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Bacteria and fungi isolates associated with harvested fish from Useh flood reservoir in Edo State

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Abstract
This study was conducted to ascertain the presence of bacteria and fungi in fish samples harvested from Useh flood reservoir. A total of 15 samples of harvested fishes over a period of 3 months, were collected and transported with clean Ziplock© bags to the laboratory for microbiological quality analysis. Isolation of bacteria was done by pour plate method. The serial dilution method by Harrigan and McCance (1976) was aseptically carried out in sterile test tubes for the ten-fold dilution. Aliquots from each tube was used to inoculate the media for isolation of fungi using the pour plate method (Bourbonnais et al., 1995). The plates were then examined after incubation and the number of colony forming units (CFU) that developed were counted. All the samples were found to harbor bacteria and fungi up to a range of 10^6 cfu/g. The total viable bacteria count of 4.39 x 10^5cfu/g, 4.41 x 10^5cfu/g and 4.37 x 10^5cfu/g were recorded in the gills, colon and skin respectively. While the total viable count of fungi were 5.17 x 10^4cfu/g, 3.33 x 10^4cfu/g and 4.67 x 10^4cfu/g in the gills, colon and skin respectively. Escherichia coli were found in all samples as a specific bacteria pathogen. The bacteria isolates identified were Klebsiella Spp., Escherichia coli, Bacillus Spp., Staphylococcus aureus, Proteus Spp., Micrococcus Spp., Pseudomonas Spp., Bacillus subtilis, Staphylococcus epidermis and Pseudomonas aerogenosa. The fungi isolates identified in fish sample during the period of study were Cladosporium Spp., Saccharomyces Spp., Cryptomonas Spp., Aspergillus nidulans, Aspergillus niger, Penicillium Spp., Trichoderma viridi, Cryptomonas Spp., Mucor mucido, Mucor Spp., Botrydiplodia Spp., Aspergillus tamarii, Neurospora Spp., Cryptomonas neoformis and Helminthosporium Spp. It was therefore observed that bacteria and fungi above 105cfu/g were present in fishes harvested from Useh flood reservoir. Bacteria count of less than 105/g is indicative of good fish quality, there is therefore the likelihood of infestation of persons with these pathogenic bacteria in this environ that consume harvested fish or make contact with the fish from Useh flood reservoir.

Key Words: *Flood, Reservoir, Bacteria, Infestation, Edo-State.*

Introduction

In a world where more than 70% of the planet is covered with water, aquatic foods may provide an essential component of the global food to improve nutrition, health, and well-being of humans (Tacon and Metian, 2013). The advantage of fish as a food is as a result of its easy digestibility and high nutritional value. Fishes are commonly viewed as safe, nutritious and advisable but aquaculture products have often been associated with positive meals protection troubles (World Health Organisation, 2007, Abisoye *et al*., 2011). In spite of these merits, fresh fish spoil very readily after capture due to high ambient temperature which accelerates the activities of bacteria, fungi, enzymes and chemical oxidation of fats in the fish. Also, fish are prone to a vast variety of microorganism pathogens, most of which are capable of inflicting ailment and

are regarded by some to be saprophytic in nature (Lipp *et al.*, 1997).

Bacteria are found everywhere in the aquatic environment and the different diseases triggered by bacteria may result in mortality in both cultured and wild fish. Most bacteria disease pathogens are commonly believed to be phase of the normal micro flowers of the aquatic environment and are commonly deemed as secondary or opportunistic pathogens in nature. Some bacteria cause only surface diseases such as skin or gill infections, especially flexibacteria, but some inflict systemic disease. The bacteria from fish can only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors affecting them, like poor water quality, overstocking, which allow opportunistic bacterial infections to occur. It has been well known that both fresh and brackish water fishes can harbour human pathogenic bacteria, particularly the coliform group (Lipp *et al*., 1997). Fungal ailments are the most serious causes of losses in aquaculture, being the most reoccurring kind of disease trouble in most types of aquaculture facilities resulting in considerable economic losses in the industries (Meyer, 1991). Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immune-compromised, or they may be secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or excessive handling or due to inadequate nutrition (Roberts, 1989; Quiniou *et al*., 1998). The loss of the fish to diseases, inflicting of illnesses or deleterious impact in man and animals (Douglas *et al.*, 2000). Many fungal species can spoil food products or produce mycotoxins or both (Anderson and Thrane, 2006). Fungal mycotoxins produce a wide variety of injurious consequences as they may result in lowered productivity, immune suppression and chronic damage to integral tissues and organs of animal and humans (CAST, 2003, Olojo *et al.,* 2010 and Efuntoye *et al.,* 2012). Fungal contamination is a common problem and adversely affects the quality of fishes, whether fresh or smoked (Prakash *et al*., 2011; Saritha and Patterson, 2012).

