Isolation and Identification of Pathogenic Bacteria in Edible Fish: A Case Study of Fletcher Dam in Gweru, Zimbabwe

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Abstract: ***Aquaculture products can harbor pathogenic bacteria which are part of the natural microflora of the environment. A study was conducted aiming at the isolation of human pathogenic bacteria in gills, intestines, mouth and the skin of apparently healthy fish, Tilapia rendali and Oreochromic mossambicus, from the Fletcher dam. Bacterial pathogens associated with fish can be transmitted to human beings from fish used as food or by handling the fish causing human diseases. Differentiation and characterization of various isolates was based on their growth characteristics on specific culture media (biochemical and gram staining reactions). The following human pathogenic bacteria were isolated Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis. All the bacterial species which were isolated from the fish were also present in the initial water samples collected. The isolation of enteric bacteria in fish serves as indicator organisms of faecal contamination and or water pollution. Their presence also represents a potential hazard to humans. The mean bacterial load of the isolates was found to be markedly higher than the recommended public health and standard value of 5.0 x 10^6 CFU/ml which has been adopted by many countries.***

Keywords: Isolation, faecal contamination, Salmonella typhi, Pseudomonas aeruginosa,

1. Introduction

Fish is a vital source of food for people and contributes about 60% of the world's supply of protein. 60% of the developing countries derive 30% of their annual protein from fish [1]. It is man's most important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population [11]. In Africa, fish supplies 17% of protein and it is one of the cheapest sources of protein in Africa[8]. The advantage of fish as food is as a result of its easy digestibility and high nutritional value. However fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature [17]. According To [4] the microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it. [8] suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat. [16] and [23] classified the bacterial pathogens associated with fish as indigenous and non-indigenous. The non-indigenous contaminate the fish or the habitat one way or the other and examples include *Escherichia coli, Clostridium botulinum, Shigella dynteriae, Staphylococcus aureus, Listeria monocytogens and Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish's habitat for example *Vibrio* species and *Aeromonas* species. The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail. Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacteium, Streptococcus spp., Vibrio spp., Aeromonas spp., Salmonella spp.* and others [17].

Other studies have also demonstrated the presence of indicator micro-organisms of faecal pollution, opportunistic and pathogenic bacteria to humans in fish samples [18]. There are often bacterial species that are facultative pathogenic for both fish and human beings and maybe isolated from fish without apparent symptoms of the disease. Human infections caused by pathogens transmitted from fish or the aquatic environments are quite common and depend on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the individual [20]. Transmission of the pathogens can be through the food or the handling of the fish. There have been great economic losses reported due to food borne illness such as dysentery and diarrhea resulting from consumption of contaminated fish. The microbial association with fish compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic and / or pathogenic in nature [18]. The risks of contracting food borne diseases by the residents from the surrounding communities that are using the fish from the Fletcher dam as a source of protein may be high. These circumstances prompted this research to investigate the occurrence of any human bacterial pathogens in the fish that was being caught from the dam.

1.1 Overall Objective

To isolate and identify the bacterial species in the fish samples which are potentially pathogenic to humans

1.2 Specific Objectives

- To isolate and quantify the human pathogenic bacteria associated with freshly caught edible fish
- To observe the organ wise distribution of the human bacterial pathogens.

2. Materials and Methods

2.1. Study Area

This study was conducted at Fletcher Dam which is situated about 15km from the town of Gweru in Zimbabwe. It is surrounded by Senga and Nehosho high density suburbs and the farming community. It is in the Midlands province in Natural region III of Zimbabwe; it has an elevation of 1 417m above sea level with 35k 079817 Northing and UTM 7839920 Easting. It is dominated by red soils and an average annual rainfall of 666 mm, summer starts in October with much of the rainfall being received between December to March. Maximum and minimum daily temperatures range from 24 to 30^{0}C and 15 to 24^{0}C respectively.

2.1 Laboratory Analysis

2.1.1 Fish samples

Forty fish samples were collected from Fletcher dam between the periods of March to July, 2013. Twenty samples each of *Tilapia rendali* and *Oreochomis mossambicus* were collected aseptically and immediately transported in a thermal bag to the laboratory and processed within 3hrs of acquisition, and samples were kept in the refrigerator (4–8^{o}C).

2.1.2 Sample preparation

Sample preparation was made using the method described by [21]. 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 water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This was done for the 40 fish samples.

2.2 Sampling

The bacterial counts on the external surfaces, intestines and tissue were estimated as follows:

2.2.1 Skin Surfaces

Sample from different locations of the skin of 40 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of Nutrient broth, MacConkey broth and Selenite F broth which are dispensed in separate tubes. 10 fold serial dilution of the bacterial suspension already inoculated in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by [24], and then incubated at 37^{o}C for 48 hrs.

