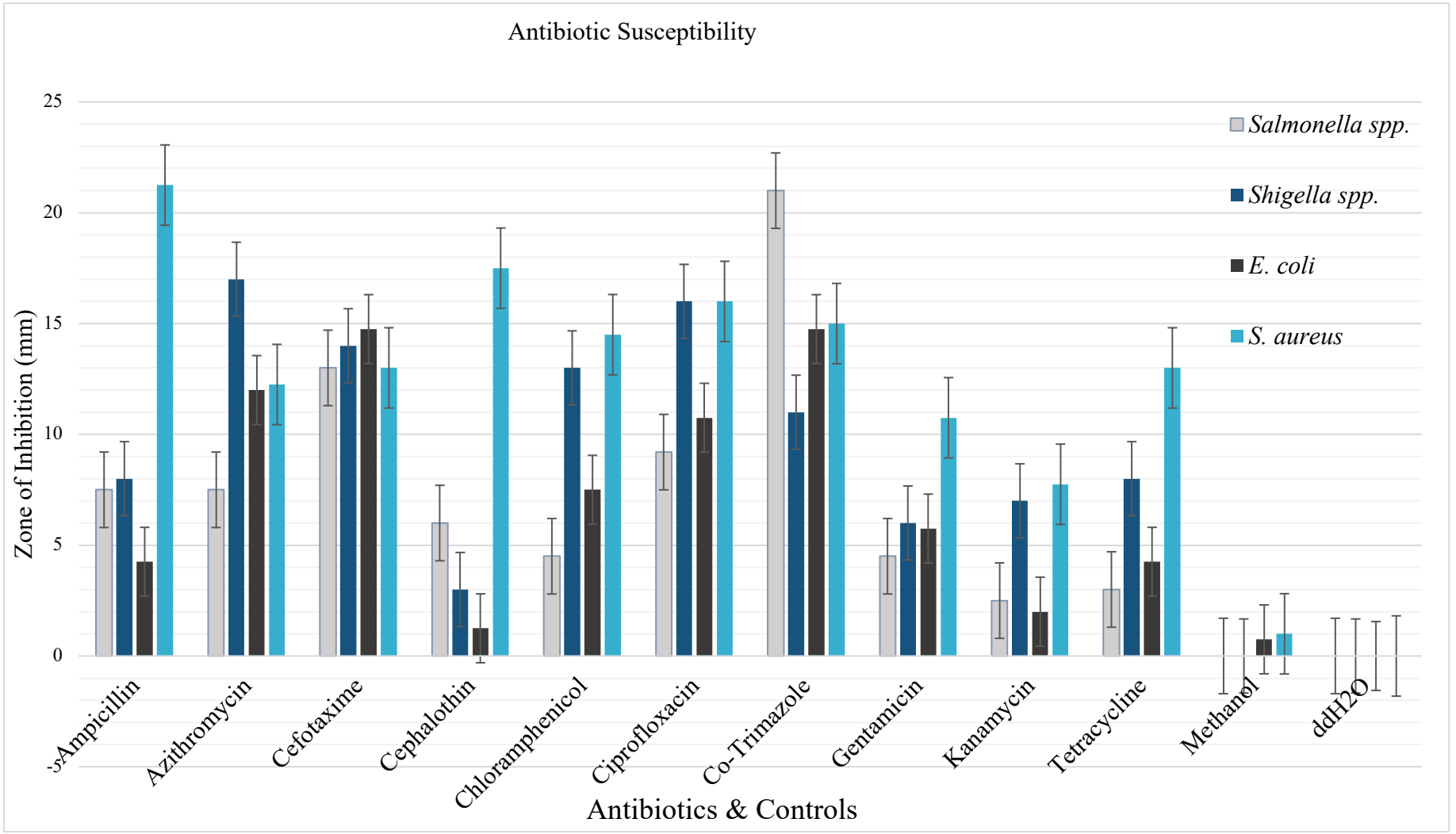

Figure 5. Susceptibility and resistance of different bacterial isolates towards used antibiotics

4. Discussion

The study successfully isolated and identified key bacterial pathogens of interest using various media and biochemical tests, revealing insights into the microbiological quality of fish in Windhoek's markets. The detection of *Salmonella* spp., *Staphylococcus aureus*, *Shigella sonnei* and *Escherichia coli* in both hake (*Merluccius spp.*) and horse mackerel (*Trachurus spp.*) highlights substantial microbiological risks associated with fish consumption. Among the 20 samples analysed, 13 (65%) showed bacterial contamination, indicating a high prevalence of pathogens in fish sold in the markets (see Appendix for detailed results). According to WHO guidelines, pathogenic bacteria such as *Salmonella* and *Shigella* should be absent in fish intended for consumption, while acceptable levels of indicator bacteria like *Escherichia coli* are very low or undetectable to ensure food safety. These pathogens are known to cause severe foodborne illnesses, including gastroenteritis, typhoid fever and dysentery, which pose significant health risks to consumers (Laxminarayan, 2013; Bantawa et al., 2019).

Bacterial identification was confirmed using the VITEK® system. It is an automated method that utilises biochemical and enzymatic activity tests to identify microorganisms (US Environmental Protection Agency et al., 2023. The high identification probabilities (98-99%) obtained from the VITEK® system emphasises the reliability of the findings and confirm the presence of these pathogens in the samples (US Environmental Protection Agency et al., 2023).The obtained results also suggest deficiencies in fish handling practices at various stages of the supply chain. Proper handling, including maintaining hygiene standards during fish processing and storage, is crucial in minimising microbial contamination. The presence of bacteria in fish could be attributed to several factors, including improper washing and unsanitary conditions during processing, as suggested by Gashe and Mhango (2010). The high bacterial loads observed may also be linked to inadequate refrigeration and cross-contamination between contaminated and non-contaminated fish, as noted by Sanjee & Karim (2016).