Fish contamination by pathogens is of most important public health concern, as some of these diseases have been specifically associated with pathogen which are resistant to antibiotics (Adebayo *et al*., 2012; Edun *et al*., 2015) and this poses a great risk to human health. Human infections by pathogens from fish are common, depending on the season, contact with fish and its habitat, dietary habits and the immune system of the person. Transmission of the pathogens therefore can be through the consumption or handling of the fish. Since water flow from different sources into this reservoir, bringing with it sewage, pesticides, home and industrial waste amongst others; capable of releasing into the water contaminates. Therefore, this study was carried out to determine the level of contamination by isolating and identifying bacteria and fungi pathogens in fish collected from Useh flood reservoir in Edo State.

Materials and Methods

This study was carried out in a flood reservoir at Useh Community, Egor Local Government Area, Edo State. The flood reservoir has a coordinate of Latitude 6°23'2"N and Longitude 5°33'54"E as shown in Figure 1.

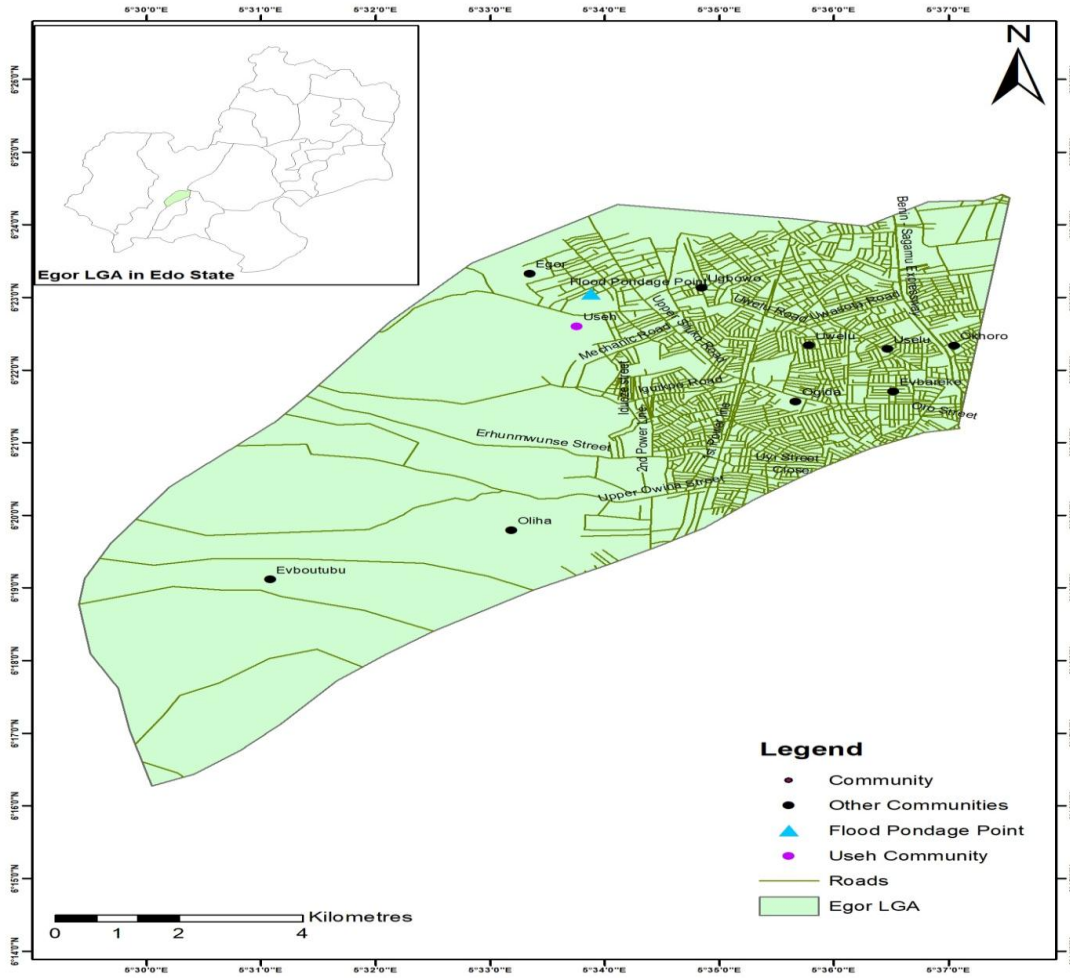

Figure 1: Map Showing Study Area
Source: Geography and Regional Planning, University of Benin, Benin City (2023)

Sample Collection and Preparations

Three fish samples were collected every three (3) weeks during the period of this study and were transported on ice to reduce to the barest minimum bacteria multiplication to the Central Research Laboratory (CRL) located in the University of Benin using a clean Ziplock© bag and was processed within 2 hours of acquisition.

All the glass wares used were washed, dried and sterilized in hot air oven at a temperature of 160°C for 1 hour according to the method described by Adibe and Eze (2004). Culture media to be used were also sterilized in an autoclave at a temperature of 121°C for 15 minutes. The fishes were killed, and part of the skin, colon and gills collected were put into 9ml of distilled water to give 1:10 dilution ratio, and shaken thoroughly. The stock solutions were serially diluted up to 10^{-5} as described by Willey *et al.* (2008).

