



Potential Public Health Risks of Pathogenic Bacteria Contaminating Marine Fish in Value Chain in Zanzibar, Tanzania

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Authors' contributions

This was a collaborative work among all authors. Author ARR designed the study, collected specimen from field, carried out and supervised laboratory work, performed statistical analysis and wrote the first draft of the manuscript. Authors PNW, SIK, RHM assisted on study design and in drafting the protocol organizing and refining the manuscript. Author AM played a role on literature citing, organising and fine tuning of the manuscript according to journal requirements. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Marine sourced food, to a large extent, provides protein and nutrients to people of Zanzibar where 85% of Zanzibar population consume fish at least five times a week. This study was carried out to investigate the safety of marine foods consumed in Zanzibar.

Study Design: A longitudinal study design was used to investigate variations in colony forming

units along the value chain of fishermen, vendors and consumers. A cross sectional study design was used to study the profile and number of microbial pathogens. A repeated cross sectional study was used for value chain analysis.

Place and Duration of Study: The study was carried out in Zanzibar, Tanzania between August 2014 and June 2015.

Methodology: A total of seven hundred and eighty fish samples were collected seasonally through the value chain from fishermen, through vendors to ready-to-eat food (consumer), between 2014 and 2015 with the aim of assessing bacterial contamination (colony forming units (CFUs), identify prevalent bacterial species and investigate if the pattern of CFUs is influenced by ambient temperatures, rainfall or human activities. The study was also intended to investigate antibiotic sensitivity profile of some of the prevalent bacteria. Sample collection was done from 8 different landing sites. Moreover, an additional 60 samples were collected from one recreation site.

Results: It was observed that bacterial load tended to increase in January-March season when ambient temperatures were high and fell down during the cool season July-September even though the difference was not statistically significant ($p>0.05$). Bacterial loads were higher in fish collected from vendors than in fishermen or consumers, again the difference was not significant ($p>0.05$). Bacterial load of fish from consumers in a recreation site (Forodhani) were highly significant ($p<0.0001$) compared with the rest of the counts in the value chain. There was no evidence that anthropogenic activities like tourism affected bacterial load. However, fish collected from town based landing sites tended to have higher bacterial loads which could be attributed to sewage disposal and human activities. Thirty one bacterial species were identified and many of them were of public health importance. Antibiotic disc sensitivity tests revealed existence of multidrug resistance among 21% of *Staphylococcus aureus* isolates.

Conclusion: To improve the hygiene situation food safety rules must be enforced, food tracing system must be strengthened and mass awareness for improvement of hygiene standards be implemented to all fish stakeholders.

Keywords: Value chain; fishermen; vendors; consumers; colony forming units; marine fish.

1. INTRODUCTION

Marine foods, especially fish, are among the most important sources of protein to humans and contribute about 60% of the world supply of protein. About 60% of the developing world derives more than 30% of their animal protein from fish [1,2]. Among the benefits of fish consumption, especially those attributed to the presence of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), include reduction of risk of suboptimal neurodevelopment in newborn, reduction of coronary heart disease, lowering of blood pressure, reduction of dementia and cognitive decline as well as depression [3].

Being endowed with a coastline that is rich with varieties of marine animals, Zanzibar depend highly on marine foods as a major source of protein and indeed fish is regularly consumed by at least 95% of the Zanzibar population [4].

Dependency on marine foods is not without challenges, however. Despite the efforts of the Revolutionary Government of Zanzibar on enactment of the law "Food, drugs and cosmetics

act" in the year 2000 that define rules to safeguard food safety to Zanzibar population, enforcement of laws especially with locally produced foods is still lagging behind. Impact of poor implementation of food hygiene rules is reflected on high number of cases of food poisoning in the Zanzibar district and zonal hospitals.

Indiscriminate use of antibiotics in humans and domestic animals can lead to antimicrobial resistance and the potential spread of resistant strains to environment and humans with public health impact [5,6]. Bacteria contaminating marine fish can therefore act as vehicle of drug resistant strains that may end up in the food chain.

The purpose of this study was to investigate the seasonal variations of bacterial load of marine fish in the value chain- from freshly harvested fish, vendor and food ready for consumption and investigate if the bacterial load is influenced by climate and human activities. Moreover the aim was to identify bacteria of public health importance and investigate antimicrobial drug resistance profiles of some of the prevalent bacteria.

