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RESEARCH ARTICLE

ECOLOGY OF ENTERIC BACTERIA IN FRESHWATER CATFISH *CLARIAS GARIEPINUS* IN GUBI DAM, BAUCHI STATE, NIGERIA

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ABSTRACT

The ecological distribution of enteric bacteria in Clarias gariepinus was studied in Gubi reservoir, Bauchi State, Nigeria. The study, showed the presence of some members of the *Enterobacteriaceae* family in the samples analyzed. From the gills, the following were isolated and identified such as *Escherichia coli* (13.3%), *Shigella* species (15.6%), *Salmonella* species (11.1%). From the intestines, the following bacteria were isolated such as *Escherichia coli* (8.5%), *Klebsiella* species (8.5%) *Pseudomonas aeruginosa* (25.4%), *Shigella* species (5.0%), *Salmonella* species (25.4%). Staphylococcus species (51.1%) *Streptococcus* species (10.2%), The *Staphylococcus* species has the highest prevalence rate followed by *Salmonella* species and *Klebsiella* species had the lowest rate. The presence of these bacteria even at low rate as shown by the study indicates that *Clarias gariepinus* in their natural habitat can harbor pathogenic bacteria of high medical importance. Proper cooking or processing prior to consumption is necessary for health safety.

INTRODUCTION

Fish is one of the best supplies of proteins, vitamins and minerals and are essential nutrients required for supplementing both infant and adult diets (FAO, 2009). Fish and fish products play a significant role in the diets of the populations of West African countries and constitute more than 60% of the total protein intake in adults especially in the rural areas. It has a relatively 10% calories content hence its role in nutrition is recognized. In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio- economic, age, religious and educational barriers. While this growth is much appreciated in terms of food security, the health risk associated with the aquaculture produce is another important concern. In recent times increased attention is given to the possibility of cultured fish as vector of human pathogenic bacteria (Apun *et al*., 1999; Islam *et al*., 2000). Enteric bacteria are rod-shaped gram-negative anaerobic bacteria that metabolize glucose to acids and can thrive under aerobic conditions. Some enteric organisms e.g. *Escherichia coli*, are part of the normal flora and incidentally cause disease while others like the *salmonellae* and *Shigellae* are regularly pathogenic for human. Fish living in natural environment are known to harbour pathogenic *Enterobacteriaceae* (Pillay, 1990). Many of these bacteria capable of causing disease are considered to be saprophytic in nature but only become pathogenic when fishes are physiologically unbalanced, nutritionally deficient, or as a result of other stressors such as poor water quality, overstocking, which allow opportunistic bacterial infections to proceed and lead to considerable economic losses in aquaculture as a results of heavy mortalities in both culture and wild fishes throughout the world (Pillay,1990). Also, Fish inhabiting waters contaminated by fecal Coliform bacteria can easily come in intimate contact with these microorganisms. Studies have shown that enteric bacteria are not part of the normal flora in the intestinal tract of fish and their presence is considered a direct result of the association of fish with sewage-polluted waters (Geldreich and Clarke, 1966; Gluntz and Krantz, 1965). These bacteria can also survive and multiply in the fish intestine with residency lasting from a few days to a few weeks (Guelin, 1952; Reasoner, 1974). Possible consequences of this association may be either infection of fish or fish acting as vectors of human disease. Among the common fish pathogens are *Staphylococcus* sp., *Aeromonas* sp., *Salmonella* sp., *Shigella* sp., *Enterococcus faecalis*, *E. coli*, Y*ersinia* sp., *V. cholerae* and other *vibrios* (Ogbondeminu *et al*., 1993). In view of the foregoing, this work is designed to examine, isolate and identify the various species of enteric bacteria associated with *Clarias gariepinus* in Gubi dam, Bauchi State, Nigeria.