The serial dilution method by Harrigan and McCance (1976) was aseptically carried out in sterile test tubes for the ten-fold dilution. Aliquots from the appropriate tubes

were then used to inoculate appropriate media during this study for isolation of fungi using the pour plate method (Bourbonnais *et al.*, 1995).

The plates were then examined after incubation and the number of colony forming units (CFU) that developed were counted and recorded. Microbial analysis of samples for fungi and bacteria was done according to method described by Robert *et al*. (1993) and colony enumeration and isolation was done according to Baker *et al*. (1998). Characterization and identification of fungi and bacteria isolates using gram staining, physiological, biological reactions and fermentation of sugar according to standard taxonomic schemes (Barnet and Hunter, 1974, Fawole and Oso, 2001). The features examined in the colonies include nature of growth, margin, plate culture reverse, shape of spores and attachment, colour of spores and culture, opacity and surface appearance. (Boussard *et al.*, 2012).

Statistical Analysis

Data was analyzed with one-way analysis of variance (ANOVA). This was used to test for difference between the mean values of the sample at 5% probability level. Duncan multiple range test (DMRT) was also used for the separation of means (Snedecor and Cochran, 1988).

Results

The experimental fish mean total viable count (TVC) ranges in the gills (1.51±2.07 to 7.30±9.43), colon (1.00±1.30 to 4.35±5.67) and skin (2.73±2.93 to 5.81±7.16), are as shown in Table 1. Bacteria counts mean ranges in the gills (1.16±1.61 to 6.92±9.07), colon (3.87±5.23 to 9.55±1.24) and skin (2.01±2.69 to 5.35±6.67). And the fungi count mean ranges in the gills (1.14±2.61 to 3.75±5.24), colon (1.79±2.71 to 4.53±6.86) and skin 2.59±3.65 to 7.21±1.62) are as shown in Table 1. The mean count in week 3 and week 9 for TVC and bacteria varied widely between weeks 3 with highest count and week 9 with the lowest count, however, analysis of variance showed that there was no significant difference ($p > 0.05$) during the period of study. Although ANOVA showed that there were no significant differences ($p > 0.05$) in the means in fungi count during the period of study, but week 6 had the lowest count in the colon and skin.

