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Microbiological Quality of Rabbit Fish (*Signus Sutor*) and Food Safety Practice of Fish Handlers from Selected Landing Sites in Zanzibar

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Abstract: The study aimed to evaluate the handling practices and microbiological contamination of pathogenic bacteria in Rabbit Fish (*Signus Sutor*) from Zanzibar, specifically at four landing sites: Malindi, Mazizini, Kizimkazi, and Matemwe. A semi-structured questionnaire and checklist were used to assess hygiene practices by fish handlers, and swabbing method was used to assess cleanliness of the boat and landing floor before fish were collected for consumption. Standard methods for microbial analysis (ISO7218:2007(E)) were used to analyze Total viable counts, *S. aureus*, *E. coli*, and *Salmonella*. The mean total viable (TVC) ranged from 2.82-3.30 log CFU/g across landing sites. *S. aureus* was the most frequently isolated bacterium, with an isolation rate of 53.33% in Malindi, 60% in both Mazizini and Kizimkazi, and 46.67% in Matemwe. *E. coli* was present but less frequently, with isolation rates of 6.67% in Malindi, 33.33% in Mazizini and Kizimkazi, and 20% in Matemwe. *Salmonella spp* was absent in all samples, with a 0% isolation rate across landing sites. There was positive correlation of 0.59 and 0.65 between fish contamination and swab samples from boats and landing floors respectively. Inadequate practices by fish handlers, such as not wearing gloves, neglecting health checks, and insufficient training, were revealed. The facilities used were also poor, with poor sanitation and low-quality raw materials. The findings emphasize the urgent need for intervention measures to enhance fish quality and safety in Zanzibar, including improvements in raw materials, infrastructure, training, and good practices throughout the fish value chain.

Keywords: Landing Sites, Rabbit Fish (*Signus surtor*), Contamination, Microbiological Quality, *E. coli, S. aureus, Salmonella* and Food Safety

1. Introduction

Marine fish are an important source of food for human diets due to their high nutritional value [41]. They are rich in omega-3 fatty acids, vitamins such as D and B2 (riboflavin), protein, calcium, phosphorus, and minerals like iron, zinc, iodine, magnesium, and potassium [50]. Marine fisheries play a crucial role in the economy and well-being of coastal communities by providing food security, job opportunities, income, livelihoods, and maintaining traditional cultural identity [24]. Fish and fishery products are a vital and affordable source of high-quality protein [40]. Globally, fish consumption per capita has increased from 9.9 kg in the 1960s to 20.2 kg in 2020 [23].

Despite of the advantages, marine fish also carry a significant risk of foodborne pathogens due to their high nutritional value and a pH close to neutral, as well as their high water activity [41]. The number of pathogens present is influenced by the microbial flora of the marine environment, the salt content of the water, its temperature, pollution levels, catching methods, and chilling conditions [41]. Marine fish can harbor pathogens that cause foodborne illnesses if they are caught in contaminated waters [14] or if proper hygiene practices are not followed during handling, processing, transportation, and storage, resulting in a decrease in fish quality [4].

Bacterial pathogens in marine fish can be classified into three main groups [33]. These groups include indigenous bacteria that naturally exist in water resources, such as virulent strains of *Aeromonas hydrophila*, *Vibrio cholerae*, *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Listeria monocytogene* and *Vibrio vulnificus*. There are also nonindigenous enteric bacteria that are present due to faecal contamination, including *Yersinia enterocolitica serotypes*, *Campylobacter spp*., pathogenic *Escherichia coli (E. coli)*, *Shigella spp*., and *Salmonella spp*. Additionally, there can be bacterial contamination during storage, processing, or preparation for consumption, such as *Clostridium perfringens*, *Staphylococcus aureus* (*S. aureus), L. monocytogenes*, and toxigenic *Bacillus cereus* strains [33]. Improper storage and handling of fishery products can also contribute to the growth of spoilage bacteria, such as *Lactobacillus spp*., *Proteus spp*., *Shewanella putrefaciens*, and *Pseudomonas spp*.

The bacteria from fish can become pathogens if there are stressors, such as poor water quality, rough handling, and overstocking. These stressors can lead to opportunistic bacterial infections [36]. Several studies have reported the presence of indicator microorganisms of faecal contamination, such as *Escherichia coli*, as well as pathogenic bacteria in humans, including *Staphylococcus aureus* and *Salmonella spp*., in fish samples [36, 41].

If the contaminated microorganism is pathogenic (disease-causing) and its count increases, it can lead to economic losses due to product degradation, followed by invasive infection on the consumer side [41]. Furthermore, if the pathogenic microorganism produces toxins, more serious foodborne poisonings (intoxication and toxic infection) can occur [12]. Detection of microbial pathogens in food is crucial for preventing and addressing health and safety issues. Food safety is a growing global health concern, and foodborne diseases are causing a major crisis in public health [22].

2. Materials and Methodology

2.1. Study Area and Handling Condition Survey

This study was carried out at Zanzibar in Unguja Island. Zanzibar has a total of 235 formal landing sites, of which 109 (49%) are in Unguja and 126 (57%) are in Pemba distributed along its coastline [49]. Unguja is made up of three Regions and seven Districts. The data was collected from four landing sites at Unguja, selected from four Districts with different geographical locations: Matemwe from North A District, Malindi Beach from Urban District, Mazizini from West B District, and Kizimkazi Mkunguni from South District. The data was collected from February to March 2023. The selected landing sites are commonly and daily used, all year round, in each of the four Districts. The map of the sampling sites is presented in Figure 1.

2.2. Research Design

A cross-sectional study design was used. The study allows data to be collected at a single point without repetition.

