The Effect of *Escherichia coli* on Catfish (*Clarias gariepinus)*

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ABSTRACT: The impact of *E. coli* on catfish (*Clarias gariepinus*) was investigated in the course of the project. The bacterium used in this study was a clinical isolate sourced from the University of Ilorin teaching Hospital (UITH), Ilorin. Various physicochemical parameters of the aquaculture which includes; dissolved oxygen, daily pH and temperature readings were also studied during the duration of the project work. *E. coli* was inoculated into *Clarias gariepinus* distributed into experiment 1 and 2 and the control was left uninoculated and observed for 7 days. At the end of the experiment, the test organism, *E. coli* was re-isolated from experiment 2 and not from experiment 1 and the control experiment. Other bacteria isolates were obtained and characterized from the fishes. They include; *Yersinia enterocolitica, Klebsiella sp., Vibro sp., Bacillus subtilis, Staphylococcus sp.* All the bacteria isolates were isolated from both the skin and the intestine of the fishes except *E. coli* which was found only in the intestine.

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1. INTRODUCTION

The importance of fish to man cannot be overemphasis in the world today. The major importance of fish to human is majorly to serve as a source of protein, and they are being converted to different forms for different purpose. The African catfish, *Clarias* gariepinus has been reared for about 20 years in Africa with mixed success; the total farmed production of this species being only 3,978 metric tones or 7.4% of the of the total farmed fish production of 69,434mt in Africa in 1994 (Balogun, 2000).

World wide, all fisheries are threatened by various factor, such as pollution, which brings about the introduction of industrial waste which comprise of various inorganic and organic waste and invasive species of bacteria. In which they are majorly enteric in nature, and their target point is the colonization of the intestine of fish and other sites in the fish. In less developed countries, which majorly include countries from the African continent, where their waste disposal method is known to be very poor, have constituted negative impact on the water bodies, thereby affecting aquatic life in water bodies and also contributing to the poor transportation system, which encourage the development of water weeds which also have effect on fish, by reducing their life span as a result of the various toxin released into the water. Fecal source of pollution have contributed to the high level of fish disease which have been experienced in recent years. The colonization of fishes by their various parasites from the faecal source of pollution has impacted disease such as Salmonellosis and others (Williams *et al*., 1989).

In every country where fish inspection programme exists, the load of faecal coliforms in farmed, feral or processed fish is evaluated to verify whether the harvest or product presents a health hazard or not (Blackwood, 1978; Fapohunda et al., 1994; Ampofo and Clerk, 2010). Their presence in fish intended for human consumption may constitute a potential danger not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from non-aquatic sources (Olayemi et al., 1991; Ampofo and Clerk, 2010).

The study of bacteria disease and its effect on catfish (*Clarias gariepinus*) and other species of fishes have been a serious concern in the fishing industries and its effect on human being when consumed. Fishes are found all over the world, they are basically found in marine and fresh water body (Colwel *et al*., 1960). Bacteria disease in marine fish come in bewildering array, and accretion rate of literature about has accelerated enormously in the past 2 decades, largely because of urgent problems in marine aquaculture and occasional epizootics in natural populations. Fish living in natural environment are known to habour pathogenic enterobacteriaceae (Pillay, 1990). Invasion

of fish muscle due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish are raised in pond with feacal coliforms, *E. coli* and salmonella of greater than 10^3 per ml in pond water, respectively (Guzman *et al*., 2004). In addition, most disease in humans is caused by opportunistic enteric pathogens, which are prevalent in the rearing environment (Jayasne *et al*., 1999).

More than 140 invasive bacteria species have being identified in great lakes and other water bodies. All these have contributed negatively to the economic impact in various countries. Parasites are of concern for several reasons. Parasite may have been keeping a nuisance species in cheek in its native habitat. In the absence of these parasites and other predator, the nuisance species can grow exponentially. On the other hand fish weakened by nonnative, such as the sea lamprey, are likely to die either from their weakened condition or from secondary infection (Welcomme, 2007). According to Ogbulie and Okpokwasilli (1999), who carried out bacteriological evaluation of various normal flora and spoilage organisms on *Clarias gariepinus* and *Heterobranchus bidorsalis*, showed higher bacteria colonization of the diseased than the apparently healthy fish.