2. MATERIALS AND METHODS

2.1 Study Area

Zanzibar being part of the United Republic of Tanzania is situated in the Indian Ocean, off the coast mainland Tanzania extending between latitudes 4 degrees and 6.5 degrees south of the equator. It enjoys a permanently humid tropical climate with four seasons- long rains between March and June, cool dry season July to August, short rains September to November and dry hot season between December and February. The average daily ambient temperature is 24.8°C while the average monthly rainfall is 138 mm [7].

Eight major fish landing sites and one recreation site in Zanzibar islands were chosen for sampling out a mixture of 207 large and smaller makeshift landing sites; four of the sites were in the northern island Pemba while four were in southern island Unguja.

2.2 Metrological Data Collection

The meteorological data on temperature and rainfall were collected during the study period between 2014 and 2015 in order to make comparison with the total viable bacterial count obtained in the fishermen, vendors and consumers.

Data on ambient temperatures and rainfall were provided by Tanzania Metrological Agency, Zanzibar office. Averages of the two years 2014 and 2015 were computed.

2.3 Research Design

Three types of study designs were used depending on type of data under investigation. A cross sectional study design was used for data on the profile and number of microbial pathogens. A longitudinal study design was used for variations of colony forming units along the value chain of fishermen, vendors and consumers. A repeated cross sectional study approach was used for the value chain analysis.

2.4 Sample Collection

Sample collection was done from 8 different fish landing sites. Moreover, additional 60 samples were collected from one recreation site. One hundred and ninety-five pooled fish samples were collected from fishermen (n=65), vendors

(n=65) and consumers (n=65) during each of the four seasons: Seven to nine samples were collected from the value chain from each site in every of the four seasons; October-December, January-March, April-June, July-September in 2014 and 2015, making a total of 780 fish samples. The samples were collected from fishermen (dhows), street vendors and consumers (ready-to-eat food). Samples from fishermen were fresh fish from sea, vendor samples were uncooked fish vended in streets and ready-to-eat foods were cooked fish from homes, restaurants and frying sites. Fresh fish samples were collected at the landing sites from boats between 7.00 am and 8.00 am local time, fish samples from vendors were collected between 2.00 pm and 3.00 pm while samples from consumers were collected at any convenient time. Each sample was preserved in sterile polythene bag and then stored in a cool box and sent to Zanzibar Veterinary Laboratory within the next one hour for processing.

2.5 Bacterial Colony Count

Sample preparation was made using the method described by Obi and Krakowiaka [8]. Briefly; about 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples were crushed into small pieces in a sterile mortar with about 10 ml sterile Phosphate Buffered Saline (PBS). From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of sterile PBS giving a 1:10 dilution followed by serial dilutions with sterile PBS. A 0.1 ml was inoculated into Artificial Sea Water Medium [9] for plate counting.

Bacteria colonies were counted manually. So as to avoid human error counts were repeated by two other laboratory technicians and mean was calculated. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and the results were recorded in cfu/g.

2.6 Culturing, Isolation and Identification of Bacteria

In the Zanzibar Veterinary laboratory each sample was homogenized separately in a sterile PBS of pH 7.2 to achieve a 10% w/v suspension of fish. The homogenized suspension was inoculated in Blood Agar and Nutrient Agar and incubated aerobically at 37°C for 24 hours. Thereafter, macro-morphological characteristics

and microscopical findings were studied. Identification of bacterial species was done using a profile of biochemical tests as described by Cheesbrough [10] followed by a Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technique in the Public Health laboratory, University of Copenhagen.

2.7 Antibiotic Drug Sensitivity Assay

Antibiotic disc diffusion sensitivity tests in Mueller-Hinton Agar, as per CLSI [11] were carried out against two selected bacterial species; *S.aureus* and *P. mirabilis*. Pure colonies of bacteria of interest were emulsified with sterile PBS and the turbidity was matched with 0.5 McFarland's turbidity standard. The inoculum was then spread on dry Mueller Hinton plate by a sterile swab and antibiotic discs impregnated on the surface. Reading by measuring zone of inhibition diameter was done after 24 hrs. Eight antibiotics (Oxoid, England) were tested against 52 representative *S. aureus* isolates collected from all fish collection sites. The antibiotics were; Nitrofurantoin (300 µg), Sulphamethazole-trimethoprim (25 µg), Erythromycin (15 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Chloramphenicol (30 µg), Ampicillin (10 µg) and Tetracycline (30 µg). *P. mirabilis* (n = 19), isolates were tested against Ampicillin (10 µg), Nitrofurantoin (300 µg) Sulphamethazole-Trimethoprim (25 µg) and Ciprofloxacin (10 µg).