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MATERIALS AND METHODS

Collection and processing of fish samples

Twenty live African catfishes (*Clarias gariepinus*) were randomly collected from the study site (Gubi dam) from three

different fishermen. Fishes were caught by cast net. Samples were collected between 8:00-10:00am GMT. The freshly caught fish samples were kept in sterile polythene bags containing ice blocks as to protect autolysis. The fish samples were transported directly to the microbiology laboratory of Abubakar Tafawa Balewa University, Bauchi within 1hour of samples collection. The different media used were MacConkey agar, Nutrient agar, Eosin Methylene Blue agar and Violet Red Bile Glucose Agar, and were prepared according to manufacturer's instruction and were stored in the refrigerator when not in use.

Sample preparation for Bacteria Isolation

The fish samples were aseptically removed from the sterile polythene bag and the fishes were disabled using a pointed scalpel to puncture its brain. A sterile knife was used to bisect the fish samples stomach in order to remove the intestine. A sterile knife was used to eviscerate the head for the gills portion then sterile forceps were used to aseptically bring out the target organs. Bacterial isolates from each specimen were obtained from the gills and intestine. Gills (1g portion), intestine (1g portion) were separately shaken in 10ml of distilled water. The samples were serially blended for some minutes. The stock solution was serially diluted in ten folds. 0.1ml of (10^{-10}), the serially diluted samples were used for the bacteriological analysis.

Detection of Bacteria

The homogenized pretreated material was incubated at 30-37^{0}c for 2-5hours. The container was shaken, 1ml of the homogenized material was transferred to 100ml of *Enterobacteriaceae* enrichment broth-mossel and then incubated for 35-37^{0}c for 18-48hours. A sub-culture was done on a plate with violet –red bile glucose agar (VRGBA, Oxoid Basingstoke, UK) and then incubated at 35-37^{0}c for 18-48hours. Then, observation was made for bacteria growth on the plate.

Identification and counting of Isolated Bacteria Pathogens

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colony appearance, size, elevation, form, edge, colour, odour, opacity, and pigmentation. Colonies were selected at random and subcultured to obtain pure isolates on fresh plates and then incubated at 37°C for 24h. The stock cultures were obtained and labeled carefully. Plate count method was used to count the isolated bacteria.

Morphological Identification, Biochemical tests and bacteriological analyses

Morphological identification of bacteria isolates was analyzed by cross reference to Bergey's manual of systematic bacteriology and the methods of Buchanan and Gibbson (1994). Biochemical methods used for the different bacteria species encountered were Gram Staining Technique, Motility Test, Catalase Test, Coagulase Test, Oxidase Test, Sugar Utilization and Fermentation Test, Citrate Utilization Test, Indole Production Test and Urease Test accordingly.

RESULTS

The enteric bacteria isolates from the gills and intestine of the sampled *Clarias gariepinus* consisted of *Klebsiella*, *Pseudomonas aeruginosa*, *Shigella* species, *Salmonella* species, and *Escherichia coli*, though other bacterial isolated from the study were *Staphylococcus* species, and *Streptococcus* species. While *Escherichia coli*, *Streptococcus* species, *Staphylococcus* species, *Shigella* and *Salmonella* species were found associated with the gills of the sampled *Clarias gariepinus*. *Escherichia coli*, *Klebsiella* species, *Streptococcus* species, *Salmonella* species, *Staphylococcus* species, *Shigella* , *Pseudomonas* species, were all found to be present in the intestine.

Table 1. Bacteria isolates found associated with the gills and intestine of *Clarias gariepinus* from Gubi dam, Bauchi state, Nigeria

	Site of occurrence	
Bacterial isolates	Gills	Intestine
Escherichia coli	+	+
Klebsiella species	-	
Steptococcus species	+	+
Staphylococcus species	+	+
Pseudomonas aeruginosa	-	+
Shigella species	+	+
Salmonella species	+	+

Key: (-) = Absent(+) = Present

Table 2. Rate of bacterial occurrence in the gills and intestine of sampled *Clarias gariepinus* from Gubi dam, Bauchi state, Nigeria

	Site / No. of occurrence		
Bacterial isolates	Gills	Intestine	Total number
Escherichia coli	6	5	11
Klebsiella sp.	0	5	5
Streptococcus sp.	4	6	10
Staphylococcus sp.	23	10	33
Pseudomonas aeruginosa	0	15	15
Shigella sp.	7	3	10
Salmonella sp.	5	15	20
	45	59	104