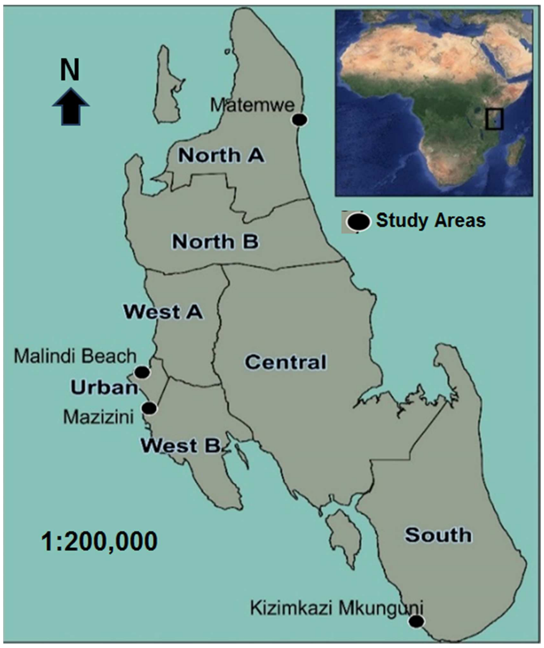

Figure 1. *Map of Unguja showing the sampling sites.*

2.3. Sample Size and Sampling

The sample size was estimated according to Dworkin [20] who suggested a minimum required sample size of 50 participants for a qualitative survey study. We collected data from a total of 165 selected respondents, comprising 83 fishermen, 19 fish handlers, 35 fish processors, and 28 fish vendors. The respondents with more than one year of experience handling fish were purposively included in the study. A questionnaire survey and direct observation were used to collect the data. All methods were performed by accepted ethics and regulatory requirements.

2.4. Sample Collection

A total of 24 swab samples were taken from both the boat and landing floor at four landing sites, to check them for microbiological contamination. A total of 36 samples of Rabbitfish were then collected under aseptic conditions from four landing sites. Nine samples were collected from each landing site. Three samples were taken directly from the marine environment, placed in zip bags, and immediately stored in a sterile cool box. The remaining six samples were collected from the boat and landing floor. These samples were placed in zip bags, appropriately marked, and immediately transferred into an ice box for transportation to the laboratory and held at -18°C until the time of analysis.

2.5. Culture, Isolation, and Identification

The culture isolation and identification of bacteria was performed by an enriched sample of fish using Peptone Buffer water of which 2g of sample was inoculated into 10 ml of buffer peptone water and incubation was done at 37°C for 24 hours. Aseptically culturing was done on Mannitol salt agar (Oxoid) for *Staphylococcus aureus*, MacConkey agar (Oxoid) for *E. coli* and XLD (Oxoid) for *Salmonella species*, and then incubated between 24 and 48 hours at 37°C. The subculture process was conducted until a pure culture of *Staphylococcus aureus* was produced on Mannitol salt agar, resulting in the growth of golden yellow colonies of medium size. Similarly, *Escherichia coli* was cultured on MacConkey agar, yielding lactose fermenting colonies that were smooth and of medium size. Bacteria were stained using the gram staining technique to ascertain their microscopic features *Staphylococcus aureus* was gram-positive, cocci in shape, grape like in clusters then identified by using enzyme test of catalase by using 3% hydrogen peroxide and coagulase test by using rabbit plasma. Consequently, *E coli* exhibited the characteristics of being gram-negative, possessing a rod-shaped morphology, and existing as individual cells. In a concise manner, the isolates were traditionally examined for their macroscopic and microscopic features, followed by biochemical analyses. *E coli* was characterized using the lactose, glucose, and sucrose concentrations of the triple sugar iron agar and the indole, methyl red, Voges Proskauer, and citrate concentrations of the IMViC test.

Microbial Load by Total Viable Count

About 2g of fish sample was chopped and placed on the 9ml of sterile normal saline, then 1 mL was serially diluted 10-fold using 10 universal bottles containing sterile normal saline. In each dilution, 1 mL was poured on the plate count medium Petri dishes in duplicate. The plates were incubated at 37°C for 24 hrs. Then, colonies were counted, and the average colony counts were used to establish the colony forming units (CFU/g).

3. Statistical Analysis of Data

The data analysis in this study involved the use of IBM SPSS Statistics (Version 25) to compute mean values and standard deviations. Differences in means related to microbial load were assessed using the Kruskal Wallis test, with post hoc Dunn's test for identifying specific group differences. Descriptive statistics were used to determine the frequency and proportion of actors in the fish production supply chain, while the Chi-square test was applied to compare proportions across different categories. A significance level of $P < 0.05$ was used to evaluate statistical significance. The prevalence analysis was conducted to assess the number of samples that tested positive for *Salmonella spp*, *E. coli*, and *Staphylococcus spp* isolation relative to the total samples analyzed.

4. Result and Discussion

4.1. Socio-Demographic Status of Respondent Along the Marine Supply Chain

Table 1 provides a detailed overview of the characteristics of respondents from four different landing sites. The majority of the participants in this study were found to be in the age range of 31-50 years, accounting for 48% of the total sample. This finding is consistent with the results reported by Adebayo *et al.* [3], who also found that 54% of fish farmers surveyed were within the same age range. The data reveals that the majority of respondents (98%) are male, suggesting a predominance of male participation in these activities. Previous studies found that most of the fish handling practices (transportation, processing and handling) are dominated by males [2, 10, 53]. In terms of education, most respondents had completed their secondary education, accounting for 49.7% of the total which aligns with the previous studies majority of them 42% have secondary [2]. According to the findings of the study conducted by Abubakar *et al.* [2], around 50.3% of the respondents reported being engaged in the activity of fisherman, thereby corroborating the primary occupation of a significant proportion of the participants. Regarding the participants' level of experience, it is noteworthy that the largest proportion of respondents (27.3%) belonged to the 5-10 years of experience category, whilst the "Above 20" category exhibited the lowest representation, accounting for just 23.6% of the respondents. This is in contrast to a study done by Sissoko *et al.* [52], where fishermen reported an average of 31.4 years of experience in fishing operations, with the vast majority (85.5% of the sample) having between 31 and 40 years of experience.