Among the few others, Stunjak *et al* (1995), who researched on the development of marine fish culture, interest in fish health is also increasing. The major reason for this is the fact that the disease or rather the mortality that occurs in such controlled cultures, cause great economic losses. By growing large quantities of fish in rather small space, natural conditions are changed, so the fish are more sensitive and prone to infectious agents.

There are many articles on viral, bacterial and parasitic disease nowadays, yet, the knowledge on these disease vary depending on whether a fish species was being cultured for a long period of time or whether, it was only being introduced in controlled culture. The objective of this study is to examine the effect of *E. coli* on catfish and to isolate and identify of microorganism from catfish (*Clarias gariepinus*).

2. MATERIALS AND METHODS

The clinical isolate of *E .coli* was got from University of Ilorin Teaching Hospital, which was later cultured in a nutrient broth, for 24hrs in order to inoculate into the samples of fishes. The other samples that were used are specie of catfish; *Clarias grariepinus*, which was purchased in one of the fish pond, along the Ilorin metropolis. The fishes were kept in each bowls that was half filled with sterile water that have being boiled and cooked before the fishes were kept inside. After the acclimatization of the fish in the bowls that was filled, the culture broth of *E. coli* was then poured directly into the bowl as a means of inoculating the organism into the fish. It was then left for 2 days, in order to ensure the infectivity of the organisms on the fish. The experimental increase lasted for 1week, during which some physicochemical parameter was taken on daily basis.

2.1. DETERMINATION OF PHYSICOCHEMICAL PARAMETRES

The pH was determined using a electrode of pH meter of the pyecamm K model which was standardized with buffer 4, 7 and 9 before being used. The reading was taken when the deflection was stable. The temperature reading was also taken every morning and evening using a thermometer. This was done, by inserting the thermometer into the experimental and control bowls. The reading was read on the calibrated thermometer, and allowed to come back to zero level, before being inserted again. The dissolve oxygen was determined during the experimental period chemically using standard techniques.

2.2. COLLECTION OF CATFISH SAMPLES FOR BACTERIOLOGICAL EVALUATION

The samples of catfish were aseptically removed from the polythene bag and were placed on a sterile trays and with the aid of sterile trays and with the aid of a sterile knife, cuts were made from the edible parts of the fishes and homogenized, and about 10g taken for microbiological analysis.

2.2.1. Skin:

Samples of raw fish from different locations were collected, of which the skin of 150 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculating into the nutrient broth.

2.2.2. Intestine:

This was done by cutting a part of intestine and muscle after sterilizing with red hot scalped and inoculation in the media (MacConkey broth tubes). The samples included intestines from 150 raw fish.

2.3. BACTERIOLOGICAL EVALUATION

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. The media used for the bacteriological analysis of catfish is Mac Conkey agar (MCA). Mac Conkey agar was weighed out and prepared according to the manufacturer's specification, with respect to the given instructions and directions and sterilized at 121°C for 15 min. at 15lb pressure. A serial dilution method was used. The sterility of each batch of test medium was confirmed by incubating one or two uninoculated plates along with the inoculated plates. The uninoculated plates were always examined to show no evidence of bacterial growth. Any

uninoculated plate that showed evidence of bacterial growth was discarded. All the samples and the test organisms were replicated on different media and the plates were then incubated at 37°C for 24 - 48 h. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics. All isolates were subjected to various morphological characterization and gram stained to determine their gram reaction. Biochemical tests were carried out as described by Jolt *et al.* (1994) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt *et al.* (1994), Cheesbrough (2006) and Oyeleke and Manga (2008).

3. RESULTS ANALYSIS

The various results that was gathered in the course the project work, which range from the determination of temperature, PH, dissolved oxygen, presence of growth of different dishes, colonial morphology and to their biochemical evidence. The temperature range during the experiment period was observed to be in the range of a minimum temperature of 25^0C to maximum of 29^0C, as typically shown in Figure 1. It has been demonstrated from research that catfish generally are able to survive such a range in their natural habitat. *E. coli* is also known to be mesophiles that can survive such temperature and also go ahead to cause infection in a water body, if it is a pathogenic strain.