Table 1: Mean TVC, Bacteria and Fungi Count in the Fish Gills, Colon and Skin at Useh Flood Reservoir

Location	**Weeks** / **Isolates**	**0**	**3**	**6**	**9**	**12**
Gills	Bacteria	5.2±7.62^{a}	6.92±9.07^{a}	4.18±5.63^{a}	1.16±1.61^{a}	4.41±5.82^{a}
	Fungi	3.73±5.22^{a}	3.75±5.24^{a}	3.49±4.55^{a}	3.48±4.70^{a}	1.14±2.61^{a}
	TVC	5.63±8.10^{a}	7.30±9.43^{a}	4.53±6.02^{a}	1.51±2.07^{a}	5.55±7.38^{a}
Colon	Bacteria	3.87±5.23^{ab}	9.55±1.24^{b}	3.94±5.10^{ab}	7.03±9.05^{a}	3.98±5.22^{ab}
	Fungi	3.61±4.83^{a}	4.53±6.86^{a}	1.79±2.71^{a}	3.07±4.83^{a}	3.65±4.60^{a}
	TVC	4.23±5.71^{ab}	1.00±1.30^{b}	4.12±5.27^{ab}	1.01±1.34^{a}	4.35±5.67^{ab}
Skin	Bacteria	4.59±5.94^{a}	5.35±6.67^{a}	4.97±6.44^{a}	2.01±2.69^{a}	4.92±6.44^{a}
	Fungi	2.59±3.65^{a}	4.61±5.55^{a}	3.41±4.60^{a}	7.21±1.62^{a}	5.54±5.71^{a}
	TVC	4.85±6.25^{a}	5.81±7.16^{a}	5.31±6.89^{a}	2.73±2.93^{a}	5.48±6.93^{a}

NB: Means with same superscript along rows are not significantly different ($p > 0.05$). Key TVC: Total viable count.

The grand mean comparison (Table 2) of the fish sample at Useh flood reservoir of bacteria count (4.37±5.69 to 4.41±7.16), fungi (3.33±4.80 to 5.17±1.23) and TVC (4.74±7.51 to 4.90±7.01) found in the gills, colon and skin during the period of study, showed that bacteria and TVC were not significantly different ($p > 0.05$), except in the fungi count, which was highest in the skin and least in the colon.

Table 2: Mean Comparison of Bacteria, Fungi and TVC in Gills, Colon and Skin of Fish at Useh Flood Reservoir

Fish parts Isolate	Gills	Colon	Skin
Bacteria	$4.39±6.44^a$	$4.41±7.16^a$	$4.37±5.69^a$
Fungi	$5.17±1.23^a$	$3.33±4.80^a$	$4.67±8.26^a$
TVC	$4.90±7.01^a$	$4.74±7.51^a$	$4.84±6.05^a$

NB: Means with same superscript along rows are not significantly different ($p > 0.05$)

The results of the biochemical tests and identification of the various bacteria isolates found in fish samples were *klebsiella Spp., Escherichia coli, Bacillus Spp., Staphylococcus aureus, Proteus Spp., Micrococcus Spp., Pseudomonas Spp., Bacillus subtilis, Staphylococcus epidermis* and *Pseudomonas aerogenosa* and the most prevalent were *Escherichia coli* and *Micrococcus Spp*. The bacteria that were predominantly found in the gills, colon and skin were *Echerichia coli, Proteus Spp.* and *Micrococcus Spp*. respectively.

The fungi isolates identified were *Cladosporium Spp., Saccharomyces Spp., Cryptomonas Spp., Aspergillus nidulans, Aspergillus niger, Penicillium Spp., Trichoderma viridi, Cryptomonas Spp., Mucor mucido, Mucor Spp., Botrydiplodia Spp., Aspergillus tamarii, Neurospora Spp., Cryptomonas neoformis and Helminthosporium Spp.* The fungi isolates were higher in the gills with *Penicillium Spp.* been predominant, while in the colon and skin, the most predominant fungi isolates were *Aspergillus niger* and *Aspergillus nidulans* respectively.