Table 1. *Socio-demographic status of respondents.*

Variables	Descriptions	Kizimkazi	Malindi	Matemwe	Mazizini	Total%
		N (%)	N (%)	N (%)	N (%)	
Age	18-30 years	14 (21%)	30 (45%)	10 (15%)	13 (9%)	67 (41%)
	31-50 years	13 (17%)	26 (33%)	24 (30%)	16 (20%)	79 (48%)
	51-60years	4 (24%)	2 (12%)	8 (47%)	3 (18%)	17 (10%)
	Above 60	1 (50%)	0 (0.0%)	0 (0.0%)	1 (50%)	2 (1%)
Gender	Female	2 (67%)	0 (0.0%)	0 (0.0%)	1 (33%)	3 (2%)
	Male	30 (18.5%)	58 (35.8%)	42 (25.9%)	32 (19.8%)	162 (98%)
Education	Certificate	0 (0%)	1 (50%)	0 (%)	1 (50%)	2 (1.2%)
	Diploma	1 (100%)	0 (%)	0 (%)	0 (%)	1 (0.6%)
	No formal	2 (6.9%)	10 (34.5%)	13 (44.8%)	4 (13.8%)	29 (17.6%)
	Primary	10 (19.6%)	18 (35.3%)	15 (29.4%)	8 (15.7%)	51 (30.9%)
	Secondary	19 (23.2%)	29 (35.4%)	14 (17.1%)	20 (24.4%)	82 (49.7%)
Main Activity	Fish handler	2 (10.5%)	11 (57.9%)	4 (21.1%)	2 (10.5%)	19 (11.5%)
	Fish processor	0 (0%)	20 (57.1%)	5 (14.3%)	10 (28.6%)	35 (21.2%)
	Fish vendor	3 (10.7%)	14 (50%)	5 (17.9%)	6 (21.4%)	28 (17%)
	Fisherman	27 (32.5%)	13 (15.7%)	28 (33.7%)	15 (18.1%)	83 (50.3%)

Variables	Descriptions	Kizimkazi	Malindi	Matemwe	Mazizini	Total%
		N (%)	N (%)	N (%)	N (%)	
Experience	10-20 years	8 (20%)	10 (25%)	14 (35%)	8 (20%)	40 (24.2%)
	5-10 years	6 (13.3%)	21 (46.7%)	9 (20%)	9 (20%)	45 (27.3%)
	Above 20	8 (20.5%)	9 (23.1%)	14 (35.9%)	8 (20.5%)	39 (23.6%)
	Below 5 years	10 (24.4%)	18 (43.9%)	5 (12.2%)	8 (19.5%)	41 (24.8%)

4.2. The Extent of Compliance with Hygienic Practices Among Fish Handlers at Landing Sites

Table 2 summarizes the hygienic practices of fishermen in four landing sites. 65% of fishermen reported to have below 10 fishermen in the fishing vessel. There was statistically significant difference in a number of fishermen in the vessel between landing sites (P< 0.05). Majority of the fishermen reported that they cleaned their vessel (60%), predominantly with saltwater 51.8%. Only a small percentage check their health regularly (32.5%). In terms of fish storage, the majority of individuals employ the vessel floor as a means of preservation, accounting for 75% of cases. Additionally, a notable proportion of individuals, approximately 10.8% and 35% respectively, utilize ice or salt to maintain the freshness of the fish. According to the findings, a significant majority of individuals, specifically 85.5%, were observed donning specialized attire while engaging in fishing activities. Conversely, a mere 5% of individuals were observed wearing gloves during the unloading process.

Table 2. *The extent of compliance to hygienic practices among fisherman at landing sites.*

Variable	Responses	Landing sites					Chi-square	P-value
		Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
Number of fishermen in the Vessel	Below 10	0 (0%)	14 (26%)	15 (27%)	26 (47%)	55 (65%)	45.684	0.000
	10 to 30	13 (48%)	1 (4%)	12 (44%)	1 (4%)	27 (34%)		
	Above 30	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (1%)		
Do you clean the fishing Vessel regularly?	Yes	10 (20%)	8 (16%)	16 (32%)	16 (32%)	50 (60%)	1.932	0.587
	No	3 (9%)	7 (21%)	12 (36%)	11 (33%)	33 (40%)		
What type of water do you use to clean the vessel	freshwater	0 (0%)	1 (50%)	0 (0%)	1 (50%)	2 (4.4%)	2.742	0.433
	saltwater	7 (16.3%)	6 (14%)	15 (35%)	15 (35%)	43 (95.6%)		
Do you check your health regularly	Yes	2 (7%)	5 (19%)	9 (33%)	11 (41%)	27 (32.5%)	2.577	0.462
	No	11 (13%)	10 (18%)	19 (34%)	16 (29%)	56 (67.5%)		
Where do you store your fish during fishing?	Container	5 (24%)	10 (48%)	4 (19%)	2 (10%)	21 (25%)	19.585	0.000
	Vessel floor	8 (13%)	5 (8%)	24 (39%)	25 (40%)	62 (75%)		
What type of storage method do you use to keep the fish fresh	None	0 (0%)	7 (16%)	23 (51%)	15 (33%)	45 (54%)	35.996	0.000
	Ice	6 (67%)	0 (0%)	0 (0%)	3 (33%)	9 (10.8%)		
	Salt	7 (24%)	8 (28%)	5 (17%)	9 (31%)	29 (35%)		
Do you have special clothes for fishing	Yes	12 (17%)	15 (21%)	24 (34%)	20 (58%)	71 (85.5%)	5.888	0.117
	No	1 (8%)	0 (0%)	4 (33%)	7 (58%)	12 (14.5%)		
Do you wear gloves during offloading the fish	Yes	1 (25%)	1 (25%)	1 (25%)	1 (25%)	4 (5%)	0.514	0.916
	No	12 (15%)	14 (18%)	27 (34%)	26 (33%)	79 (95%)		
Where do you store your fishing gear during fishing?	away from fish	11 (15%)	15 (21%)	27 (38%)	19 (26%)	72 (87%)	10.925	0.012
	same place with the fish	2 (18%)	0 (0%)	1 (9%)	8 (72%)	11 (13%)		

Hygiene adoption among fish handlers at various ports is summarized in Table 3. There was a statistically significant variation (p<0.05) in the types of vessels used to handle fish at different landing places, with 63% of fish handlers reporting using plastic baskets. There is a consistent practice of using salt water for washing vessels among fish handlers, with an overall prevalence of 96%. Hand washing practices were reported to be conducted before handling fish by 74%. Wearing gloves during fish handling is not widely practised among fish handlers, with 89% not using them.

