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Evaluation of pathogenicity of motile *Aeromonas* species in African catfish

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Abstract
This study was conducted to evaluate the ability of motile *Aeromonas* species (MAS) to induce skin lesions and cause mortality in African catfish (ACF). Eight motile *Aeromonas* isolates (*A. hydrophila* – 4 isolates, *A. caviae* – 2 isolates and *A. sobria* – 2 isolates) were used for the study. Ninety apparently-healthy ACF juveniles were randomly assigned into 9 groups of 10 fish each. Fish in groups 1 – 4 were infected with *A. hydrophila* while those in groups 5 – 6 and 7 – 8 were infected with *A. caviae* and *A. sobria*, respectively. Infection was achieved by immersing fish in water containing $1x10^8$ colony forming units (cfu)/ml of experimental isolate. Fish in group 9 were immersed in water containing sterile phosphate buffered saline (PBS) and served as the control. Fish in the infected and control groups were monitored daily for 15 days for signs of ill health including development of skin lesions and mortality. Skin lesions were processed for re-isolation of MAS. Skin lesions were found in 40 – 90% of experimentally-infected fish while none of the uninfected had skin lesion. Mortality rate in infected fish ranged from 20 – 90% while none of the uninfected died. Development of skin lesions as well as mortality in the ACF was associated with experimental infection with *Aeromonas* isolates. This study has shown that MAS could serve as the primary cause of skin lesions in cultured ACF.

Keywords: Motile *Aeromonas* species, African catfish, Pathogenicity, Nigeria.

1. Introduction
African catfish (ACF) also called African sharptooth catfish, *Clarias gariepinus* (Burchell) is an economically important fish species in West African countries including Nigeria [1]. Aquaculture in Nigeria, particularly in the Southeastern region, is dominated by ACF production. This dominance is related to the aquaculture attributes of the ACF which include adaptability to tropical environment, suitability for monoculture and polyculture with other freshwater fish species, ability to withstand handling stress, disease resistance, high fecundity, high weight gain, palatability and nutritional quality [1, 2, 3]. It is also partly due to cost and bias associated with other animal protein sources [4, 5]. The need to pursue and advance production techniques for ACF is increasing in response to its demand for human consumption, ornamental fish trade, and to support restocking schemes for conservation or sport [6].
African catfish are teleosts with their entire body surfaces, fins and barbells covered with skin made up of living non-keratinized stratified squamous epithelial cells [7, 8]. Skin is the organ of interaction with the environment and it plays crucial homeostatic role which include protection, sensory perception, communication, excretion, locomotion, osmoregulation, respiration, thermoregulation and antimicrobial activity [8, 9, 10]. It serves as the first site of attachment for a plethora of microorganisms in the aquatic environment [8, 10]. Attachment of microorganisms to fish skin often induces skin lesions which, irrespective of the size, results in colonization by many opportunistic pathogens, life-threatening osmotic stress, increased energy costs from locomotion due to impairment of mucus production, swimming imbalance, increased predation due to colour change and deficiency in oxygen uptake [11, 12, 13, 14, 15]. Thus, skin lesions adversely affect performance and productivity of the affected fish.
Infectious diseases caused by aerobic bacteria are responsible for substantial financial loss to fish farmers worldwide [3]. Amongst the aerobic bacterial organisms that impede African catfish production, motile *Aeromonas* species (MAS) namely *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* have been reported to be indigenous