Furthermore, *P. mirabilis* isolates were tested for production of extended spectrum beta-lactamase (ESBL) using Ceftazidime (30 µg) and Cefotaxime (30 µg) with their perspective combination of clavulanic acid.

2.8 Statistical Analysis

The data for bacterial load in different sampling sites in the value chain in different seasons were subjected to analysis of variance (ANOVA). Bacterial load between urban and rural based towns were compared using t- test, $p < 0.05$ was judged indicative of significant difference.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Metrological findings on the study site

The highest temperature was experienced in January to March (25°C) while the lowest was experienced in July to September (22.05°C) (Fig. 1).

Two rainfall seasons were experienced in the study site; the long rains (Masika) between March and July and short rains (Vuli) in November to December. The highest rainfall was recorded in April to June season (357 mm) and lowest in July to September (73 mm) (Fig. 2).

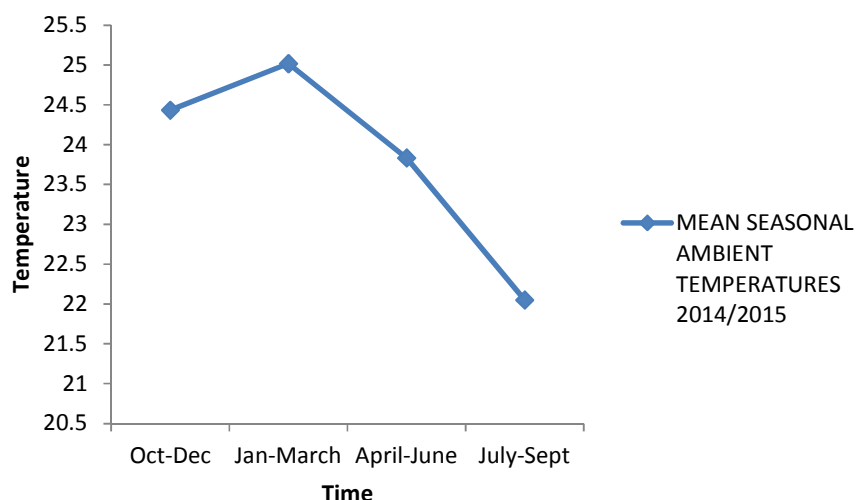


Fig. 1. Mean season's ambient temperature for 2014/2015
(Data was kindly provided by the Tanzania Metrological Agency, Zanzibar office)

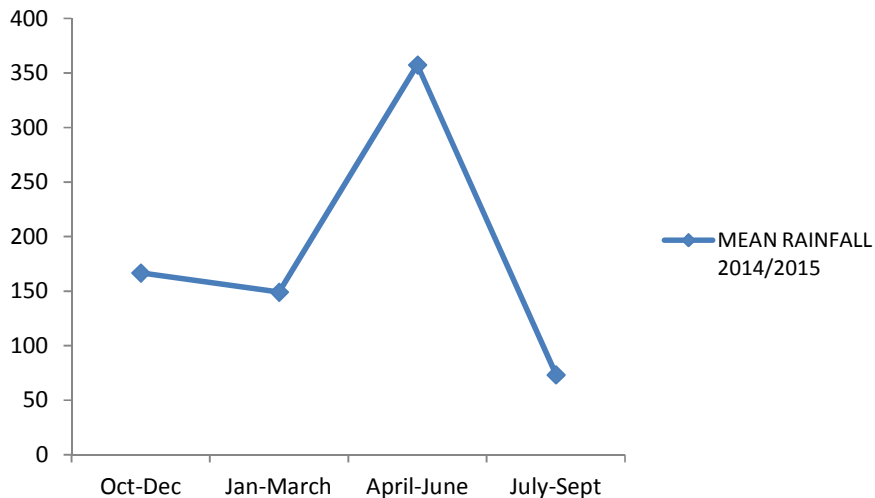


Fig. 2. Pooled mean rainfall for years 2014/2015
(Tanzania Metrological Agency, Zanzibar office)

3.1.2 Bacterial load in different sampling sites

Bacterial load (total viable counts) was determined in samples from fishers, vendors and consumers throughout the season. On the average the bacterial load in colony forming units (CFU) was highest in vendors compared to fishers and consumers in all seasons (Table 1, Fig. 3). Bacterial load was high during the hottest season (January to March) and low during the cool season (July to September), when the rainfall was high. There was low number of bacteria in cooked marine foods from consumers throughout the seasons. The difference in bacterial load observed along the value chain was not significant ($p>0.05$). Bacterial load between urban based towns (Mkoani, Tumbe, Malindi and Mkokotoni) and rural based towns (Chake, Kiuyumbuyuni, Pwanimchangani and Mkunduchi) were compared by t-test and no significance was found ($p>0.05$).