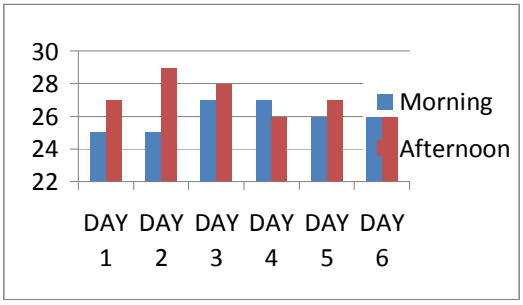

Figure 1: Temperature variation for duration of the experiment

The pH values in course of experiment have being shown from table 3, that the range of 6.39 to 6.67 have observed. The pH values obtained have been discovered to be weakly acidic, as a result the uneaten feed which then dissolve in water and metabolic activity of the host in water, which predispose the host to infection.

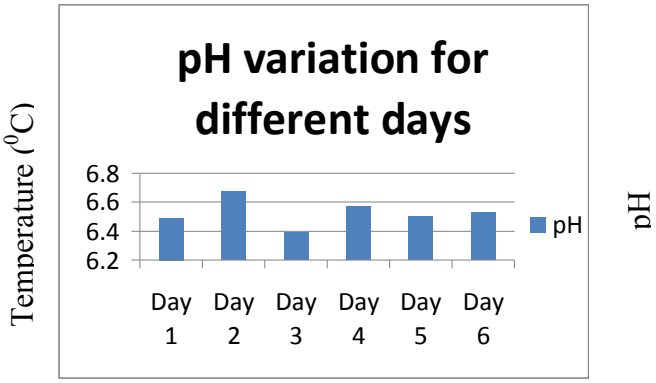

Figure 2: pH variation for duration of the experiment

The result from table 4 has shown that there have been variations in the trapped dissolved oxygen. The dissolved oxygen in the experimental bowl have a more depleted dissolve oxygen level, as compared to the control bowl that have D.O slightly higher than the experimental samples, after the readings with 3 days intervals. This is as a result of the condition such as infectivity, in which the rate of depletion will be increased due to increased respiration of the host in the experimental bowls. This is one of the most indicative physicochemical parameter, that indicate infectivity in fishes in open water bodies during pollution of the water with wide range pathogenic enteric organisms such as *E. coli.*

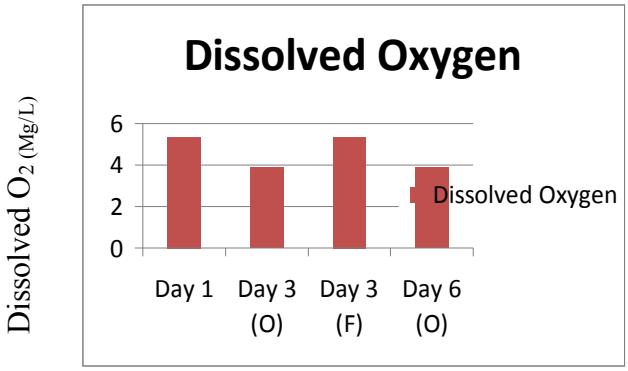

Figure 3: Dissolved oxygen variation for duration of the experiment

This work have revealed that more of gram negative bacteria have being isolated as compared to gram positive bacteria, which is lower in number. According to Ogbulie and Okpokwasili (1999), who reported also that wide range of gram negative bacteria bacteria have being isolated from species *Clarias gariepinus* and *Clarias bidorsalis*. From the table below, it has been shown that the intentionally inoculated clinical isolate, which is *E. coli*, was only isolated from experimental sample 2, indicating that

the test organism was infective, but not able to initiate a disease process. It was isolated from the intestine majorly, and also revealing that it is a coliform found in the intestine of human and animals.

