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ORIGINAL ARTICLE

Occurrence of Klebsiella Species in Cultured African Catfish in Oyo State, South-West Nigeria

Adeshina, I.[1]*; Abdrahman, S. A.[2] and Yusuf, A. A.[3]

[1]Department of Aquaculture and Fisheries, University of Ilorin. [2]Department of Veterinary Microbiology and Parasitology, University of Ilorin. [3]Department of Life Sciences, Deakin University, Australia. *Corresponding Authors: Email: padibra4all@yahoo.co.uk; Tel No:+2347054994559.

SUMMARY

Over the years, the increase in population, incomes, and demograph has lead to the increase in the consumption of fish as a result of its nutritional values. *Clarias gariepinus* is the most cultured fish species in Nigeria and attracts significant economic value making it a species of interest. Fish is capable of habouring diseases and therefore, must be safe and free of infectious pathogens if aquaculture should fulfill its potential. Infectious disease has being one of the major causes of death worldwide in recent times. *Klebsiella* species in the family of enterobacteriacea causes urinary tract infections, pneumonia, septicemias and soft tissue infections in human. They are opportunistic pathogens found the gastrointestinal tract of the host and spread rapidly thereby capable of causing nosocomial outbreaks. The study examined the prevalence of Klebisiella species in fish tissues in Oyo State, Nigeria. A total of seven hundred and fifty-six (756) fish organs from one hundred and eight (108) fish were examined for *Klebsiella* investigation. Fish of body weight ranges between 300g to 1300g were used for this study using stratified methods. The samples were examined using standard methods, biochemical characterization methods were employed in this study and data obtained were analysed using descriptive statistics. The results show that all the 66 isolates were gram-negative, did not haemolysis sheep blood, catalase positive, oxidase negative, non-motile, and ferment lactose, sucrose and mannitol sugars. Percentages of klebsiella species occurrence were 22.22% in forest zone and 40.72% in savannah zone. Fish with total length 51-60cm had highest incidence (52.17%), and body weight of 601-900g had highest incidence (57.14%). Generally, skin, gill, liver and muscle were the major location of Klebsiella species occurrence ranges from 11% to 14.81%. Therefore occurrence of Klebsiella species in higher in savannah zone than forest zone. The distribution of the klebsiella in fish in Oyo State shows that it is a problem present in fish tissues.

Key words: Klebsiella, Occurrence, Diseases, infection.

INTRODUCTION

One of the fastest growing sectors is aquaculture in recent times, representing about 50 percent of the fish supply across the globe (FAO, 2005) and developing countries like Egypt and Nigeria are not lagging behind. Food fish supply has increase (3.2%) with world per capita of fish consumption (19.2 kg). Aquaculture has become business in Nigeria and serves as a source of animal protein to the populace. Significant reduction in captured fisheries, aquaculture is providing about 70% of fish supply in Nigeria especially in Oyo State where the aquacultural practices is high. Over the years, the increase in population, incomes, and demograph has lead to the increase in the consumption of fish as a result of its nutritional values (FAO, 2014). *Clarias gariepinus* is the most cultured fish species in Nigeria and attracts significant economic value making it a species of debate.

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. RASFF in 2012 reported that import rejection of seafood was approximately 15% out of total product rejection (Anonymous 2013). In international trade, the major reason for product rejection is bacterial pathogens (Ababouch *et al.,* 2005). 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.

Infectious disease has being one of the major causes of death worldwide in recent times. 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 diseases in fishery sector, it could cause biological damage such as reduction in fish growth, decrease nutritional values and transmission to the consumers (zoonotic). Faruk *et al.,* (2004) reported that the loss from fishery sector linked to fish diseases is estimated by Word Bank to be US$ 3 million per annum.