In Pemba and Unguja the bacteria were further quantified where the overall bacterial load was high at Mkoani in Pemba and Malindi in Unguja while the lowest bacterial load was observed at Mjini kiuyu in Pemba and Makunduchi in Unguja (Table 2). There was a high bacterial count in consumers at Forodhani which is the famous recreation site in Zanzibar.

3.1.3 Anthropogenic activities

There are two high tourists season in Zanzibar; July to September when tourist numbers swell to the highest and December to January that

experience relatively diminished number of tourists. Low numbers of CFUs were observed during high tourist season in July-September (Fig. 3).

3.1.4 Isolation and identification of bacteria

A total of 31 species of bacteria were isolated from 264 out of the 780 fish samples along the marine foods value chain (Table 3). Most of the isolates have high prevalence in vendors and consumers as shown in Fig. 4. Bacteria of public health significance, among others include *Aeromonas punctata caviae*, *Pseudomonas aureginosa*, *Staphylococcus aureus* *Klebsiella pneumoniae* *Proteus mirabilis* *Edwardsiella tarda* *Citrobacter freundii* *Eschericia coli*, *Bacillus cereus* and *Enterococcus fecalis*.

Table 1. Mean Bacterial load (cfu/ml) in fishers, consumers and vendors in Unguja and Pemba islands

Study site	Bacterial load (cfu/g)	
	Unguja Island	Pemba Island
Fishermen	7.2×10^5	5.6×10^5
Consumers	6.3×10^4	6.3×10^4
Vendors	9.2×10^5	7.3×10^5

3.1.5 Antibiotic drug sensitivity

Antibiotic resistance was observed in the two selected bacterial species of public health importance *S. aureus* and *P. mirabilis*

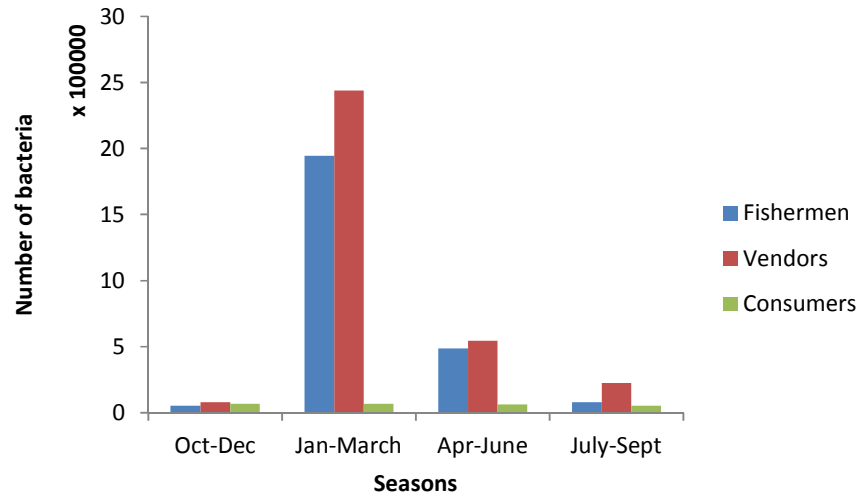


Fig. 3. Mean number of colony forming units in fish from fishermen, vendors and consumers