Table 3. *The extent of compliance to hygienic practices among fish handlers at landing sites.*

Variable	Landing sites						Chi-square	P-value
	Responses	Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
Type of vessel to handle fish	Plastic basket	10 (83%)	1 (8%)	0 (0%)	1 (8%)	12 (63%)	17.705	0.039
	Plastic basin	0 (0%)	1 (33%)	2 (67%)	0 (0%)	3 (16%)		
	traditional basket	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)		
	Others	0 (0%)	0 (0%)	2 (67%)	1 (33%)	3 (16%)		
What type of water do you use to wash the vessel	freshwater	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0.768	0.857
	saltwater	10 (56%)	2 (11%)	4 (22%)	2 (11%)	18 (96%)		
Do you check your health regularly	Yes	5 (45%)	2 (18%)	2 (18%)	2 (18%)	11 (58%)	3.71	0.295
	No	6 (75%)	0 (0%)	2 (25%)	0 (0%)	8 (42%)		

Variable	Responses	Landing sites					Chi-square	P-value
		Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
Do you wash your hands before handling fish	Yes	9 (64%)	0 (0%)	4 (29%)	1 (7%)	14 (74%)	7.982	0.046
	No	2 (40%)	2 (40%)	0 (0%)	1 (20%)	5 (26%)		
Do you wear gloves during fish handling	Yes	1 (50%)	1 (50%)	0 (0%)	0 (0%)	2 (11%)	4.039	0.257
	No	10 (59%)	1 (6%)	4 (24%)	2 (12%)	17 (89%)		
How long does it take to handle fish	<5 min	7 (70%)	0 (0%)	2 (20%)	1 (10%)	10 (53%)		
	5-10 min	3 (38%)	2 (25%)	2 (25%)	1 (12.5%)	8 (42%)		
	>10 min	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	4.297	0.637

Table 4 presents a summary of the degree to which fish processors at landing sites have adopted hygienic practices. The frequency of health checks exhibits variability, as 49% of individuals engage in frequent monitoring of their health, but the remaining 51% do not partake in such practices. The utilization of gloves during the processing phase is infrequent, as a significant proportion (83%) of fish processors refrain from their usage. The practice of hand washing before fish processing is observed to be more widespread, as shown by 69% of the participants in the sample reporting its implementation. A majority of processors (51%) place fish on benches during processing, while surface utilization is less common. Lastly, the time taken to process fish varies, with no significant statistical difference observed (p>0.05).

***Table 4.** The Extent of compliance to hygienic practices among fish processors at landing sites.*

Variable	Responses	Landing sites					Chi-square	P-value
		Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
Do you check your health regularly	Yes	12 (70%)	3 (18%)	2 (12%)	0 (0%)	17 (49%)		
	No	8 (44%)	7 (39%)	3 (17%)	0 (0%)	18 (51%)	2.574	0.276
Do you wear gloves during fish processing	Yes	3 (50%)	3 (50%)	0 (0%)	0 (0%)	6 (17%)	2.263	0.323
	No	17 (59%)	7 (24%)	5 (17%)	0 (0%)	29 (83%)		
Do you wash hands before processing fish	Yes	16 (67%)	7 (29%)	1 (4%)	0 (0%)	24 (69%)		
	No	4 (36%)	3 (27%)	4 (36%)	0 (0%)	11 (31%)	6.695	0.035
Do you wash fish before processing	Yes	17 (57%)	8 (27%)	5 (17%)	0 (0%)	30 (86%)		
	No	3 (60%)	2 (40%)	0 (0%)	0 (0%)	5 (14%)	1.108	0.575
Do you wash fish after processing	Yes	20 (59%)	9 (26%)	5 (15%)	0 (0%)	34 (97%)		
	No	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (3%)	2.574	0.276
Where do you place fish while processing	on the bench	12 (67%)	2 (11%)	4 (22%)	0 (0%)	18 (51%)		
	on the floor	8 (57%)	5 (36%)	1 (7%)	0 (0%)	14 (40%)		
	on the surface	0 (0%)	3 (100%)	0 (0%)	0 (0%)	3 (9%)	11.25	0.024
Time taken to process fish	Less than 5 min	2 (50%)	1 (25%)	1 (25%)	0 (0%)	4 (11%)		
	5- 10 min	6 (55%)	2 (18%)	3 (27%)	0 (0%)	11 (31%)		
	10- 20 min	5 (63%)	3 (37%)	0 (0%)	0 (0%)	8 (23%)		
	over 20 min	7 (58%)	4 (33%)	1 (8%)	0 (0%)	12 (34%)	3.904	0.69

Table 5 shows the degree of Hygienic Practices Adoption among Fish sellers at Landing Sites. A majority of the fish vendors, specifically 61%, indicated that they engage in regular health check-ups. The prevalence of glove usage among fish vendors during fish selling activities is limited, as indicated by a significant majority of 93% of respondents who reported not wearing gloves. The transportation technique for fish exhibits variation, with bicycles being the predominant mode, accounting for 46% of instances. The placing of fish throughout the selling process exhibits variability, with plastic baskets being a frequently employed method, accounting for approximately 36% of cases. The prevalence of shielding fish from direct sunlight is notably higher among sellers, with around 82% of them adhering to this practice. Furthermore, the predominant method employed for preserving the freshness of fish during storage predominantly entails the utilization of ice, as reported by approximately 89% of respondents. The duration of time spent selling fish varies, with a significant percentage (50%) in the "<3 hours" category.

***Table 5.** The extent of compliance to hygienic practices among fish vendors at landing sites.*

Variable	Responses	Landing sites					Chi-square	P-value
		Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
Do you check your health regularly	Yes	8 (47%)	5 (29%)	2 (12%)	2 (12%)	17 (61%)		
	No	6 (55%)	1 (9%)	3 (27%)	1 (9%)	11 (39%)	2.306	0.511
Do you wear gloves during fish selling	Yes	0 (0%)	1 (50%)	1 (50%)	0 (0%)	2 (7%)		
	No	14 (54%)	5 (19%)	4 (15%)	3 (12%)	26 (93%)	3.374	0.337

Variable	Landing sites						Chi-square	P-value
	Responses	Malindi	Mazizini	Matemwe	Kizimkazi			
		N (%)	N (%)	N (%)	N (%)	Total%		
How do you transport fish	by feet	4 (50%)	2 (25%)	2 (25%)	0 (0%)	8 (29%)		
	by bicycle	9 (69%)	2 (15%)	2 (15%)	0 (0%)	13 (46%)		
	by motorcycle	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)	3 (11%)		
	open board truck	0 (0%)	1 (50%)	0 (0%)	1 (50%)	2 (7%)		
	public transport	0 (0%)	0 (0%)	0 (0%)	2 (100%)	2 (7%)	26.509	0.09
Where do you place fish while selling	plastic basket	7 (70%)	2 (20%)	0 (0%)	1 (10%)	10 (36%)		
	plastic basin	3 (43%)	1 (14%)	1 (14%)	2 (29%)	7 (25%)		
	traditional basket	3 (60%)	0 (0%)	2 (40%)	0 (0%)	5 (18%)		
	on the bench	1 (17%)	3 (50%)	2 (33%)	0 (0%)	6 (21%)	13.118	0.157
Do you cover fish from direct sunlight	Yes	12 (52%)	4 (17%)	5 (22%)	2 (9%)	23 (82%)		
	No	2 (40%)	2 (40%)	0 (0%)	1 (20%)	5 (18%)	2.678	0.444
storage method used to keep fish fresh	Ice	13 (52%)	5 (20%)	4 (16%)	3 (12%)	25 (89%)		
	Salt	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)	3 (11%)	1.22	0.748
What hours are spent selling the fish	< 3 hrs	8 (57%)	3 (21%)	3 (21%)	0 (0%)	14 (50%)		
	3-7 hrs	4 (40%)	3 (30%)	2 (20%)	1 (10%)	10 (36%)		
	>7hrs	2 (50%)	0 (0%)	0 (0%)	2 (50%)	4 (14%)	9.65	0.14

