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Susceptibility response of *Claria gariepinus* to infection with *Pseudomonas aeruginosa* and prophylactic treatment with chloramphenicol

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ABSTRACT: Susceptibility responses of cat fish – *Clarias gariepinus* to infection with *Pseudomonas aeruginosa* vis-à-vis treatment with chloramphenicol were investigated in the present study. Thirty healthy and physically active fishes weighing between 200-350g sourced from Challawa Gorge Dam, Karaye, Kano State were used for the study. These were divided into three aquarium tanks A, B, C (ten in each case). Fishes in A were infected with 1.0ml (about 1.2 x 10^6cfu/ml) of *Pseudomonas aeruginosa* pyrenterally through linea alba by ventral medial furrow. This was followed immediately with 0.5ml chloramphenicol (MIC 125µg/ml) solution. The dose was repeated twelve hourly for five days. Similarly, fishes in B were infected but no treatment with chloramphenicol. Fishes in C were injected with ringers solution only and these served as the control. At the end of the 21 day experimental period, a 70% mortality was observed in the infected and anti-microbially treated fishes of tank A in addition to generalized symptoms of poor feeding, sluggishness (loss of vigour) and skin depigmentation. However, there was 90.5% mortality in the infected but untreated fishes of tank B. In the control group, 10% mortality was observed which may be due to short comings in the husbandry techniques employed. It could be concluded therefore, that *Pseudomonas aeruginosa* is highly pathogenic to *Clarias gariepinus* and the bacteria showed lower sensitivity to one of the drugs of choice-chloramphenicol. It is thus recommended that anti-microbial should be screened against this infective bacteria in order to improve on the quality and yield of fish production.

Key words: Pathogenicity, Antimicrobial sensitivity, *Pseudomonas aerugionosa*, *Clarias gariepinus*, chloramphenicol.

Introduction

Clarias gaeirpinus is a cat fish found among fish communities in tropical fresh water. In terms of nutrition, Lowe (1975) described fish protein as one of the best especially in quality and even relative availability to consumers. This has generated an imputes toward improving production since the last three decades (Holk, 1967).

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However, one very important problem militating against profitable management of fish is that of epidemic. Fish like other animals, remain susceptible to microbial diseases especially bacterial and viral types (Schiewer, 1983). The susceptibility of fish to such diseases can be associated with their subjection to intensive culture practices.

Protection of fish against a number of important bacterial diseases by the use of specified anti-microbial could be a vital step forward in the quest for a rapid development in fish and fisheries industry. The use of chloramphenicol in this connection has been shown to be very efficacious (Lionel, 1972). Nitrofurans, sulphamera zines and the tetracyclines have also been very valuable in the control and prevention of fish diseases. Further research is however needed especially on the pathogenicity of infection with pseudomonas species, the main farmed fish species in Nigeria.

This study aims to evaluate the response of *Clarias gariepinus* to infection with *Pseudomonas aeruginosa* with a view to assessing the effect of treatment and prophyluxis with chloramphenicol on this very valuable farmed fish water species, such studies could improve the husbandry practices of this species and therefore result in intensification of their culture.

Materials and Method

Thirty apparently healthy adult *Clarias gariepinus* with initial mean weight of 200-350g were collected from Challawa George Dam in Turawa vollage of Karaye Local Government, Kano, Nigeria in the month of November, 1999.

The fishes were individually monitored to ensure that they were healthy and physically very active with respect to feeding, movement and responses to touch, light and temperature in rectangular glass aquarium tanks with dimension 62cm x 32cm x 32cm. Stocking rate adopted was ten fishes per tank. Fish were fed ad libitum with a standard diet made up of a cocktail of ingredient sourced from waste of agro-allied activities in the Kano area, as described by Adikwu (2001). Fish were acclimatised on this diet for two weeks and complete water change in the tanks was affected once every 3 days before the commencement of the infectivity trials.

Bacterial culture

The bacteria used to infect the fishes in this study was *Pseudomonas aeruginosa* (Pyocyanea). This pathogen was obtained from the Microbiology laboratory of the Microbiology Unit of the Department of Biological Sciences, Bayero University, Kano, Nigeria. The culture was resuscitated on nutrient agar (Oxoid) start at 37°C throughout the period of study. The cultural characteristic of the organism were confirmed in the same laboratory, following the methods described by Dodds and Gillies (1984), Jeans (1978) as well as Mackie and MacCartney (1989).

The bacteria was standardized before injecting into the fish from overnight broth culture in nutrient broth (oxoid) and diluting it with Ringers solution to achieve (1.2 x 106cfu/mol) bacteria count. This was arrived at by serial dilution and pour plate technique (Duguid, 1980).

Anti-microbial Agent Used

A standard concentration of chloramphenicol solution 50mg/ml (MIC 125µg/ml) was used as the antibiotic for chemoprophylactic assessment in this investigation.

Infection and Treatment Procedure of the Fishes

The thirty fishes were divided into three different aquarium tanks, A,B,C (ten in each case). All the fishes in tank A were injected with 1.0ml syringe through the ventral body wall at the point of the linea alba by the ventral medial furrow (Lagler, 1972). Treatment was followed immediately by injecting 0.5ml each of chloramphenicol solution. The dose was repeated twelve hourly for five days.

Similarly, the next 10 fishes of tank B were injected with the same quantity of *P. aeruginosa*. However, no treatment with chloramphenicol was given.

The fishes in tank C were injected with only 1ml of Ringers solution without bacteria or chloramphenicol. This serves as the control.

The behaviour of the fishes in all the three groups was monitored as regards to vigour, feeding habit, tendency towards mating, swimming and respiratory rate were monitored. Also morbidity, colour change of the skin and mortality rates were observed on a daily basis for a period of twenty eight days.