Table 2. Mean bacterial load (Cfu/g) in Pemba and Unguja Islands

SITE (Towns)	Fish value chain			
	Fishermen	Vendors	Consumers	Mean
Chake ^a	5.4 X 10 ⁵	6.3 X 10 ⁵	6.2 X 10 ⁴	4.1 X 10 ⁵
Mkoani ^a	6.1 X 10 ⁵	8.4 X 10 ⁵	6.6 X 10 ⁴	5.1 X 10 ⁵
Tumbe ^a	1.3 X 10 ⁵	6.9 X 10 ⁵	6.2 X 10 ⁴	3.0 X 10 ⁵
Kiuyumbuyuni ^a	5.0 X 10 ⁵	7.5 X 10 ⁵	6.9 X 10 ⁴	4.4 X 10 ⁵
Malindi ^b	1.2 X 10 ⁶	4.2 X 10 ⁵	7.2 X 10 ⁴	5.6 X 10 ⁵
Mkokotoni ^b	6.3 X 10 ⁵	2.6 X 10 ⁵	6.9 X 10 ⁵	3.4 X 10 ⁵
Pwanimchangani ^b	5.4 X 10 ⁵	9.6 X 10 ⁴	6.7 X 10 ⁴	2.3 X 10 ⁵
Makunduchi ^b	5.1 X 10 ⁵	1.4 X 10 ⁵	7.1 X 10 ⁴	2.4 X 10 ⁵
Forodhani ^b	-	-	5.5 X 10 ⁷	5.5 X 10 ⁷

Key: ^a Pemba, ^b Unguja

(Table 4). Multidrug resistance (MDR) was observed in 21% of the *S. aureus* isolates. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [12]. It is noteworthy that 21% of the *P. mirabilis* isolates were ESBL producers.

3.2 Discussion

Fish serve as an important source of food protein in Zanzibar. In this study assessment of bacterial load in fish along the value chain in Zanzibar was carried out. It was evident that the bacterial loads were higher in fish collected from fishermen and vendors while low in fish collected from consumers. This was probably due to fish processing activities such as boiling and frying at consumer level. The low bacterial load obtained from fish collected from consumers may be as a

result of using contaminated utensils as described by Reij and co-workers [13] in their study. It is noteworthy that the Forodhani site had high bacterial counts in fish collected from consumers. This may be because Forodhani is a popular recreation site in Zanzibar and the high bacterial count could be due to cross contamination and or repeated store-sell trends. The relatively high bacteria count in vendors and recontamination in consumers can be attributed to the same reasons pointed by Adebayo et al. [14] and Reij et al. [13] which are poor hygiene, deficient or absence of hand washing, contaminated surfaces and unclean, insufficiently or inadequately cleaned equipment. High bacteria count in fish might raise concern not only due to food poisoning risks but also increased possibility of spoilage and reduced shelf life with resultant high food losses [15,16].

Table 3. Profile of bacteria isolated along the marine foods value chain

	Bacteria isolated	Frequency of isolation	Prevalence (%)
1	<i>Staphylococcus aureus</i>	66	24.5
2	<i>Micrococcus luteus</i>	25	9.3
3	<i>Escherichia coli</i>	23	8.6
4	<i>Staphylococcus epidermidis</i>	19	7.1
5	<i>Proteus mirabilis</i>	19	7.1
6	<i>Bacillus megaterium</i>	14	5.2
7	<i>Staphylococcus sciuri</i>	11	4.1
8	<i>Micrococcus lylae</i>	8	3
9	<i>Bacillus cereus</i>	7	2.6
10	<i>Enterococcus faecalis</i>	6	2.2
11	<i>Enterococcus casseliflavus</i>	6	2.2
12	<i>Pseudomonas stutzeri</i>	5	1.9
13	<i>Enterobacter cloaca</i>	5	1.9
14	<i>Pseudomonas aurogenosa</i>	5	1.9
15	<i>Morganella morganii</i>	4	1.5
16	<i>Acinetobacter baumannii</i>	4	1.5
17	<i>Aeromonas punctatacaviae</i>	4	1.5
18	<i>Klebsiella aerogenes</i>	4	1.5
19	<i>Klebsiella pneumoniae</i>	4	1.5
20	<i>Pseudomonas fluorescens</i>	4	1.5
21	<i>Micrococcus roseus</i>	4	1.5
22	<i>Acinetobacter haemolyticus</i>	3	1.1
23	<i>Lysinibacillus fusiformis</i>	3	1.1
24	<i>Aeromonas hydrophila</i>	3	1.1
25	<i>Proteus vulgaris</i>	3	1.1
26	<i>Citrobacter freundii</i>	2	0.7
27	<i>Streptococcus faecalis</i>	2	0.7
28	<i>Lysinibacillus sphaericus</i>	2	0.7
29	<i>Bacillus thuringiensis</i>	2	0.7
30	<i>Edwardsiella tarda</i>	1	0.4
31	<i>Enterobacter aerogenes</i>	1	0.4
Total		269	100