Table 6 presents a summary of the observation checklist inquiries pertaining to the hygienic conditions seen during fishing and landing activities at various sites. Several key observations emerge from the data. The number of fishermen on the boat varies significantly ($p<0.05$), with a higher percentage of boats having below 10 fishermen (73%). The cleanliness of the boat also varies, with a higher percentage (70%) of clean boats. The cleanliness of the fishermen themselves varies, with 85% of fishermen not being clean. Moreover, the practice of icing fish on the boat is minimal (15%). Similarly, salting fish on the boat is not common (2%). The source of ice also varies, with a higher percentage (83%) of ice not being made from clean water. The performance of hygienic practices by fish handlers varies across sites, with an overall rate of 40%. Lastly, there is a noticeable association between fish handling activities and fish contamination, with 75% of respondents acknowledging contamination.

Table 6. *Observation checklist questions for hygienic conditions during fishing and landing.*

Variable	Responses	Malindi	Mazizini	Matemwe	Kizimkazi		Chi-square	P-value
		N (%)	N (%)	N (%)	N (%)	Total%		
Number of fisherman in the boat	below 10	4 (14%)	7 (24%)	8 (28%)	10 (34%)	29 (73%)		
	above 10	6 (55%)	3 (27%)	2 (18%)	0 (0%)	11 (27%)	9.404	0.024
Is the boat clean?	Yes	4 (14%)	6 (21%)	8 (29%)	10 (36%)	28 (70%)		
	No	6 (50%)	4 (33%)	2 (17%)	0 (0%)	12 (30%)	9.524	0.023
Are the fisherman clean?	Yes	2 (33%)	4 (67%)	0 (0%)	0 (0%)	6 (15%)		
	No	8 (24%)	6 (18%)	10 (29%)	10 (29%)	34 (85%)	8.627	0.035
Does the fish iced in the boat	Yes	0 (0%)	2 (33.3)	2 (33.3)	2 (33.3)	6 (15%)		
	No	10 (29%)	8 (24%)	8 (24%)	8 (24%)	34 (85%)	2.353	0.502
The ice made from clean water	Yes	0 (0%)	2 (29%)	2 (29%)	3 (43%)	7 (18%)		
	No	10 (30%)	8 (24%)	8 (24%)	7 (21%)	33 (83%)	3.29	0.349
Do fish handlers perform hygienic practices?	Yes	5 (31%)	6 (38%)	3 (19%)	2 (13%)	16 (40%)		
	No	5 (21%)	4 (17%)	7 (29%)	8 (33%)	24 (60%)	4.167	0.244
Does fish handling activities contaminate the fish	Yes	5 (17%)	7 (23%)	8 (27%)	10 (33%)	30 (75%)		
	No	5 (50%)	3 (30%)	2 (20%)	0 (0%)	10 (25%)	6.933	0.074

In this study, landing sites were identified as the crucial points for contamination sources, including the operator's practices, equipment and the nature of the area. This makes the assessment of contamination sources and potential pathways within the fish supply chain possible, providing a basis for targeted interventions aimed at enhancing food safety and quality.

The findings of this investigation revealed that a portion of handlers did not adequately implement essential hygiene measures, as seen by the data presented in Tables 2 to 5. Regular hand washing, health checks, the utilization of gloves, and wearing of specialized attire are seen as vital procedures for food workers. However, the findings revealed that a substantial percentage of fishermen (67.5%), fish handlers (42%), fish processors (51%), and fish vendors (39%) did not habitually undergo health checks. These findings align with a study by Mwasulama *et al*. [39], which revealed that personnel were not subjected to health checkups, while contrasts with a previous study by Abubakar *et al*. [2], where over half (50.3%) of fishermen regularly checked their health. In Tanzania, food laws mandate that all food handlers undergo health checks upon employment and every six months to ensure the safety and quality of food [54]. The Codex Alimentarius Commission [16] also recommends that food handlers with any disease that could be transmitted through food handling should not be allowed to handle food.

The practice of wearing gloves during fish handling was notably low, with only 5% of fishermen, 11% of fish handlers, 17% of fish processors, and 7% of fish vendors complying with the habit. These results were significantly lower than the findings of Çakıroğlu and Uçar [17], Al-Shabib *et al*. [7] and Hashanuzzaman *et al*. [27] who reported that almost 80%, 97% and 32% of food handlers frequently wore gloves while handling food respectively. Furthermore, Grema *et al*. [25] found that the majority of fish processors (91.9%) used gloves during fish handling, which contradicts this study.

In contrast, a considerable proportion of fish handlers (74%) and fish processors (69%) exhibited a relatively high adherence to the practice of hand washing before handling. The percentages observed in this study align with the results reported by Hashanuzzaman *et al*. [27] and Abubakar *et al*. [2], who found that hand washing rates among fish handlers were 64% and 64.7%, respectively. In a study conducted by Grema *et al*. [25], it was shown that a significant proportion of fish processors engaged in hand washing practices both before (91.9%) and after (97.3%) handling fish. These rates were found to be higher compared to the present study. Additionally, in the present study, the majority of fishermen (61%) and fish handlers practised washing their vessels before placing fish in them. This contrasts with a study by Yohans *et al*. [60], which found that fish handlers (80%) did not wash or clean their boats before and after use. However, it was important to note that the water used for vessel cleaning was often saltwater from nearby marine areas, which can be a source of contamination. In this study, fishermen (51.8%) and fish handlers (96%) used salt water for cleaning, which aligns with Ouedraogo *et al*. [45], more than sixty percent (60%) did not have access to potable water.