Results

The responses of the fishes to infection with *P. aeruginosa* and the corresponding chemprophylaxis with chloramphenicol are presented in Table 1. It was noted that 60% of the infected but treated *C. gariepinus* (group A) maintained their vigorous activity in the first three days. The same applied to feeding and respiration parameters. The skin colour was normal. However, six days latter, 40% of the fishes in this category became pale and later darken. Swimming activity was normal within the first three days. But at the end of the first 10 days, only 80% swam normally. Later, the percentage of normal swimmers after the 18^{th} day decreased to 30%. In all, the percentage mortality was observed to be 70%.

Group B, comprised of the fishes injected with pathogen but left untreated throughout the study period. In the first 49 hours of the infection, 90% of the fishes appeared sluggish and only 10% was active. The feeding behaviour was subnormal. respiratory rate was also abnormal when compared with the control groups. The skin colour darkened in all the fishes. Mortality occurred progressively: three in the first four days, then one in the seventh day, in the ninth day, another set of four fishes died. The eight fish died during the 17^{th} day of infection. However, the remaining two (20%) fishes were observed to recover from the infection but gradually. On the twenty-fifth day, the ninth fish died out. But the only surviving one has reverted to its full activity even after the experimental period.

The control group (C) were normal throughout the study period and even after. However, one fish (10%) died at the end of the 3^{rd} week. The mortality recorded in the control group could be due to shortcomings in husbandry techniques employed.

Table 1: Some observable behaviour and three weeks mortality rate in *Clarias gariepinus* on infection with *Pseudomonas aeruginosa* before and after treatment with chloramphenicol.

Group	Vigour	Feeding	Respiration	Swimming Activity	Skin Colour	Mortality Rate (%)
A	60% were active	40% feed normally	60% respire normally	Initially normal, but later only 70% could swim	Normal but later became darkened	70%
B	90% became sluggish, only 10% active	Only 20% feed normally	Respiratory rate fell below normal	Very slow and later failed to swim	Changed from light to dark	90%
C	100% active	Normal	Normal	Normal	Normal	10%

Groups: A. Group of 10 *C. gariepinus* infected with *P. aeruginosa* but treated with chloramphenicol.
B. Group of 10 *C. gariepinus* infected with *P. aeruginosa* but untreated.
C. Control group.

Discussion

Chloramphenicol used to be the recommended drug that was used as an additive in the preparation of fish feeds. However, evidences yielded that many pathogens including *P. aeruginosa* arecurrently showing resistance tendency towards this very important anti-bacterial agent (Mukhtar, 1998).

In the present study, it was shown that even though the fishes were treated with a repeated dose regmen of chloramphenicol, 70% of them died probably due to methods and place of keeping them. Thus when compared with the control, there was 30% cure. In terms of infectivity rate, the findings demonstrated that up to 100% of the test animals have shown a full blown pathological responses which led to a mortality rate of 90%. It therefore appears that the infectivity of *P. aeruginosa* in *C. gariepinus* is very high.

Many workers (Jean, 1978; Dodds and Grillies, 1984, Duguid, 1989, Grivan, 1989 and Mukhtar, 1998) have ascerted that *P. aeruginosa* (pyocycanea) is a hardy organism that has developed intrinsic resistance to many antimicrobials, chloramphenicol inclusive. This is thus a key to explain why there was a poor recovery in *C. gariepinus* after being laboratory infected and then followed by chemoprophylaxis using chlorophenicol. The degree of damage by this agent to the fish haematological parameters could also have a possible contribution to such an observed effect. This aspect was not however assessed in this research.

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References

Adikwu, A.I. (2001). Biological nutritive evaluation of Sorghum bran in the African catfish – *Clarias gariepinus* (Burchell, 1822). African Journal of Applied Zoology and Environmental Biology. 3: 5 – 12.

Duguid, G.L. (1989). Disease of fresh water fishes caused by Bacteria of genera *Aeromonas pseudomonas* and vibrio. USFWSOW of Cultural Method Res. FDL 40, Washinbgton D.C., GPO, pp. 4 – 8.

Gillies, R.R. and Dodds, A.P. (1984). bacteriology illustrated, 5th Edition. Churchill Livingstone, New York, pp. 2, 89.

Govan, P.L. (1989). Routine haematological method for use with fish blood. J. Fish Biology, 5, 771 – 781.

Holk, M. (1967). Medical Laboratory Mannual for Tropical Countries, vol. II, Microbiology Tropical Health Tech. The Ford Press, London.

Jeans, W.A. (1978). Fish as potential vector of human bacterial. Diseases Special Publication. American Fish Society, No. 5, pp. 284 – 290.

Lagler, I. (1972). Parasites, infections and diseases of fish in Africa cifa. tech. paper 7, pp. 216.

Lionel, E. mandesley (1972). Disease of fish. Symposia of Zoological Society of London, pp. 1-10. Academic press, London.

Lowe, D.R. (1975). Studies on viral disease of Japan fishes infectious pancrenic Neurosis of rainbow trout fish isolated from epizootics in Japan. Bull. Japan Society of Scientist Fish. 223 – 229.

Mackie, R.B. and Maccartney, D.H. (1989). Some blood parameters of the rain bow tout (Salmogairnineri) I Kamploops variety. J. Fish Biol. 3, 1 – 8.

Schiewer, B.F. (1983). Remarks on some facets of Epizoology of Bacteria. Fish Disease Devs. in Microbial. 9: 97 – 100.

Mukhtar, M.D. (1998). Screening of drinking water sources of Kano State for antimicrobial resistant enterobacteria and pseudomonas aerugi. XXVth Annual Regional Conference of the West African Society for Pharmacology, 20-24th Oct., 1998. ABU, Zaria, CHE 12, pp. 69.