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Organ distribution and biochemical characteristics of *Klebsiella species* in farm raised heteroclarias (*Clarias gariepinus* female x *Heterobranchus longifilis* male) fish in Ilorin metropolis

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Abstract

Fish is capable of harbouring disease and therefore, must be safe and free of infectious pathogens if aquaculture should fulfill its potential. Infectious disease has been one of the major causes of death worldwide in recent times. This study examined the occurrence of *Klebsiella species* in fish organs in Ilorin metropolis. A total of 108 organs (gill, intestine and liver) were collected and examined for *Klebsiella* investigation. The samples were examined using standard techniques, biochemical characterization were used in this study and data obtained were analyzed using descriptive statistics. The results shows that all the 108 organ collected were *Klebsiella species* positive.

Keywords: *Klebsiella*, Distribution, Disease and infection

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Introduction

Aquaculture has become a business in Nigeria and serves as a source of animal protein to the populace. Significant reduction in captured fisheries, aquaculture should be promoted to enhance the production of fish to the populace. The occurrence of new pathogens and resurfacing of old ones especially infectious ones has been the reasons for this trend WHO 1984. Apart from economic losses associated with infectious disease in fishery sector, it could cause biological damage such as reduction in fish growth, decrease nutritional value and transmission to the consumers (zoonotic) (Adeshina *et al*., 2016).

Fish is capable of harboring diseases hence it safety is important. According to Mead *et al*. (1999) diseases caused from food were estimate to be 76 million illnesses, 325,000 hospitalizations and about 5,000 deaths in the US yearly. Diseases from known pathogens were estimate to be about one-fifth of the cases. Therefore, fish must be safe and free of infectious pathogens if aquaculture should fulfill its potential. The excessive spread of pathogens from fish and other seafood are a major concern issue globally. Rapid Alert System for Food and Feed (RASFF) reported that fish is second only to vegetable in terms of alerts recorded between 2009 and 2012.

Klebsiella is a genus of non-motile, Gram-negative, oxidase negative, rod-shaped bacteria with a prominent polysaccharide-based capsule, belongs to the family *Enterobacteriaceae* (Ray & Ryan 2004; Archana & Sumathy 2018; Pavan *et al.* 2022). *Klebsiella* species are classified as one of the opportunistic bacteria that normally inhabit the gastrointestinal tract of healthy humans and animals. People with immunosuppression are most likely to be infected with *K. pneumonia* (Riwu *et al*. 2022; Paczosa & Mecsas 2016; Liu *et al.* 2021). *Klebsiella pneumoniae*, an opportunistic bacterial pathogen, is known for its high frequency and diversity of antimicrobial resistance genes. *K. pneumoniae* is a well-known opportunistic human pathogen recently linked to outbreaks of disease in aquatic organisms (Vaneci-Silva *et al*. 2022; Barati *et al.* 2016). Xu *et al.* (2022) reported the genetic diversity of *K. pneumoniae* strains in 14 species of edible aquatic animals.

Hybrid catfish crossbreeding *Clarias gariepinus* female and *Heterobranchus longifilis* male is the most cultured fish species in Ilorin metropolis due to the qualities it possesses and also attracts significant economic value. In semi-intensive systems, fry of hybrids

(mean weight 7.5 g) within 24 weeks reached an average harvest weight of 880 g (Sobczak *et al*. 2022). Fish is capable of habouring diseases hence it safety is important. According to Mead *et al.* (1999) diseases caused from food were estimate to be 76 million illnesses, 325,000 hospitalizations and about 5,000 deaths in the US yearly. Diseases from known pathogens were estimate to be about one fifth of the cases. In international trade, the major reason for product rejection is bacterial pathogens.

Therefore, fish must be safe and free of infectious pathogens if aquaculture should fulfill its potential. The excessive spread of pathogens from fish and other seafood are a major concern/dangerous issue globally. Rapid Alert System for Food and Feed (RASFF) reported that fish is second only to vegetable in terms of alerts recorded between 2009 and 2012. Over the years, the increase in population and incomes has led to the increase in consumption of fish as a result of its nutritional value. *Klebsiella* spp. are gram-negative microorganisms found in different environments and are part of the natural microflora of soils, effluents, and natural water environments and in some plants. Among the species found in this genus,

Heteroclarias is one of the most cultured fish species in Kwara state especially in Ilorin metropolis due to its hardy behavior and faster growth. Therefore, *Klebsiella species* in relation to the catfish, by isolating the microorganisms present on the heteroclarias gill, liver and intestine. The research is to be carried out due to how it affects the fish and human health and to see what to put into consideration to avoid being affected with the bacteria called *Klebsiella species*. *Klebsiella* species isolate can survive in the fish (immunity of fish) and observation of the public health hazard that bacteria i.e., test organism and natural flora exposes the people to, samples of heteroclarias were analyzed using standard techniques. The objective of this study is to determine the distribution and biochemical characteristics of *Klebsiella* species present in organs of farm raised hybrid catfish in Ilorin metropolis.

Material and methods

Fish organs collection

A total number of thirty six (36) fish were collected and taken to the lab to check the length (total length and standard length), weight and dissect for the collection of the organs. Three 3 organs (gill, intestine and liver) were collected and weighed into a sterile universal bottle making a total number of one hundred eight samples and subjected to *Klebsiella* isolation. Fish within the range of 150g-1000g were used for this study as the group comprises of stage of fish that are consume by most individuals or processed for economic value.