Discussion

The quantity of bacteria and fungi found in the gills and skin of the fish were similar all through the study except for slight changes in the bacteria count in the colon. This could be as a result of fishes taken from the flood reservoir receiving continuous untreated human, industrial, household and other anthropogenic waste that can help enrich the microbial ecology of the reservoir, therefore, according to Edun *et al.* (2015) and Asemota *et al.* (2019), such organisms that live in such aquatic environments are likely to gather these pathogenic microbes present in the environment since they living and feed on the same kind and quality of diet. The quantity of bacteria found were more in the colon than in the gills and skin, just as earlier reported by Buras *et al.* (1987) that in general fish usually contain high numbers of bacteria in the digestive tract, gills and flesh. Trust (1975) had earlier reported that gills could harbor high bacteria population of up to 10^6cfu/g in fishes. But this study is not in agreement with this observation, as the colon harboured more bacteria. The quantity of fungi was found more on the gills than the colon and skin. *Escherichia coli* were present in all samples thus agreeing with the report of Saritha and Patterson (2012), and according to Lambu *et al.* (2022), many aquatic foods not properly processed and prepared have been linked to

high concentration of *Escherichia coli* and *Micrococcus spp.*, that can affect food safety if consumed.

The bacteria genera isolated in fishes used for this study were *Pseudomonas, Bacillus, Aeromonas, Flavobacterium, Enterobacter, Klebsiella, Escherichia, Proteus, Staphylococcus and Micrococcus*. Haniffa and Abdulkader (2011) reported that bacteria especially motile aeromonads are frequently isolated from both healthy and diseased fish as well as from other aquatic animals. Similarly, Tiamiyu *et al*. (2011) stated that *Salmonella spp.* may be present naturally in tropical aquatic environments, and results from their studies also support this finding. As they isolated and identified *Staphylococcus aureus*, *Bacillus spp.*, *Salmonella spp.* and *Streptococcus spp.* from the skin of *Clarias gariepinus* while working at Ibadan. It is also a well-established fact that aquatic birds spread *Salmonella Spp.* and other pathogens in the environment (Fenion, 1983; Beveridge, 1989). The fungal organisms isolated from apparently healthy fish in this research were somewhat similar. The genera *Penicillium, Aspergillus, Cryptomonas, Cladosporium, Saccharomyces, Trichoderma, Neurospora, Botrydiplodia and Mucor* species found in the fish at Useh flood reservoir has previously been categorized by Refai *et al.* (2010) as normal myco-biota of freshwater fish. Even though these isolates are normal mycobiota, can however, be opportunistic fungi that can cause diseases (Refai *et al.*, 2004), especially under favourable predisposing condition. This finding agreed with the reports of other researchers that fungal infections are among the most serious types of disease problems in all types of aquaculture facility causing economic losses in fish farming in tropical Africa, and in other developing countries (Meyer, 1991). *Bacillus spp.*, *Escherichia coli*, *Salmonella spp.*, *Streptococcus spp.* and *Staphylococcus aureus* have been implicated in fish-borne diseases of humans (Babu, 2000). Ikpi and Offem (2011) in a study carried out on bacterial infection of mudfish *Clarias gariepinus* isolated and identified *Staphylococcus aureus, E. coli* and *Pseudomonas fluorescens* however dominating. This study agrees with the findings of Ikpi and Offem as *Escherichia coli* was dominant in this study. *Staphylococcus epidermis*, isolated in this study is among the predominant microorganism that has been previously isolated from both gills and skin of *Clarias gariepinus* (Hassan *et al*., 2010). And these microbial activities according to Nrior *et al*. (2017) are responsible for the high spoilage rate in dead aquatic species.

Conclusion and Recommendation

The study shows that Useh flood reservoir is a highly contaminated habitat, hence the high contamination of the fish with bacteria and fungi. The total viable count of bacteria and fungi in the gills, colon and skin may have resulted from stagnancy of the water and the anthropogenic, human and household wastes entering the reservoir. Stagnant water can result in high incidence of bacteria and fungi on flora and fauna found in such waters. Therefore, since these fish are able to reproduce without intervention in this reservoir, and their consumption cannot be stopped, it is therefore advisable that consumers of fishes harvested from Useh flood reservoir be encouraged to properly wash and cook harvested fish, else their consumption will result in public health issues. Anthropogenic activities and indiscriminate dumping of waste should be monitored to prevent further contamination of the flood reservoir, pending when other intervention to mitigate the pollution level is put in place.

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