Table 4. Antibiotic assay profile of the selected bacteria

Antibiotic tested	<i>Staphylococcus aureus</i> (n=52)		<i>Proteus mirabilis</i> (n=19)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Tetracycline (TET)	29(55.8)	23 (44.2)	-	-
Ciprofloxacin (CIP)	52 (100)	0 (0)	19 (100)	0 (0)
Chloramphenicol (CHL)	52 (100)	0 (0)	-	-
Gentamicin (GEN)	52 (100)	0 (0)	-	-
Nitrofurantoin (NIT)	52 (100)	0 (0)	7 (36.8)	12 (63.2)
Sulphamethaxazole-trimethoprim (SXT)	52 (100)	0 (0)	12 (63.2)	7 (36.8)
Erythromycin (ERY)	41 (78.8)	11 (21.2)	-	-
Ampicillin (AMP)	41 (78.8)	11 (21.2)	19 (100)	0 (0)
Ceftazidime	-	-	15(78.9)	4 (21.1)
Cefotaxime	-	-	16(84.2)	3 (15.8)
AMP, ERY, TET*	-	11 (21.2)	-	-
NIT, SXT**	-	-	-	7 (36.8)

Key: * *S. aureus* isolates resistant against the three named antibiotics
 ** *P. mirabilis* isolates resistant against the two named antibiotics