Furthermore, the use of ice during the transportation of fish appeared to be lacking, as only 10.8% of fishermen across all sites adopted this practice. This finding aligns with the study conducted by Yohans *et al*. [60], which revealed that 100% of fish handlers transport fish without using ice. However, it differs from the studies conducted by Singh *et al*. [51] and Edirisinghe *et al*. [21], which reported that 96.67% and 47% of fishermen, respectively, use ice to preserve their fish before transportation.

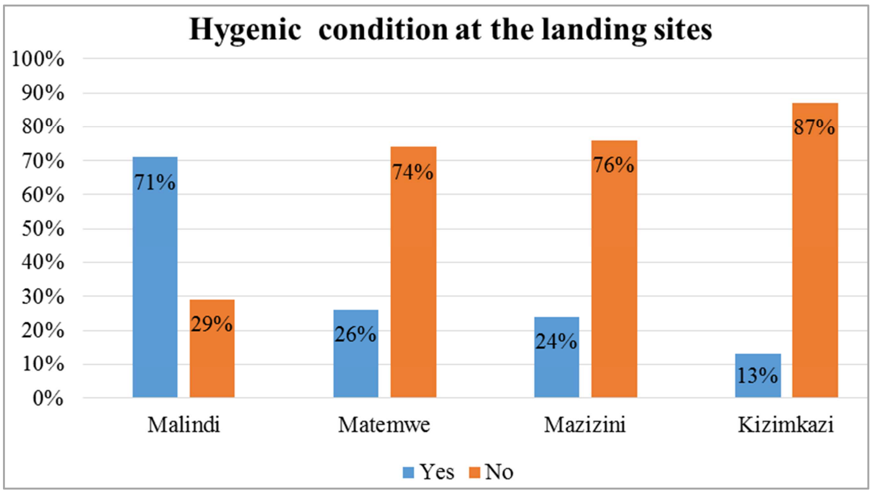

Figure 2. *Observation checklist questions for hygienic condition of the landing sites infrastructure and sanitary.*

Figure 2 provides an observation checklist outlining the hygienic conditions and infrastructure at landing sites. Among the sites, Malindi stood out with 71% of good practices. This was attributed to its new construction, well-maintained infrastructure, proper waste disposal, clean toilets, hand washing facilities, and adequate water supply. However, there were still issues at Malindi, such as fish handlers lacking proper training and the underutilization of the available ice factory. In contrast, Kizimkazi Mkunguni lacked dedicated fishing infrastructure, proper drainage, and sufficient water resources. As a result, it had poor building and sanitation conditions, with only 13% of good practices. Mazizini had 24% of good practices, while it's fishing buildings suffers from water scarcity and animals roaming around the premises. Inadequate sanitation was also a problem, as there were no cleaning personnel and no control of food vendors (including fruits, vegetables, and shops). Finally, Matemwe had 26% of good hygiene practices. The fishing building facilities lacked a drainage system, water, toilets, and waste disposal containers. This poses a risk to fish contamination during extended storage. Additionally, the presence of animal and vegetable vendors with shops could be a vehicle for microbiological hazards that could subsequently cross-contaminate the premises and products.

Previous studies had reported inadequately designed premises without a good drainage system, lack of control of food vendors (including fruit and vegetable), animal roaming, lack of adequate knowledge on proper food handling, and no hygienically designed toilets with hand-washing facilities and practices for fish processing as by Mwasulama *et al*. [39] site and building layout of Kayabo processors. Another study by Akintola and Fakoya [6] also highlighted similar issues. Additionally, the results of the study showed that, similar to Table 6, the majority of fishermen were not clean (85%), fish was not properly iced on the boat, and 60% of fish handlers did not practice proper hygiene.

4.3. Assessment of Microbiological Contamination

Table 7 shows the percentage of bacterial species isolated (*S. aureus*, *E. coli* and *Salmonella)* from fish muscle samples at four landing sites. *S. aureus* was the most commonly isolated bacterium, with isolation rates of 53.33% in Malindi, 60% in both Mazizini and Kizimkazi, and 46.67% in Matemwe. *E. coli* was also present, though less frequently, with isolation rates of 6.67% in Malindi, 33.33% in Mazizini and Kizimkazi, and 20% in Matemwe. *Salmonella spp* was absent in all samples, recording a 0% isolation rate across landing sites.

Table 7. *Percentage of occurrence of bacteria in fish muscle at coastal landing sites.*

Landing sites	No. of samples	No. of positive sample		
		S. aureus	*E. coli*	*Salmonella spp*
Malindi	15	8 (53.33%)	1 (6.67%)	0 (0%)
Mazizini	15	9 (60%)	5 (33.33%)	0 (0%)
Kizimkazi	15	9 (60%)	5 (33.33%)	0 (0%)
Matemwe	15	7 (46.67%)	3 (20%)	0 (0%)

Numbers in Parentheses Are the Percentage (%) of Bacterial Species Isolated

The finding from Table 7 revealed that, three distinct bacterial species were identified, where *S. aureus* emerged as the most frequently isolated bacterium from cultured fish, displaying relatively high isolation rates across all landing sites, ranging from 46.67% to 60%. This finding aligns with previous research conducted by Rabia *et al*. [46] who found that *S. aureus* was a prevalent bacterium in the fish value chain, accounting for 66% of isolates, which was more than other bacterial species. However, Ali [8] reported a very high prevalence of 100% for *S. aureus*, which is significantly higher than the results of this study. Choudhary *et al*. [19] isolated *S. aureus* in 33.33% of raw fish samples, which is lower than the findings of the current study. Rong *et al*. [47] reported a prevalence of 37.2% for *S. aureus* in fish samples, also lower than the findings of this study.