Table 1 shows the distribution of the isolates on different part of the fish. It showed that more of bacteria isolated were more of the gram negative bacteria compared to gram positive bacteria, which is lower in number. From Table 1, it has been shown that the intentionally inoculated clinical isolate, *E. coli* was only isolated from experimental sample 2, indicating that the test organism was infective, but not able to initiate a disease process. It was isolated from the intestine majorly, and also revealing that it is a coliform found in the intestine of human and animals.

Table 1: Distribution of the isolates on different part of the fish

Isolate	Skin	Intestine
E. coli	_	+
Y. enterocolitica	+	+
Staphylococcus sp.	+	+
Klebsiella sp.	+	+
Bacillus subtilis	+	+
Vibrio sp.	+	+

Key: + = present; − = absent

4. DISCUSSION

The result that has been collated from this project has shown that the use of a clinical isolate on catfish was infective against it. The basic purpose of the experiment was archived, by infecting a host (*Clarias gariepinus*) with a member of the enterobacteriaceae (*E. coli*), which was also isolated and identified with other group member of the family. The physicochemical parameter was majorly indicative that the bacterium was infective. The temperature during this experiment was majorly observed to be on the rise, which was fluctuating between the ranges of 25-29^{o} C. From Fig (1), it was observed that highest mean temperature was notice on day 2, during the afternoon and the lowest temperature was also on day 1 and day 2, in the morning. This increase on day 2 can be correlated to the high intensity from the sun, as major climatic factor, which brought about the increase. The lowest temperature can also be attributed to the reduced sunlight which will impact the water containing the fish and organism. The organism in particular (*E. coli*) is known to thrive well, because it is mesophilic in nature. The fish in particular does not show any sign of disease under this condition. Since fish are cold-blooded, every aspect of their physiology is controlled by temperature, which constitute to the infectivity of fishes. Their metabolism, or the amount of energy they need to survive and grow, is also temperature-dependent. Every species of fish is adapted to live within a certain thermal range. Temperature at the high end of this range causes metabolic inefficiency. Fish living at these higher temperatures simply cannot eat enough to meet their energy demand. On the other hand, temperature at lower end of this range cause decreases activity and appetite, which decrease growth and weight gain (Shuter and meisner, 1992).

Climate change with the presence of the organism in the water body constitute to the depletion in the dissolved oxygen of the catfish (*Clarias gariepinus*), which could also reduce production of catfish, especially those without benefit of pond aeration systems. When fish “breathe in water”, they are actually using the dissolve oxygen gas trapped in water. The dissolved oxygen concentration in water is temperature-dependent: as water temperature increase, oxygen level decrease (Goldman and Horne, 1983). Water temperature is the most important predictor of dissolve oxygen concentration in shallow ponds (Hargreaves and tucker, 2003). Catfish are usually grown in ponds, and the management of waste from uneaten feed, fecal matter, and fish metabolites is largely left to nature. Most of these natural decomposition processes require dissolve oxygen. Therefore, the maximum stock capacity of a catfish pond receiving feed fertilizer is determined by available dissolved oxygen (Hargreaves and Tucker, 2003). In Fig. 3, the result of the observation have being very significant, in which during day 1 and day 3 (F), the mean dissolve oxygen have shown an appreciable increase. As a result, indicating that the dissolve oxygen have not being used up by the fish. This is the most significant way to determine the effect of *E. coli* on fish. This reason and facts have constituted to the major decrease in dissolve oxygen in the experimental sample while that of the control have being observed to be higher.

Clarias gariepinus can live in water having a pH range from about 5-10.The desired pH range for fish production is between 6.5-9.0.The pH of pond water is influenced by amount of carbon dioxide present. Much of the carbon dioxide (CO_2) in water is the result of animal and plant respiration. Carbon dioxide is used in photosynthesis. Therefore, carbon dioxide concentration in water increase at night and decrease during daylight hour. Since carbon dioxide is acidic, the pH of water is usually highest in the late afternoon and lowest just before sunrise (Tucker *et al.,* 1990). Due to the ability of *E. coli* to infect *Clarias gariepinus*, in other word increase the respiratory rate of *C. gariepinus*, during the infective period. Also in addition, as indicated in Fig. 2, many of the dissolve and uneaten food and some other factors such as metabolic waste contribute to the low pH, unlike when

natural or organism free water is determined and most at times falls within weakly acidic to neutral pH.