Additionally, variations in contamination levels across different locations may reflect the influence of local practices and environmental conditions, supporting the observations made by Mitiku et al. (2023) regarding the role of environmental factors, water quality and storage facilities in microbial load differences. These factors highlight the need for interventions tailored to specific markets to address localized contamination risk factors, as emphasized by Sheng & Wang (2021).

The presence of bacterial loads in both hake and horse mackerel points to the need for improved decontamination and handling techniques. Other studies have demonstrated that physical methods like freezing and thermal processing, along with chemical disinfectants, can reduce bacterial loads but may not eliminate pathogens (Sanjee & Karim, 2016; Mir et al., 2022).

Furthermore, the resistance profiles of *Salmonella* spp., *Staphylococcus aureus* and *E. coli* observed in this study are consistent with global trends in antibiotic resistance (Laxminarayan, 2013; Acar & Moulin, 2017;). For example, resistance to commonly used antibiotics, such as penicillin and tetracycline, reflects findings from other studies reporting increasing resistance in foodborne pathogens (Bantawa et al., 2019; Kumar & Babu, 2021). The disc diffusion method used for susceptibility testing provided crucial insights into the effectiveness of various antibiotics, underlining the growing challenge of antibiotic resistance in managing foodborne infections (CLSI, 2022).

5. Conclusion and Recommendations

The study identified bacterial contamination in fish from Windhoek's markets, with *Escherichia coli*, *Staphylococcus aureus*, *Shigella sonnei*, and *Salmonella* spp. detected. Contamination was highest in Katutura 1 and Katutura 2, while none of the selected bacterial pathogens were detected in samples from Pioneers Park. Horse mackerel samples had higher contamination than hake samples. Antibiotic resistance was observed, particularly against Ampicillin and Cephalothin, highlighting hygiene deficiencies in fish handling and storage. To improve food safety, stricter hygiene protocols, better training for fish handlers, and routine microbial assessments are necessary. Expanding the sample size and including multiple sampling points along the supply chain would enhance the study's reliability and help identify contamination sources. Implementing a traceability system is also recommended to monitor contamination trends and enforce better handling practices.

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Appendix 1

Table A. Summary table of isolated and identified key bacterial fish

	Sample Code	*Salmonella*	*Shigella*	*Staphylococcus aureus*	*E. coli*
Kleine Kuppe	1aS1-Original	Absent	Present	Present	Absent
	1aS1-Duplicate	Absent	Present	Present	-
	1aS2-Original	Absent	Absent	Absent	Absent
	1aS2-Duplicate	Absent	Absent	Absent	-
	1bS1-Original	Absent	Absent	Absent	Present
	1bS1-Duplicate	Absent	Absent	Absent	-
	1bS2-Original	Absent	Absent	Absent	Absent
	1bS2-Duplicate	Absent	Absent	Absent	-
Pioneers Park	2aS1-Original	Absent	Absent	Absent	Absent
	2aS1-Duplicate	Absent	Absent	Absent	-
	2aS2-Original	Absent	Absent	Absent	Absent
	2aS2-Duplicate	Absent	Absent	Absent	-
	2bS1-Original	Absent	Absent	Absent	Absent
	2bS1-Duplicate	Absent	Absent	Absent	-
	2bS2-Original	Absent	Absent	Absent	Absent
	2bS2-Duplicate	Absent	Absent	Absent	-
Olympia	3aS1-Original	Absent	Absent	Present	Absent
	3aS1-Duplicate	Absent	Absent	Present	-
	3aS2-Original	Absent	Absent	Absent	Absent
	3aS2-Duplicate	Absent	Absent	Absent	-
	3bS1-Original	Absent	Absent	Absent	Absent
	3bS1-Duplicate	Absent	Absent	Absent	-
	3bS2-Original	Absent	Absent	Absent	Absent
	3bS2-Duplicate	Absent	Absent	Absent	-
Katatura 1	4aS1-Original	Absent	Present	Absent	Absent
	4aS1-Duplicate	Absent	Present	Absent	-
	4aS2-Original	Absent	Absent	Absent	Present
	4aS2-Duplicate	Absent	Absent	Absent	Absent

	4bS1-Original	Absent	Absent	Absent	-
	4bS1-Duplicate	Absent	Absent	Absent	Absent
	4bS2-Original	Absent	Absent	Absent	Absent
	4bS2-Duplicate	Absent	Absent	Absent	-
Central Windhoek	5aS1-Original	Absent	Absent	Absent	Present
	5aS1-Duplicate	Absent	Absent	Absent	-
	5aS2-Original	Absent	Absent	Absent	Absent
	5aS2-Duplicate	Absent	Absent	Absent	-
Katatura 2	6bS1-Original	Present	Absent	Present	Present
	6bS1-Duplicate	Present	Absent	Present	-
	6bS2-Original	Present	Present	Present	Absent
	6bS2-Duplicate	Present	Present	Present	-

Note: Table A summarises the bacterial species identified in fish samples from various locations in Windhoek. *Shigella sonnei* and *S. aureus* were sporadically detected in Kleine Kuppe, while Pioneers Park samples showed no bacterial presence. Olympia had S. aureus in one sample, while Katutura 1 showed occasional *Shigella sonnei* and *E. coli*. Central Windhoek samples only had *E. coli* in one instance. Katutura 2 exhibited the highest diversity, with all four bacteria (*Salmonella, Shigella sonnei, S. aureus*, and *E. coli*) detected in various samples. This highlights location-based variability in bacterial contamination