In contrast, *E. coli* was present but less frequently, with isolation rates varying between 6.67% and 33.33% across the landing sites. The findings of this study were in line with previous research conducted by Awot *et al*. [9], who reported a 9.4% prevalence of *E. coli* among 96 fish samples obtained from fish meat retailing shops in Mekelle City, Ethiopia. Similarly, a comparable pattern emerged in the study by Tilahun and Engdawork [55], where 80 (23.3%) *E. coli* isolates were identified in fish samples collected from Lake Hawassa in Southern Ethiopia, demonstrating alignment with the observations of our current study. However, it's important to note that the prevalence of *E. coli* in this study was lower when compared to the previous findings of Kumar *et al*. [30], who estimated a higher prevalence of 47% for *E. coli*, including faecal coli forms, in tropical seafood. Additionally, Gupta *et al*. [26] also reported a higher occurrence of *E. coli*, specifically 48.95%, in their study involving 96 raw fish samples. In contrast, this study's results were lower than those reported by Wendwesen *et al*. [59], who found that 42.5% of raw fish samples in Arba Minch town, Ethiopia, tested positive for *E. coli*. These variations underscore the importance of considering regional factors and local conditions when assessing *E. coli* prevalence in fish products.

The findings from this study were consistent with an absence of *Salmonella spp*., with a 0% isolation rate observed across all landing sites. This aligned with previous research, as demonstrated by studies conducted by Aboagye *et al*. [1] Bediang *et al*. [11] and Valenzuela *et al*. [56], which similarly reported a lack of *Salmonella spp*. in their samples, with an isolation rate of 0%. In contrast, this bacteria was reported 5% in retail fish samples in Turkey by Onmaz *et al*. [44] and Wendwesen *et al*. [59] who reported 7.5% from frozen of raw fish samples of Nile tilapia. The higher results were reported by Budiati *et al*. [15], Jegadeeshkumar *et al*. [29] and Kumar *et al*. [31] at rates of 90%, 43.8%, and 30% in fish samples, respectively.

The study results indicated a notable similarity in the high levels of identification of *Staphylococcus aureus* and *Escherichia coli*, in contrast to *Salmonella*, which was consistent with findings from different studies. In line with the research conducted by Wendwesen *et al*. [59] from 40 frozen raw samples of tilapia fillet examined, 65% had *S. aureus*, 42.5% had *E. coli* and 7.5% had *Salmonella spp*. Additionally, Muhammad *et al*. [38], revealed that *Staphylococcus aureus* and *Escherichia coli* were the most prevalent pathogenic bacteria. Similarly, Rabia *et al*. [46] showed a similar pattern, with *Staphylococcus aureus* having a high prevalence rate of 66% and *Escherichia coli* at 23%, closely resembling the results of our current study.

The presence of pathogenic bacteria in fish, such as *Staphylococcus spp*. and *E. coli*, indicated potential risks to consumers, as these are not naturally part of fish's microflora. The presence of *S. aureus* in food indicated potential contamination from food handlers' skin, mouth, or nose [34]. Several factors contribute to the introduction of *S. aureus* into seafood, including inadequate hygiene and sanitation during fish handling, transportation, and storage, as well as contamination by asymptomatic carriers of coagulase-positive *S. aureus* [18, 32]. Cross-contamination via utensils has also been extensively documented [13, 42]. Most of the isolated bacteria are of significant public health concern, as they can cause both local and systemic infections and food poisoning. *Staphylococcus aureus* is particularly worrisome due to its ability to produce enterotoxins associated with food poisoning and gastroenteritis, and although it can be eliminated by heating, its heat-tolerant toxins can still pose health risks [46].

The presence of *E. coli* in the examined fish samples indicates faecal and environmental contamination, linked to inadequate hygiene practices during fish handling. Notably, fish handlers were found to exhibit insufficient hygiene measures, leading to the presence of *E. coli* in the fish samples. Furthermore, the landing floor and equipment used for selling the fish were observed to be notably dirty, and the water used for rinsing the fish was directly sourced from the sea at the landing site, which likely introduced *E. coli* bacteria to the fish. Such bacterial contamination in food or processing equipment clearly underscores the urgent need for improved sanitation practices during fish handling, as emphasized by [58]. *E. coli*, a well-known enteric bacterium, is capable of causing gastroenteritis. It, along with other coliforms and bacteria, serves as an indicator of hygienic conditions during fish handling and processing. Ideally, these organisms should be absent in freshly caught fish. The contamination of fish products with pathogenic *E. coli* can occur during various stages, including handling and production processes, or as a result of harvesting fish from polluted waters with inadequate sewage treatment and irresponsible waste disposal [5, 18, 28].

The TVC were quantified at four landing sites ranging from 2.82 to 3.30 log CFU/g (Table 8). The results showed that Malindi had the lowest count at 2.82 ± 0.83 log CFU/g, followed by Matemwe (2.87 ± 0.70 log CFU/g), Kizimkazi had 3.03 ± 1.34 log CFU/g, and Mazizini had the highest at 3.30 ± 1.65 log CFU/g.

There was a statistically significant variation in the bacterial count between the different sample types (p = 0.002). Using Dunn's test, it was noted that there was a statistically significant difference (p 0.05) in bacterial count between the fish samples from the water and from the landing floor (Table 9). Matemwe was the sole landing site where there were statistically significant differences between locations within the site (P = 0.039), with the greatest mean found on the landing floor (3.76 log CFU/g) and the lowest found in fish taken straight from the sea (2.31 log CFU/g). There were no significant differences at other sites, although Kizimkazi showed a tendency towards significance, as presented in Table 10.

Table 8. *Bacterial count from four landing sites (log CFU/g).*

Landing sites	N	Mean ± SD (log CFU/g)
Malindi	9	2.82 ± 0.83^{a}
Kizimkazi	9	3.03 ± 1.34^{a}
Matemwe	9	2.87 ± 0.70^{a}
Mazizini	9	3.30 ± 1.65^{a}

Mean bacterial counts among landing sites with similar subscript letters along the column are not significantly different at p>0.05.