Escherichia coli, the predominant species of the faecal coliforms, has been found in the intestinal tract of fish (Newman et al., 1972; Ampofo and Clerk, 2010), on the gills, in the muscles and on the skin (Ogbondeminu, 1993), when sewage water has been used to rear fish (Ampofo and Clerk, 2010). Salle (1964) reported that the most heavily contaminated parts are the intestines and the skin. Presence of *E. coli* in water or food indicates the possible presence of causative organisms of many gastro-intestinal diseases (Frazier, 1958; DHSS, 1991; Ampofo and Clerk, 2010). Raj and Liston (1961) found that some pathogenic and potentially pathogenic microorganisms including *E. coli, Staphylococ- cus* and some anaerobes survived when uncooked and precooked fish foods were stored at freezing temperatures.

According to Ogbulie and Okpokwasili (1999), who reported also that wide range of gram negative bacteria bacteria have being isolated from species *Clarias gariepinus* and *Clarias bidorsalis*. Studies by Roberts (1978) showed that bacteria belonging mostly to the genera *Aeromonas, Corynebacterium, Pseudomonas* and *Vibrio* cause infectious diseases in fish.

In the course of this study, the infectivity of the catfish was basically due to the inoculums load of culture, which was sufficient enough to cause infection. The virulence of the various bacteria isolates, which was used in the inoculation of other catfishes, by different project members, was observed to be very high, especially those inoculated with *Pseudomonas sp.* and *Salmonella* sp., in which the experimental catfish died before the expiration of the exercise, as a result of the infectivity level, unlike bacteria species like E. *coli* and *Proteus* sp., which were active and healthy and did not die, not until the conclusion of the study. Most strains of *E. coli* are harmless, and are occasionally responsible for product recall (Hudalt *et al*., 2001). The harmless strain is part of the normal flora of the gut, and can benefit their host by producing Vitamin k_2 and by preventing the establishment of pathogenic bacteria within the intestine (Reid *et al*., 2001). In this study, *E. coli* was found in the intestine majorly and since it is found in association with water as well as intestine, its presence in the intestine to a reasonable extent is vindicated This because the can acquire it from the immediate environment.

Indication that *E. coli* is probably a normal flora of the fish (*Clarias gariepinus*). The other experimental study that used *Pseudomonas* and *Salmonella* species, experienced peeling of their outermost skin and reduced appetite of the fishes, which the skin peeling was not experienced in, fishes inoculated *E. coli* and P*roteus* sp. The presence of these six genera in the fish is, therefore, a threat to the fish industry as fish, which do not succumb to the attack may still be subjected to spoilage.

The presence of the coliform group of bacteria, mainly *Escherichia* and *Klebsiella* in fish and fish products presents a health hazard to humans (Caldreich and Clarke, 1966; van DUIJN, 1973; Fapohunda et al., 1994; Ampofo and Clerk. 2010). Allen and Hepher (1979) have stated that most of the epidemics attributed to wastewater sources are from raw sewage gaining access to food eaten directly by man, or from contamination of water supply systems by untreated sewage (Ampofo and Clerk, 2010).

5. CONCLUSION

From this study, it was made clear that *E. coli* was found in the fish during the isolation and biochemical identification, but whether the particular strain of *E. coli* was a very virulent strain was not really determined to be Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC) and diffusely adhering *E. coli* (DAEC) in which, if they are consumed by humans, when proper cooking is not done, might lead to disease like Traveler's diarrhea and other *Escherichia coli* infections. Public health must therefore be of prime concern when dealing with fish farming and its products in countries with less restriction on release of waste into water bodies, and in use of untreated wastewater for aquaculture (Ampofo and Clerk, 2010). The digestive tract and intraperitoneal fluid of fish in this study showed concentrations of pathogens. Handling and cleaning of such contaminated fish can result in contamination of the hands of farm workers and through them to their family members and others.

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