Klebsiella is a genus of non-motile, facultative anaerobes, gram-negative, oxidase-negative, and rod-shaped bacteria belonging to the family of enterobacteriaceae (Ryan and Ray, 2004). It is a ubiquitous bacteria which could be found almost everywhere. It has been isolated in water, soil, vegetation, animals (including fish) and in human (Bagley, 1985; Brisse *et al.,* 2006). It is typically has straight rod shape with rounded /pointed ends, appears singly in pairs or in short chains, grows between 35 and 37^0C. Brisse *et al.,* (2004) reported that Klebsiella species produces capsule or slime layer. The presence of O and K antigens helps in its pathogenicity and has become serious pathogens in nosocomial infections. It causes urinary tract infections, pneumonia, septicemias, and soft tissue infections. The genus has six species which are *K. granulomatis, K. oxytoca, K. michiganensis, K. pneumoniae K. quasipneumoniae,* and *K. variicola*. All the members capable of causing illness, however, the three major members of the Klebisiella genus that causes human infection are *K. pneumonia, Klebsiella granulomatis*. and *K. oxytoca* . They are opportunistic pathogens found the gastrointestinal tract of the host and spread rapidly thereby capable of causing nosocomial outbreaks.

In Malaysia, about 15-40% illness were reported annually, 66% of alcoholism group were affected in USA with mortality rate more than 50% of incidence (Qureshi, 2015). Klebsiellae cause as many as 14% of cases of primary bacteremia, second only to *Escherichia coli* as a cause of gram-negative sepsis. The US Centers for Disease Control and Prevention report that *Klebsiella* strains were responsible for 3% of all pathogenic epidemic outbreaks. It has a worldwide neonatal septicemia outbreaks including central Africa, and south Africa. However, in Ilorin, January and June 2000, about 300 cases of infection as a result of Klebsiella infection were recorded (Akanbi II, *et al.,* 2004),there making it a concern issue.

Pathogenic and zoonotic bacteria will cause infection in humans and animal including fish that consume or ingest it. Farms with no biosecurity measure tend to be exposing to this challenges which is the characteristics of the most farms in the study areas. Contamination of hands and surfaces during cleaning and evisceration of fish is a common route for pathogenic infection in process fish while, water, and soil are also sources of contamination in the fishery value chain. In raw fish, Diana and Manjulatha, (2012) reported 80% of Klebsiella infection in fish in India. Prior to this work, there no significant work on occurrence of Klebsiella spp.in fish in Nigeria. Since fish is capable of carrying the pathogens it is imperative to investigate it occurrence in fish if aquaculture would remain a profitable venture, hence the need for this study. The aim of the study is to examined the prevalence of Klebisiella species in fish tissues.

H_0= There is no significant different in the distribution of Klebsiella species among the fish tissues.

MATERIALS AND METHODS

The study was carried out in Oyo State, located in the South-West geopolitical zone of Nigeria. It lies within Latitudes $7^0$3'N and $9^0$12' N and longitudes $2^0$47' and $4^0$23'' (MANRRD, 2013). The two ecological-vegetation division (forest and derived savannah zones) of Oyo State was adopted for this study (MANRRD, 2013; OYSG, 2015). Forest zone comprises of 11 local government areas in Ibadan metropolis in the southern part of the state while derived savannah zone comprises 22 local government areas of Oyo, Ogbomosho, Saki and Ibarapa in the northern part of the state comprises of four agro-ecological zones (Ibadan/Ibarapa, Oyo, Ogbomosho and Saki zones) (Adeola, *et al.,* 2012). Farms in each zones were be sub-grouped into Large, Medium and Small scales using stratified methods as described by Fagbenro and Adebayo (2007). A total number of thirty-six (36) farms (3 Large, Medium and Small scales farms) were selected.

Fish organs collection

A total number of one hundred and eight (108) fish were collected [($3 \times 9 \times 4 = 108$) i.e No. of fish/farm x No. of farm/zone x No. of zones]. Therefore, forest (Ibadan/Ibarapa zone) had 27 fish ($3 \times 9 = 27$) and savannah (Oyo, Ogbomosho, Saki zones) had 81 fish ($3 \times 9 \times 3 = 81$). Seven (7) organs (liver, kidney, gill, intestine, muscle and spleen) were aseptically collected and weighed into sterile universal bottles while skin samples were collected using skin swab making a total of making seven hundred and fifty-six samples and subjected to *Klebsiella* isolation. Muscle was collected using sterile scissors after dissection to ensure no skin contact. Fish of body weight ranges between 300g to 1300g were used for this study as the group comprises of stage of fish that are consume by most individual or processed for economic value.