(authochtonous) in the aquatic environment [16], hence they are associated with the fish as normal commensal flora [16, 17]. However, studies have shown that MAS are associated with skin condition (in both wild and cultured fish) called motile aeromonad septicaemia also known as red sore, red pest, haemorrhagic septicaemia or epizootic ulcerative disease (EUD) or syndrome (EUS) [18, 19, 20]. This disease is of economic importance because it mainly affects young fish and accounts for epidemic outbreaks which results to massive mortality in different parts of the world [20, 21]. Nevertheless, MAS have been associated with skin lesions in naturally-diseased fish species cultured in different parts of the world [3, 20, 22, 23, 24, 25], including African catfish cultured in Bangladesh [18], India [26], Malaysia [27] and Nigeria [28, 29]. In these studies, the MAS were isolated with or without other pathogens from the skin lesions. Motile *Aeromonas* species have been suggested to be secondary invaders of skin injury that resulted from physical causes (such as mishandling and cannibalism), toxins, non-specific toxaemias/septicaemias or from other skin infections [8, 17, 30]. Thus, the role of MAS as primary cause of skin lesions in cultured fish is still controversial [31, 32, 33].

In nature, there is a wide range of susceptibility to infectious organisms between fish species, a fact that has been confirmed experimentally [6, 34]. Experimental studies conducted to establish the role of MAS as primary cause of skin lesions in fish were done using fish species cultured in different parts of the world such as America [35], Asia [30, 36] and Europe [31, 32]. It is well recognized that susceptibility to disease is not a fixed parameter and is influenced by a complex series of interactions involving the host, pathogen and environmental factors [6, 34]. No study has investigated the role of MAS as the primary cause of skin lesions in African catfish. This study therefore investigated the effect of experimental infection with MAS on the development of skin lesion and mortality in African catfish.

2. Materials and methods

2.1 Handling of experimental fish

Ethical clearance and valid approval were obtained from the University of Nigeria, Nsukka Ethics Committee for Medical and Scientific Research (MSR) before the commencement of the experiment. Fish used in this study were handled according to the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* (CCAC *Guide)* [37].

2.2 Bacterial cultures and preparation of inocula

Stocked cultures of *A. hydrophila*, *A. caviae* and *A. sobria* isolated from skin lesions of naturally-infected ACF cultured in Southeast Nigeria [29] were used for the study. The isolates were sub-cultured on nutrient agar, incubated at 37 ^{o}C for 24 hours and their identity confirmed using biochemical characteristics. Colonies were homogenized in sterile phosphate buffered saline (PBS) and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard equivalent to $1x10^{8}$ cfu/ml.

2.3 Experimental infection

One hundred healthy 8-week-old ACF post-fingerlings obtained from a reputable commercial fish farm were used for the study. After acclimatization for 7 days, 90 juvenile catfish were randomly assigned to 9 groups of 10 fish per group. Eight groups were infected by immersing fish in water containing $1x10^{8}$cfu/ml of the isolates at the rate of 1ml/L as follows: groups 1 – 4 - *A. hydrophila*, groups 5 and 6 - *A. caviae*, while groups 7 and 8 were infected with *A. sobria*. Fish in group 9 was similarly inoculated with 1ml/L of sterile PBS and served as the uninfected control group. The fish were not fed throughout the period of the experiment to ensure that the water was not further contaminated [30, 38], and 50% of the water was replaced at 24 hour interval to further ensure good water quality [3, 36]. The ammonia, pH and oxygen concentration of the water was kept at acceptable limit during the course of the study [39].

The experimentally-infected fish were observed daily for 15 days. Clinical signs, skin lesions, mortalities, and the nature of water were noted and recorded. Skin lesions from experimentally-infected fish were processed for re-isolation of infective bacterial isolates. Positive culture was confirmed if the morphology and biochemical characteristics of the re-isolated strain were identical with those of the isolates used in the experimental infection. No colony growth after 48 hours incubation was regarded as negative culture.

2.4 Statistical analysis

The data collected for rates of induction of skin lesions and mortality were subjected to descriptive statistics and expressed in percentages.