Table 3. Percentage of bacteria occurrence on the gills and intestine of sampled *Clarias gariepinus* from Gubi dam

Bacterial isolates	Gills (%)	Intestines (%)
Escherichia coli	13.3	8.5
Klebsiella species	0.0	8.5
Streptococcus species	8.9	10.2
Staphylococcus species	51.1	16.9
Pseudomonas aeruginosa	0.0	25.4
Shigella species	15.6	5.0
Salmonella species	11.1	25.4
	100	100

DISCUSSION

The variation in bacterial occurrence at different sites of the sampled fishes have been observed previously (Trust and sparrow, 1974; Yoshimzu and Kimuna 1976; Spanggaard *et al*., 2000) and were confirmed by the results. The dominating bacteria in the study: *Staphylococcus* species and

S*almonella* species belong to a few phylogenetic groups, and they were the dominant bacteria. The overall presence bacteria isolates consisting of fermentative gram-negative, rod-shaped bacteria belonging to the family *Enterobacteriaceae* agrees with the previous studies (Trust and Sparrow, 1974; Nieto *et al*., 1984; Spanggaard *et al*., 2000). It is generally contended that the fish intestine does not have a stable microflora although; the gastrointestinal tract provides an ecosystem distinctly from the surrounding water. However, other investigators have not detected any similarity between bacteria groups isolated from the water intestine or fish diet. Bacteria such as *Escherichia coli, Salmonella* species, *Staphylococcus* species, *Pseudomonas aeruginosa*, were isolated from the sampled *Clarias gariepinus* at different sites in the course of the investigation. These microorganisms are often designated as coliforms i.e. they are normal inhabitant in large intestines of human and other animals and consequently present in feces (Pelczar *et al*., 1998). Thus, the presence of these indicator organisms belonging to the *Enterobacteriaceae family* is an evidence of fecal pollution of human or animal origin.

If these indicator organisms is present in water, e.g. *Escherichia coli* then the way is also open for human intestinal pathogens to gain entrance into the water since they also occur in feces (Pelczar *et al*., 1998). *Staphylococcus* species especially the *staphylococcus aureus* is known to cause intoxication because they produce toxins which cause gastroenteritis in their human consumers. Pathogenic effect of bacteria can be directly related to the toxins they produce (Stewart and Amerine, 1992). Some Genera including *Pseudomonas species* have been identified with fish spoilage. The spoilage causing bacteria in the fish are part of the natural flora of the fish of the external slime and intestinal content. When the fish dies, the bacteria invade the fish flesh. This is possible because the fish has lost its natural defence mechanisms. The bacteria in the intestine and gills/gut multiply and rapidly invade the fish flesh. The bacteria (pseudomonas species) feed on the fish flesh which break down with the aid of their enzymes (Nogachi *et al*., 1987). Thus, the abundance of food leads to an exponential growth in bacteria resulting in the presence of heavy slime on the skin and gills surface.

The presence of *Staphylococcus* species in all the target organs investigated, is an indication of possible food poisoning as it may cause gastroenteritis in the unwary human consumption (Austin and Al-zahrani, 1998). Although, some of these microorganisms produce endospores and endospores are heat resistant which may survive inadequate heat treatment during cooking or smoking of these *Clarias gariepinus*. This is due to the fact that the endospores formed are extremely resistant to dessication, disinfections, chemicals, radiation and heat (Pelczar *et al*., 1998). Enteric population or presence could be advantageous as seen in the digestive process of fish such as microbial breakdown of chitin, collagen, cellulose and these organisms could also supply fatty acids and other vitamins to the host (Ringo *et al*., 1995). Also, the presence of these bacteria prevents colonization of the fish by other microbes that might otherwise be pathogenic. The presence of species such as *Klebsiella pneumoniae* is known to be found in the environment inhabiting plants. The presence of *Salmonella* species in the sampled *Clarias gariepinus* and at moderate rates were attributed to high temperatures in water bodies which promoted the growth of salmonella species as well as contamination.

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