2.2.2 Intestines, Gills & Tissues

1g of the fish sample was dissected out, blended and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9mls of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37^{o}C for 48 hrs as described by [24]. Coliform organisms and gram negative enteric bacteria counts were determined using pour plate method with MacConkey agar, EMB Agar respectively. Mueller-Hinton Agar for *Pseudomonas spp*. *Salmonella spp*. and *Shigella spp*., were enumerated using Salmonella Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for pathogenic *Vibrio spp*. The plates were incubated at 35+2^{o}C for 24h. The observed colony growth were counted using Coulter™ Colony counter according to plate count method. Identification of the organisms was done using the phenotypic and biochemical characteristics as described by [6] and [24].

2.3 Estimate of mean colony forming unit per gram (CFU g^1)

The mean colony forming unit per gram (CFU g^{-1}) denoted by (x) was calculated as $\Sigma f\chi/\Sigma f$, where Σfx is the sum of the products of number of colonies and the colony forming unit per gram; while Σf is the summation of the number of colonies.

3. Results

In this study, the total plate count (TPC) for all the fish samples ranged between 8.60 x 10^6 and 25.60 x 10^6 cfu/g as shown in table 1. Out of the 120 fish samples analysed for TPC, the skin had the highest number of bacteria with 25.60 x 10^6 cfu/g in *Tilapia rendali* and *Oreochomis mossambicus* had 23.60 x 10^6 cfu/g respectively. The gills had the lowest isolation with 8.60 x 10^6 cfu/ml in *Tilapia rendali* and *Oreochomis mossambicus* 9.60 x 10^6 cfu/g. The Coliform count was highest in *Oreochomis mossambicus* 24.66 x 10^6 cfu/g as compared to other fish.

Table 1: Mean count of the bacteria present at different parts of examined sampled fishes

Fish	*Parts*	*TPC*	*Coliforms*	*P.aueruginosa*	*V. cholerae*	*E.coli*	*S.aureus*	*S.dysentariae*	*S.typhi*	*E.faecalis*
		cfu/g	*cfu/g*	*cfu/g*	*cfu/g*	*cfu/g*	*cfu/g*	*cfu/g*	*cfu/g*	*cfu/g*
		10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6
T.rendali	Intestines	8.6	15.70	19.49	6.30	8.2	4.18	3.86	5.25	8.68
	Gill	8.60	12.68	18.78	-	6.26	3.85	2.84	3.36	3.44
	Skin	25.6	17.1	26.89	1.81	9.72	4.47	1.96	0.94	4.20
	Mouth	13.6	14.2	15.3	2.94	4.34	2.34	2.44	3.10	4.70
O.mossambicus	Intestines	19.03	24.66	22.12	1.31	8.44	4.18	3.35	4.44	9.44
	Gill	9.60	11.07	20.65	-	6.12	3.58	2.48	3.22	5.12
	Skin	23.60	14.64	36.6	0.07	8.12	4.47	1.48	2.13	7.14
	Mouth	14.4	12.20	16.64	3.40	5.24	2.84	2.60	0.85	4.47

Table 1 revealed the isolation of *Pseudomonas spp*. with the skin having the highest number in *Oreochomis mossambicus* (36.60 x 10^6 cfu/g) and (26.89 x 10^6 cfu/g) in the *Tilapia*, The *Vibrio spp*. isolated had the lowest count of 0.07 x 10^6 cfu/ml from the skin as compared with the skin of other fish samples. The intestine is the most colonized part of the

examined areas in the fish with *Tilapia* having the highest count of 6.30 x 10^6 cfu/g while the lowest count was exhibited in the *Oreochomis mossambicus* (1.31 x 106 cfu/ml). The gills likewise showed possible colonization but in the lowest count as compared to other parts. No isolation of *Vibrio spp*. on the gills of both fishes.

E. coli isolation showed the highest count in *Oreochomis mossambicus* for skin (26.91 x 106 cfu/g), followed by *Tilapia rendali* (18.70 x 106 cfu/g). The intestine and gills were also heavily populated by *E. coli* with the highest exhibited in the gills of *Tilapia rendali* (21.54 x 106 cfu/g), followed by *Oreochomis mossambicus* (19.10 x 106 cfu/g). Likewise, the intestine of *Tilapia rendali* exhibited the highest colonization rate of 17.64 x 106 cfu/g and *Oreochomis mossambicus* with 17.18 x 106 cfu/g (Table 1). *Staphylococcus spp*. had a low isolation rate in all samples analysed as generally compared with other isolated organisms except *Vibrio spp*. that had the lowest counts.