3. Results

3.1 Effect of experimental infection on development of skin lesion

By day one post-infection (p.i.), fish in all the infected groups showed signs of sluggish, "head-up-tail-down" movement and constant rubbing of body against the tank. Increased water turbidity in all the infected groups was also observed from day one post-infection. In all the infected groups, some of the fish became very pale (discoloured from normal grayish to pinkish) with hyperaemic spots at the base and tips of the fins (Figure 1) with or without skin lesions. Extensively distributed haemorrhagic skin ulcers were observed at day 4 p.i. in groups 1, 2 and 3 (Figure 2). In these groups, there were also severe hyperaemic patches of the fins. By day 5 p.i., erosive lesions were observed on the head, fins and tail of some of the fish in groups 5 and 6 (Figure 3). At day 6 p.i., extensive widely distributed erosive lesions occurred in fish in groups 5, 6, 7 and 8. Atrophy (rotting) of the barbells was observed in fish in all the infected groups (Figure 2) except group 4. None of these clinical signs were observed in fish in the control group. Bacterial agents recovered from the skin lesions of experimentally-infected fish were found to be same as those used in the experimental infection.

Fig 1: Skin discolouration and hyperaemic spots (arrows) in African catfish experimentally-infected with motile *Aeromonas* species

Fig 2: Haemorrhagic ulcerative skin lesions (arrows) and fin rot (arrow heads) in African catfish experimentally-infected with motile *Aeromonas* species

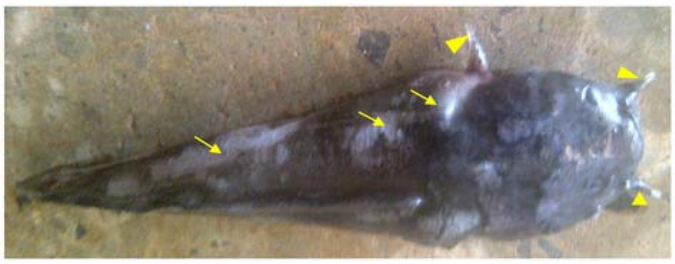

Fig 3: Erosive skin lesions (arrows), barbells and fins atrophy (rotting) (arrow heads) in African catfish experimentally-infected with motile *Aeromonas* species

By day 15 p.i. (end of experiment), 90% of fish in group 4 had skin lesion as against 60% in groups 1, 2 and 3. Fifty percent of those in groups 7 and 8 developed skin lesion, 40% had skin lesions in groups 5 and 6 while none in the control group had skin lesion (Table 1).

Table 1: Number of fish with skin lesions following experimental infection with *Aeromonas* species

Group	Cummulative number of fish with skin lesions at days post-infection																Percentage
	Number of fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	10	0	0	0	1	1	1	1	1	1	2	2	2	3	4	6	60
2	10	0	0	0	1	1	1	1	1	2	3	4	4	5	6	6	60
3	10	0	0	0	1	1	1	1	1	2	2	2	6	6	6	6	60
4	10	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	90
5	10	0	0	0	0	2	2	2	3	3	3	3	3	4	4	4	40
6	10	0	0	0	0	2	2	2	2	3	3	3	4	4	4	4	40
7	10	0	0	0	0	0	2	2	2	2	3	4	4	5	5	5	50
8	10	0	0	0	0	0	2	2	3	3	4	4	4	5	5	5	50
9	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Groups 1 – 4 – infected with *A. hydrophila*; Groups 5 and 6 – infected with *A. caviae*; Groups 7 and 8 – infected with *A. sobria*; Group 9 - control

3.2 Effect of motile *Aeromonas* species on fish mortality

By day 3 p.i., 9 fish with extensively distributed haemorrhagic skin ulcers (Figure 2) died in group 4. By 15 days post-infection (dpi) (end of experiment), the cumulative mortality rates recorded were 90% (group 4), 40% (group 1), 30% (groups 2, 3, 5, 7 and 8) and 0% (group 9) (Table 2).