Table 9. *Bacterial count from four sample sources.*

Sample source	N	Mean ± SD (log CFU/g)
Fish from boat	12	2.70±0.68^{ab}
Fish from landing floor	12	3.87±1.51^{a}
Fish directly from water	12	2.45± 0.60^{b}

Mean values with different superscript letters in a column are significantly different (p<0.05*)*

Table 10. *Bacterial count from different sample sources within each landing sites.*

Location	Sample source	Mean ± SD (Log CFU/g)	P value
Malindi	Fish from boat	2.60±1.09^{a}	P=0.561
	Fish from landing floor	3.29±0.40^{a}	
	Fish directly from water	2.56±0.95^{a}	
Kizimkazi	Fish from boat	3.16±0.95^{a}	P=0.06
	Fish from landing floor	3.93±1.98^{a}	
	Fish directly from water	2.01±0.28^{a}	
Matemwe	Fish from boat	2.53±0.15^{ab}	P=0.039
	Fish from landing floor	3.76±0.28^{a}	
	Fish directly from water	2.31±0.15^{b}	
Mazizini	Fish from boat	2.52±0.16^{a}	P=0.561
	Fish from landing floor	4.50±2.69^{a}	
	Fish directly from water	2.88±0.58^{a}	

Mean values with different superscript letters in a column are significantly different (p<0.05)

To assess the overall quality of fish, the total bacterial count (TVC) is employed as a general indicator. The microbiological limit for TVC, which distinguishes between fresh fish of good and bad quality, is set at 5.00 log CFU/g according to TZS 118:2007 and EAS 828:2016 standards. In a recent study, the mean bacterial counts in samples from various locations were examined. The findings indicated that the mean TVC values were consistently below the specified standards, with average log CFU/g values of 2.82, 3.03, 2.87, and 3.30 in Malindi, Kizimkazi, Matemwe, and Mazizini, respectively, for *Signus surtor* of fish samples. Similar trends were observed in the other locations. Importantly, all mean TVC values remained below the recommended limit, indicating good fish quality. These results suggest that rapid sampling and limited exposure to contamination contributed to maintaining low levels of bacterial contamination in the fish samples.

This study aligns with previous research conducted in similar contexts. In a study conducted in Parangipettai coastal waters by Velmurugan *et al.* [57], it was found that the total bacterial population in freshly caught fish was relatively low, ranging from log 3 to log 3.15. After two hours, the bacterial count increased to log 3.88 to log 3.945, and at the landing center, it ranged from log 4.3 to log 4.5, all of which indicated a low mean bacterial count below recommended limits. Similarly, another study by Onjong *et al.* [43] showed that the means of total bacterial counts (TVC) in fish samples from different locations and supply chains were not significantly different. However, the TVC mean shows significance depending on the fish's value chain stage, similar findings of the current study. The mean TVC in the samples was 4.44 log CFU/g, slightly lower than the established fish quality standard. In contrast, this study reported a lower microbial load compared to Mitiku *et al.* [37], who reported log 5.06 in raw fish samples, and Wendwesen *et al.* [59], who reported 6.7 log CFU/g in frozen raw Nile Tilapia fish samples in Arba Minch town, Ethiopia.

The findings of a correlation analysis between boat swabs and boat contamination, as well as between floor swabs and floor contamination, are presented in Table 11. The Pearson correlation coefficients for both correlations exhibit a significant positive association, with a coefficient of 0.591 for boat swabs and 0.654 for floor swab. The observed coefficients indicate a significant positive linear correlation between the swab samples collected from boats and the corresponding levels of pollution. The associated p-values, 0.043 for boat swabs and 0.021 for floor swab, are both less than the common significance level of 0.05, revealing that these relationships are statistically significant. These findings imply that the contamination on boats and floors is likely influenced by the presence and extent of microbial or particulate matter, as indicated by the swab samples, highlighting the importance of effective cleaning and maintenance practices in these environments.

Table 11. *Pearson Correlation (R) and Significance (P) for Fish Contamination and Swab Samples from Boat and Floor Surfaces.*

Sample source	Mean ± SD (log CFU/g)	Fish contamination		Swab samples
				Boat swab
Fish from water	2.45± 0.60	Boat contamination	Pearson correlation	0.591
Fish from boat	2.70±0.68		Sig (2 tailed)	0.043*
Fish from Landing floor	3.87±1.51			Floor swab
Swab from boat	3.03± 1.04	Floor contamination	Pearson correlation	0.654
Swab from landing floor	5.19±1.49		Sig (2 tailed)	0.021*

The value of boat contamination= (fish from boat - fish from water) and Floor contamination = (fish from floor- fish from boat).

The cleanliness of the examined places were assessed by using the swab method to evaluate their hygienic conditions. Swab samples were randomly collected from both boat and landing floor areas before the fish were kept at the landing sites, resulting in TVC values were 3.03±1.04 for boat swabs and 5.19±1.49 log CFU/g for landing floor swabs, as presented in Table 11. This study reveals variations in the mean of fish and swab samples collected, with swab samples revealed a high level of contamination, which were similar to other studies by Roy et al. [48] Swab samples collected from crates and areas where fish was kept in Baghajatin ranged from 4.51±0.11 log CFU/g in October to 4.11±0.07 log CFU/g in January, while in Garia, the TPC (Total Plate Count) varied from 4.66±0.16 log CFU/g in October to 4.18±0.02 log CFU/g in January. Furthermore, Margaret and Edgar [35] study reported that swab samples collected from boats at Butiaba had counts 2.76 x 10^5 times higher than fish samples, and at Walukuba, swab samples from fishing boats had counts 2.5x10^5 higher than raw fish, while Bugoigos swab samples from fishing boats 1.44 x10^5 also had higher counts than fish samples.

The findings of this study highlight that raw fish obtained from four landing sites exhibited a low total viable count, indicating a relatively low level of bacterial contamination. It was revealed that contamination levels increased as the fish were transported on boats and subsequently reached the landing area, reflecting potential contamination risks associated with improper hygiene practices and the use of ice prepared from contaminated water for fish preservation. Higher microbial counts in some samples could be attributed to extended handling times, overcrowding, inadequate sanitation during harvest or processing, and delays in freezing. Furthermore, leaving fish at landing sites without proper cooling measures, as observed in this study, can significantly boost microbial activity and increase the microbial load, potentially raising food safety concerns for consumers when fish spend extended periods at these sites.

5. Conclusion

This study addresses a critical issue in Zanzibar's seafood industry by focusing on the microbiological quality of Rabbit Fish (*Siganus Sutor*) and the food safety practices among fish handlers at four landing sites. The study revealed that the collected fish is safe for human consumption. However, it also reveals that the poor hygienic conditions at the landing sites result from factors such as inadequate sanitation, improper handling, and the use of contaminated water in ice production. The contamination observed on boat and landing surfaces underscores the urgent need for comprehensive disinfection to eliminate microbial risks. The study emphasizes the necessity of immediate improvements in food safety and quality control to safeguard public health and the reputation of Zanzibar's seafood industry. To achieve this, it is essential to implement enhanced hygiene and storage procedures, provide training and support to fish handlers, strengthen regulatory oversight, and promote awareness of food safety standards.

Conflicts of Interest

The authors declare no conflict of interest.

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