The human bacterial pathogens that were isolated and identified include *Escherichia coli, Pseudomonas aueriginosa, Shigella dynteriae, Staphylococcus aureus, Enterococcus faecalis and Salmonella typhi* as indicated in the table.

4. Discussion

A high population of bacteria in food indicates the general quality of the food and the degree of spoilage it might have undergone. The occurrence of total bacterial counts of many of the samples investigated having > 5X10^6 CFU/g raises concern about the hygienic status of the production and point of sale environment. Although only a few infectious agents in fish are able to infect humans, some exceptions such as salmonella exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products [27].

The results from this study and according to published microbiological guidelines as cited by [14] suggest that the microbiological quality of the fish examined is unacceptable and pose a potential risk to public health. The diversity of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. These opportunistic and pathogenic bacteria were also previously isolated by several other researchers from fish [18]. The fish in this study harbored human disease causing organisms that cause diseases such as food poisoning, diarrhea, typhoid fever and Shigellosis. [8] suggested that when present in food, pathogens such as *S. aureus, Salmonella, Shigella* and *Pseudomonas* are most likely to cause food-borne diseases.

The high incidence of *Salmonella* in the fish from the dam is a major health concern. In addition to salmonellae, the presence of diverse enteric bacteria in fish indicates the contamination representing a potential hazard to human health especially those who are sick or are on immunosuppressive drugs. Stringent regulations and monitoring activities coupled with food safety training of suppliers (fishermen and traders) and ultimately the consumers on various aspects of Good Hygiene Practice (GHP), Good Manufacturing Practice (GMP) and HACCP is strongly recommended.

The presence of faecal coliforms in fish demonstrates the level of pollution of their environment because Coliforms are not the normal of bacteria in fish. Of the organisms that were isolated and identified that is *S.typhi, S.aureus, S.dysentariae* and *E.coli* are non-indigenous pathogens that contaminate fish or fish habitats in one way or the other [16] and [23]. The isolation of *Salmonella, Shigella, and E.coli* indicate faecal and environmental pollution [28]. Coliforms such as *E.coli* are usually present where there has been faecal contamination from warm blooded animals [5]. The organism *E.coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers it is an indicator of mishandling [10]. *E.coli* is the only species in the coliform group that is found in the human intestinal tract and in the other warm blooded animals as a commensal and is subsequently excreted in large quantities in faeces [13].

The presence of *S.aureus* and *E.coli* was attributed to the contamination of the fish samples by the sometimes raw sewage that is discharged directly into the tributaries feeding the dam. While the Fletcher dam was used as a case study, it is representative if the many peri urbanely located small dams dotted around the country which suffer the same fate of high levels of effluent discharge in open water bodies. Of concern is the fact that the high bacterial loads found in the raw fish at the source point are most likely to have a multiplier effect as the caught fish are poorly handled and stored until they are consumed. In similar studies, *Escherichia coli, Pseudomonas aueriginosa, Shigella dynteriae, Staphylococcus aureus and Salmonella typhi were* isolated from the gills, intestines, muscle and skin of *Megalaspis cordyla* and muscles of *Priacanthus hamrur* from Royapuram waters in India by [25]. This was attributed to the heavy load of sewage disposal into the seas which could act as a suitable environment for the growth and survival of the human pathogens. Members of the genus *Pseudomonas* are found in the soil and natural sources of water and are important phytopathogens and agents of human infections being considered opportunistic pathogens [25].

5. Conclusion

Seven human bacterial pathogens i.e. *Escherichia coli, Pseudomonas aueriginosa, Shigella dynteriae, Staphylococcus aureus, vibrio cholera, Enterococcus faecalis* and *Salmonella typhi* were isolated from the two fish species *Tilapia rendali* and *Oreochromic mossambicus* from the Fletcher dam. The presence, in large populations of these bacterial pathogens indicates high levels of faecal contamination in the dam. The presence of enteric bacteria may be attributed to faecal contamination due to improper sewage disposal and or water pollution. The fish act as a reservoir of human pathogens and the presence of highly pathogenic agents such as *Salmonella, Shigella* species and of opportunistic pathogens is a potential health risk/hazard to human beings and may cause diseases to susceptible individuals especially the immune-compromised consumers.

Moreover the recoveries of various organisms, which are potentially pathogenic to humans, in the fish suggest that if they are improperly handled, undercooked or consumed raw may contribute to the spread of the pathogens in the community. Further examination of fish especially for the presence of pathogens, during handling, storage and up to the very point of consumption is needed for the protection and maintenance of community health by keeping food borne diseases to a minimum.

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