Table 2: Mortality rate following experimental infection with *Aeromonas* species

Group	Cummulative mortality at days post-infection																Percent mortality
	Number of fish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	10	0	0	0	0	0	0	0	0	1	1	2	2	3	4	4	40
2	10	0	0	0	0	1	1	1	1	2	3	3	3	3	3	3	30
3	10	0	0	0	1	1	1	1	1	2	2	2	2	3	3	3	30
4	10	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	90
5	10	0	0	0	0	2	2	2	3	3	3	3	3	3	3	3	30
6	10	0	0	0	0	0	0	0	1	2	2	2	2	2	2	2	20
7	10	0	0	0	0	0	0	1	1	1	1	1	1	3	3	3	30
8	10	0	0	0	0	0	0	0	0	3	3	3	3	3	3	3	30
9	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Groups 1 – 4 – infected with *A. hydrophila*; Groups 5 and 6 – infected with *A. caviae*; Groups 7 and 8 – infected with *A. sobria*; Group 9 - control

4. Discussion

The rubbing of the body against the tank walls by the infected fishes observed by day 1 p.i. suggests that there was skin irritation following attachment and colonization by the infective organisms [20]. Yarmidici and Aydin [17] also observed body rubbing against tank walls in Nile tilapia infected with *A. hydrophila* isolate after 8 hours of infection. The fact that fish in all the MAS-infected groups were observed to rub their bodies against the tank walls suggests that all the *Aeromonas* strains used in this study attached to the African catfish skin and established infection at the same rate and time interval. The incubation period of MAS infection depend on fish species and resistance, environmental conditions and the season [17]. The incubation period of MAS-associated infections varies between 2-4 days in natural infections and 8-48 hours in experimental infection models [17, 40, 41]. In the present study, clinical signs started manifesting day 1 p.i. which is within the range indicated for experimental infection [17, 41]. Increased water turbidity observed by day 2 p.i. may be due to excessive skin mucus secretion by the infected fish

following attachment of the organisms. Attachment of pathogens to fish skin results in increased epidermal mucus production – a mechanism employed to wash-off adhered pathogens [8]. The increased water turbidity could also have resulted from excessive mucus secreted from the skin and gastrointestinal tract of the infected catfish [13]. *Aeromonas* strains have been reported to produce cytotoxins and enterotoxins which destroy mucosal lining of the skin and gastrointestinal tracts resulting in excessive release of mucus [16, 38, 42]. Experimental studies in other fish species reported defecation of mucus by fish infected with MAS [17]. The fact that the increased water turbidity was observed in all the infected groups, suggest that all the *Aeromonas* strains established infection in the African catfish. However, blood released from haemorrhagic ulcerative skin lesions by day 2 p.i. could also have contributed to increased water turbidity observed. As the water turbidity was increasing, there was every likelihood that the water quality would have been reduced, hence the need for removal and change of 50% of the water at 24 hour interval [3, 36].

Discolouration of some of the fish from normal grey colour to pinkish could be as a result of depigmentation of the skin melanophores and/or chromatophores [13]. In Czech Republic, Rehulka [31] reported depigmentation of skin in Rainbow trout experimentally-infected with *A. sobria* and *A. caviae*. Loss of skin colour was reported in Asian stinging catfish experimentally-infected with *A. hydrophila* [43]. Paleness of gills in Rainbow trout experimentally-challenged with MAS was related to anaemia [31]; and the skin and gills of catfish (teleosts) are histologically similar [8]. The fact that discoloured fish were observed in all the infected groups further suggests that all the *Aeromonas* strains established infection in the African catfish.

The erosive and heamorrhagic ulcerative skin lesions observed in the present study have also been reported in experimental studies with MAS in catfish species in other parts of the world by earlier investigators [19, 43]. These skin lesions were similar to those reported in naturally-diseased catfish species [3, 15, 28, 29]. The findings in this study as well as those of Angka [43] and Islam *et al*. [19] indicate that MAS could be the primary cause of skin lesions observed in naturally-infected catfish species. Cytotoxin produced by these *Aeromonas* species could be responsible for the induction of skin lesions [16, 38].