Isolation of Klebsiella species

Each organ were carefully collected and weighed into a sterile universal bottle making a total of 108 samples. The sample organs collected were taken to the laboratory and subjected to microbiological analysis under sterile conditions. 10mls of sterile water was added to each samples and leave for 30mins to get the solutions of the samples. 1ml of each samples were then added to 9mls of nutrient broth and incubated at 37°C for 24 hours. The culture media were then streaked on macConkey agar prepared plates using wire loop and then incubated for 24hrs. The wire loop was being sterilized on

spirit lamp before usage. The suspected colonies were sub-culture on nutrient agar to get pure colonies. All the media were prepared according to manufacturer's instructions.

Confirmation tests for Klebsiella species isolation

All the 108 samples were suspected to *Klebsiella species* based on colony chacteristics. The suspected colony were confirmed using biochemical characterization. Confirmation was by biochemical test which include gram stain reaction, oxidase reaction, catalase reaction and motility test (using hanging method) at room temperature.

Statistical analysis

The data were analyzed using descriptive statistics using IBM statistical package for social science version 20.

Result

The result of the biochemical test carried out on all the isolates, it helps to confirmed that all the isolates were *Klebsiella species*. All the 108 isolated were gram negative, catalase positive, oxidase negative, non-motile as shown in (Table 1). Table 2 shows the collection of the samples based on sex, weight and the length (standard length and total length). Table 3 shows the prevalence of *Klebsiella* species among the fish organs which shows that all the organs collected were positive.

Table 1: biochemical characterization of the suspected organisms

Tests	positive	negative	Remarks
Gram stain	0	108	
Catalase reaction	108	0	Shows sign of catalase formation
Oxidase reaction	0	108	No color was formed
Motility	0	108	No movement of the organisms

Table 2: Collection of the samples based on sex, length and weight

Parameters	No. of samples	No. of infection	Percentage of infection
Sex			
Male	16	16.00	44.4
Female	20	20.00	55.6
Total	36	36.00	100.00
Total length (cm)			
21-30	6	6.00	16.7
31-40	14	14.00	38.9
41-50	16	16.00	44.4
Total	36	36.00	100.00
Standard length (cm)			

Parameters	No. of samples	No. of infection	Percentage of infection
21-30	18	18.00	50.0
31-40	14	14.00	38.9
41-50	4	4.00	44.4
Total	36	36.00	100.00
Weight (kg)			
150-350	16	16.00	44.4
351-550	2	2.00	5.6
551-750	16	16.00	44.4
751-950	0	0.00	0.00
950 and above	2	2.00	5.6
Total	36	36.00	100.00

Table 3: Distribution of Klebsiella species in fish by organs

Organ	No. of sample	No. of postive	% of infection	Intensity of infection
Gill	36	36	100	0.24
Intestine	36	36	100	0.40
Liver	36	36	100	0.52
Total	108	108	100	1.16

Discussion

This study describes the distribution and biochemical characterization of *Klebsiella species* in Ilorin metropolis. All the fish collected in ilorin metropolis irrespective of it sex, length and the weight are carriers of *Klebsiella species*. Organs used were *Klebsiella species* positive which suggest that all these organs have contact with water, feed, soil, vegetable etc thereby makes it the susceptible sites. The result obtained in this study is in agreement with the work of Olayemi *et al.* (1991) and who observed more than 50% in Ilorin. In the study of Takyi *et al.* (2012) about 30% of *Oreochromis niloticus* gill examined in Akosombo, Ghana and 44% in Ashaiman had *Klebsiella species* which is higher than the result in the present study but shows a similar trend. In Akosombo and Ashaiman, 11% and 28% respectively of *O. niloticus* examined by Takyi *et al.* (2012) were positive for *Klebsiella species* which is in agreement with the result of this present study. Therefore from the results, the possibility that *Klebsiella* are comfortable in sampled fish farms in Ilorin metropolis occurs which may cause a threats to the health of the fish in the case of physiological and environment imbalance and consumers. Based on the morphological assessment of the fish there is no sign of *Klebsiella* species from the fish. Therefore there is no outbreak at the study area as a result of this bacteria yet, though cases might go unreported. The isolation of the *Klebsiella species* from the fish suggest that this potential human pathogens are present on fish farms in ilorin metropolis. The result further suggest that fish and fish products from this farms could pose a serious health threat to humans when this bacteria are consumed in large quantities (Ampofo and Clerk, 2010).

Conclusion

The distribution of *Klebsiella species* in fish in Ilorin metropolis shows that is a predominant bacteria present in the organs. *Klebsiella species* is present in the organs in this study area and may pose some threat because this organs are consumed by human and animal. Since *Klebsiella species* are commonly found in water receiving effluents from paper mill, pulp, sugar cane, textile industry etc and most of the fish farmers in this study area depends on dam, stream water etc as their source of water for their fish farming activities, a study should be carried out on the usage of the water without affecting or posing any threat to the fish health and effort should be made to prevent, control and manage it occurrence in the fish should be develop in the study area to prevent outbreak. Therefore, it is important to handle fish with proper hygiene and best public health measures since fishes are used for consumption. Also fish handlers should avoid contact with the fish and fish water when they have injuries as this microorganisms may be present. However, fish should always be handle with utmost care and hygiene.

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