Isolation of *Klebsiella species*

The samples were brought to the laboratory and subjected to microbiological analysis under sterile conditions. Samples organs were nutrient broth. One gram of each samples were added to universal bottles containing 9ml of nutrient broth (Lab M) and incubated at 37^0C for 24 hours (USDA, 1990). The culture media were then streaked on Eosin Methylene Blue (EMB) (LAB M) prepared plates using wire loop and incubated for 24hrs. The wire loops were sterilized on spirit lamp before usage. The suspected colonies were sub-culture on EMB agar and incubated for 24 hours to obtain pure colonies. All the media were prepared according to manufacturer's instructions.

Confirmation tests for *Klebsiella* species isolates

Out of seven hundred and fifty-six (756) samples tested, only sixty-six samples were suspected to Klebsiella spp based on colony characteristics. The suspected colony were confirmed usinf biochemical characterization. Confirmation was by biochemical tests that include gram stain reaction, beta-haemolysis, catalase reaction, oxidase, motility (hanging method) at room temperature, sugar or carbohydrate fermentation (Lactose, Sructose and Mannitol) as described by Collee *et al.*, (2006).

Statistical analysis

The data will be analysed by descriptive statistics (percentage) and correlation using IBM Statistical Package for Social Science (SPSS) version 20.

RESULTS

The result of the biochemical tests confirmed that the suspected isolates were klebsiella species. All the 66 isolated were gram-negative, did not haemolysis sheep blood, catalase positive, oxidase negative, non-motile, and ferment lactose, sucrose and mannitol sugars (Table 1).

Table 2 shows that the distribution of klebsiella species by sex. Out 16 males from forest zone, 25% were positive, and 39.00% in savannah zone. Among the females, 18.18% and 42.50 were positive from forest and savannah zones respectively. Considering the total length, fish with total length of 41-50cm had occurrence of klebsiella species (33.33% and 36.59% in

Table 1: Biochemical characterization of the suspected of the suspected organisms

Tests	No. of organisms tested	No. of positive	No. of negative	Remarks
Gram stain	66	0	66	Showing a red colour
Haemolysis	66	0	66	No clear zone was observed which indicated no haemolysis
Catalase reaction	66	66	0	Shows sign of catalse formation
Oxidase reaction	66	0	66	No reaction
Motility	66	0	66	No movement of colony or individual
			Sugar test	
Lactose	66	66	0	Fermented but produced no H_2S
Sucrose	66	66	0	Fermentation occurred by change of colour from pink to yellow
Mannitol	66	66	0	Fermented and produced acid

Table 2: Occurrence of Klebsiella species by length, weight and sex

Parameter	No. of samples		No. of infection		Percentage		Intensity of infection	
	F	S	F	S	F	S	F	S
				SEX				
Male	16	41	4	16	25.00	39.02	0.25	0.39
Female	11	40	2	17	18.18	42.50	0.18	0.43
Total	27	81	6	33	22.22	40.74	0.22	0.41
				TOTAL LENGTH (cm)				
30-40	13	17	2	6	15.38	35.29	0.15	0.35
41-50	12	41	4	15	33.33	36.59	0.33	0.37
51-60	2	23	0	12	0.00	52.17	0.00	0.52
Total	27	81	6	33	22.22	40.74	0.22	0.41
				BODY WEIGHT (g)				
300-600	17	43	6	13	35.29	30.23	0.35	0.30
601-900	8	35	0	20	0.00	57.14	0.00	0.57
≥ 900	2	3	0	0	0.00	0.00	0.00	0.00
Total	27	81	6	33	22.22	40.74	0.22	0.41