In this study, the hyperaemic spots observed at the base and tips of fins in some of the infected fish by day 2 p.i. have also been reported at day 1 p.i. in Nile tilapia [17] and Indian catfish [3] experimentally-infected with *A. hydrophila*. Rehulka [31] noted hyperaemic zones in Rainbow trout infected i/p with *A. caviae* and *A. sobria*. In Switzerland, Wahli *et al*. [32] also observed similar lesions in cultured Perch experimentally-infected with *A. sobria*. In natural infections, hyperaemic spots on fins were observed at the acute stages of motile aeromonad septicaemia in common carps cultured in East India [20]. Thus, this clinical finding in the present study indicates that all the *Aeromonas* strains used caused hyperaemic skin spots in the catfish. Atrophy (rotting) of the barbells and fins observed in this study has also been reported in naturally-diseased catfish cultured in East India [20]. Yarmidici and Aydin [17] observed fin rot in Nile tilapia infected with $1x10^8$cfu/ml of *A. hydrophila* at 3 days post-infection. Wahli *et al*. [32] observed lateral fin rot in cultured Perch infected with *A. sobria*.

The highest percentage (90%) of fish with skin lesion observed in group 4, suggests that *A. hydrophila* strain used to infect the group may be more pathogenic than the other strains and species used in the other groups. Some authors reported that *A. hydrophila* is the most pathogenic among the three MAS, being the most frequently isolated species from naturally-diseased fish [16, 22, 44]. Higher percentage of fish with skin lesion in the *A. sobria* groups (50%) compared with the *A. caviae*-infected groups (40%) indicates that *A. sobria* had a more damaging effects on fish skin than *A. caviae*. The variation in the rate of induction of skin lesion by these *Aeromonas* species may also be related to the strains used in this study.

The increase in fish mortality observed when the infected groups were compared against the control further suggests that the *Aeromonas* strains were pathogenic to the African catfish. It may also have resulted from septicaemia/toxaemia caused by the infective and invading *Aeromonas* species [33]. The cumulative mortality rate recorded in the *A. hydrophila*-infected groups ranges from 30-90%. This rate was higher than that recorded in the *A. caviae*-infected (20-30%) and *A. sobria*-infected (30%) groups. This finding further suggests that the *A. hydrophila* strains used in this study were more pathogenic than the other species. The highest mortality rate (90%) recorded in group 4 when compared with the other groups further supports the fact that the *A. hydrophila* strain is very virulent. In Bangladesh, Sarkar and Rashid [36] reported 100% and 60-80% mortality at day 15 p.i. in Catfish (*Heteropneustes fossilis* and *Clarias batrachus*), Carps (*Labeo rohita*, *Catla catla* and *Cirrhinus cirrhosus*) and Perch (*Anabas testudineus*) infected with $6.7x10^7$ and $6.7x10^6$cfu/ml of *A. hydrophila*, respectively. Hossain *et al*. [45] reported 100% and 40% mortality by 4-9 dpi when Climbing perch was challenged i/m with $9.2x10^7$ and $9.2x10^6$cfu/fish of *A. hydrophila* isolate, respectively. The variation in the pattern and mortality rates may be related to the species of fish challenged, immune status of the fish, strain of *Aeromonas* species, experimental conditions, dose of the infective pathogen given, route of administration of the pathogen and duration of the experiment. In this study, immersion challenge was done using $1x10^8$cfu/ml of each of the *Aeromonas* strain at 25 ^oC and observed for 15 days. The mortality may have resulted from the alteration of homeostasis of the fish consequent upon skin damage [13]. Skin lesion affecting as little as 10% body surface area of fish can result in very high (near 50%) acute mortality [11].

5. Conclusion

The MAS used in this study were able to induce skin lesions and cause mortality in experimentally-infected African catfish cultured in Southeast, Nigeria. This indicates that MAS are potential primary causes of skin lesions in this species of catfish.

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