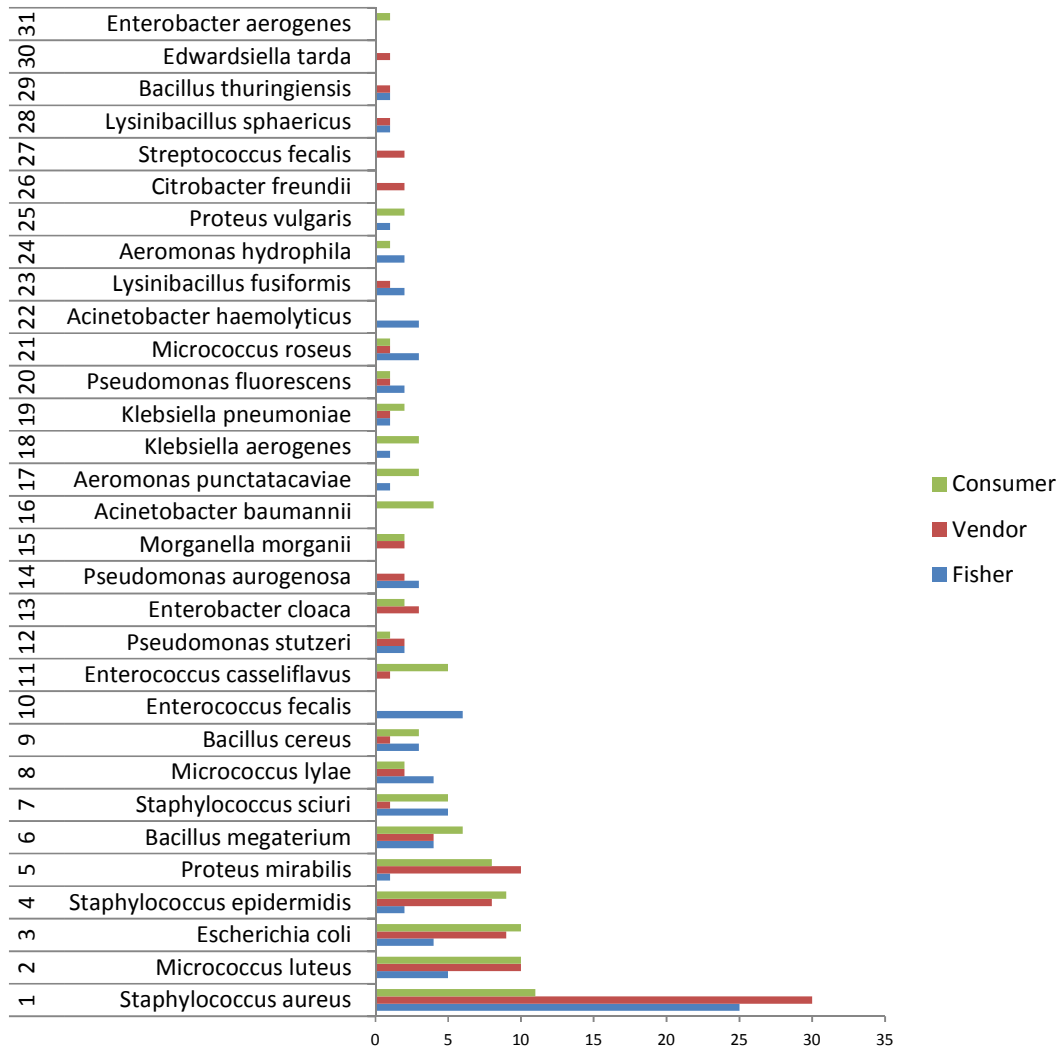


Fig. 4. The prevalence of bacteria along the marine foods value chain

A small variation was noted in seasonal basis, where a relative high bacterial load in fish was observed in January- March season when the ambient temperature was high and the bacterial count was low in cool season (July-September) and rainy season (April-June). This is expected due to higher rate of bacterial multiplication in hotter climate as was observed by Velmurugan et al. [17]. On the other hand, no evidence was found that anthropogenic activities like tourism had influence on bacterial load as July to September (which is the highest tourist season) had the lowest bacterial counts along the value chain and, therefore, the resident human population, probably with its consequent increase of sewage disposal could be an influencing

factor. This is supported by the fact that urban based landing sites had relatively higher bacterial load compared to rural based sites.

Most of the isolated bacteria in fish value chain are of public health importance causing infection resulting in local or systemic infections and food poisoning. *Staphylococcus aureus* is one of the most prevalent public health bacterial species isolated in fish value chain and this is in agreement with the work of Ibrahim et al and Tiamiyu et al. [18,19] who found predominance of *S. aureus* in fish foods but in contrast to Dib et al. [20] who didn't isolate any *S. aureus* in fish foods. Despite the fact that *S. aureus* can be totally eliminated by heating its heat-tolerant

toxins are still capable of causing food poisoning. The high numbers of *S. aureus* isolated from this study could potentially cause food-borne diseases in humans and, additionally they may harbor other virulence factors such as biofilm formation-associated genes, antibiotic resistance, or lysogeny [21]. As was found in this study *P. mirabilis* was among the four most prevalent bacteria isolated by Moshood and TengkuHaziyaamin [22]. *Edwardsiella tarda* is a gram-negative enteric pathogen which in this study had low prevalence but is an etiological agent of both gastrointestinal and extraintestinal infections in humans [23].

The presence of multidrug resistance as detected in *S. aureus* is indicative of circulating antibiotic resistance genes in the bacterial biome contaminating sea foods in Zanzibar. Sources of genes could be seafood stakeholders as well [24]. The resistance genes can be transmitted to other bacteria through horizontal gene transfer and may ultimately circulate in human hosts. Despite the fact that the existence of ESBL *P. mirabilis* producing isolates from fish is of important to note, this study has also revealed high susceptibility of *P. mirabilis* isolates to ceftazidime (84%) and cefotaxime (78.9%) which is in agreement with the reports from United states, Canada and United kingdom [25-28].

Bacterial contamination predisposes fish foods to spoilage, exposes the public to bacterial pathogens and encourages bacteria antibiotic resistance. There is therefore great need to improve hygienic standards of marine foods in Zanzibar right through the value chain from freshly harvested fish to ready-to-eat food.

4. CONCLUSION

This study revealed that there is seasonal increase in microbial load in fish through the value chain from fishermen to vendors, hot seasons favoring the increase though not statistically significant. Ready to eat foods have the lowest bacterial numbers except in the recreation site where traders reserve foods to sell the following days. Most of the isolated bacterial species were found to have public health importance and could be responsible for food spoilage and poisoning. Multidrug antibiotic resistance was found in *S. aureus* isolates which could be transmitted to other bacteria and ultimately end in human hosts. To minimize marine food contamination it is recommended that personal hygiene practices be implemented

by fish foods stakeholders, these practices could include hand washing with soap and warm water and abstention from contaminating habits of coughing, sneezing, smoking, chewing gum, handling garbage, touching hair. The public health sector has to ensure continuous surveillance of sea foods through the value chain especially in recreation areas. Food regulatory enforcements and establishment of food tracing and traceability systems will further improve hygiene standards. Mass awareness on safe food handling to all fish stakeholders is emphasized.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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