Note: F = Forest zone, S = Derived savannah zone

Table 3: Distribution of Klebsiella species in fish by organs

	Forest zone				Derived savannah zone			
organ	No. of sample	No. of Positive	%	Intensity of infection	No. of sample	No. of Positive	%	Intensity of infection
Gill	27.00	3.00	11.11	0.11	81.00	12.00	14.81	0.15
Liver	27.00	3.00	11.11	0.11	81.00	6.00	7.41	0.07
Kidney	27.00	0.00	0.00	0.00	81.00	6.00	7.41	0.07
Intestine	27.00	0.00	0.00	0.00	81.00	9.00	11.11	0.11
Skin	27.00	3.00	11.11	0.11	81.00	6.00	7.41	0.07
Muscle	27.00	0.00	0.00	0.00	81.00	12.00	14.81	0.15
Spleen	27.00	0.00	0.00	0.00	81.00	6.00	7.41	0.07
Total	189.00	9.00	4.76	0.05	567.00	57.00	10.05	0.10
				Overall				
Gill	108	15	13.89	0.14				
Liver	108	9	8.33	0.08				
Kidney	108	6	5.56	0.05				
Intestine	108	9	8.33	0.08				
Skin	108	9	8.33	0.08				
Muscle	108	12	11.11	0.11				
Spleen	108	6	5.56	0.05				
Total	756	66	8.73	0.09				

forest and savannah zones respectively) while 51-60cm had 52.17% in savannah zone. Fish with 601-900g had 57.14% of klebsiella species in savannah zone and more that 35.20% in forest zone. The prevalence of klebsiella species among the fish organs were presented in table 3. Addition of number of infection in both forest and savannah areas shows that 66 out of 756 samples representing 8.73% was

Table 4: Correlation among the infection, sex, weight and length

		Infection	Sex	Weight	Length
Infection	Pearson Correlation	1	1.000**	.860	.779
	Sig. (2-tailed)		.	.341	.431
	N	8	2	3	3

positive for Klebsiella species. The result shows that skin, gill and liver had 11.11% occurrence in forest zone. In savannah zone muscle and gill (14.81%), intestine (11.11%) while liver, kidney and skin (7.41%) were observed. However, in overall comparison, 13.89% of the gills 8.33 of the liver, intestine and skin, 5.56% of the kidney and spleen, and 11.11% of the muscle were positive for Klebisella. Table 4 shows that the is strong positive and strong correlation among infection, weight and length but no correlation with respect to sex.

DISCUSSION

The high percentage recorded in this study in fish with 51.60cm suggests that the large size may promote infection as it covers more space. The higher space covered exposed the fish to more contact with the environment. Also, in with 601-900g suggest possibility of infection with length-weight relationship. Among the organs, higher percentage recorded in gill, skin and muscle suggest that these organs are the major organs that 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 Akanbi II *et al.,* (2004) 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.

The result of Takyi *et al.,* (2012) on *Clarias gariepinus* shows that liver of C. gariepinus had 8.3% of klebsiella species which similar to the result of this study. Thus, occurrence of this pathogenic bacterium of fish tissues *Clarias gariepinus* in Oyo State is of concern. (Ampofo and Clerk, 2010). Therefore from the result, the possibility that Klebsiella are comfortable in sampled fish farms in Oyo State occurs which may pose a threats to the health of the fish in the case of physiological and environment imbalance and consumers. Presently, there is no outbreak as a result of Klebsiella from fish yet but cases might be gone unreported. The isolation of bacteria species from the genus Enterobacterecease especially Klebsiella from the fishes suggests that these potential human pathogens are present on fish farms in Oyo State. The result further suggests that fish and fish products from these farms could pose serious health threat to humans when these bacteria are consumed in large quantities (Ampofo and Clerk, 2010). Farm workers usually handle fish and farm equipments with little or no biosecurity and care for their health may be sources of contamination and re-contamination.

CONCLUSION

The distribution of the klebsiella in fish in Oyo State shows that it is a problem present in fish tissues. Skin, gill and muscle are the major organs of klebsiella load in this study area and pose some threat because these organs are consumed by human. Effort to prevent, control and manage it occurrence in the fish should be development in the study

area to prevent outbreak.

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