# AN ASSESSMENT OF THE PATHOGENICITY OF *Edwardsiella tarda* AND TREATMENT OF EXPERIMENTAL EDWARDSIELLOSIS IN CULTURED *Clarias gariepinus* (CUVIER 1822)

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**ABSTRACT**
*The pathogenicity of Edwardsiella tarda isolated from pond water, and chemotherapeutic control of experimental edwardsiellosis in cultured Clarias gariepinus were investigated. The E. tarda isolate was identified based on colony and cellular morphology, as well as biochemical characteristics. A total of 120 fish were randomly assigned to four groups (A, B, C and D) of 30 fish, each comprising of 15 fish replicated once. Fish in groups A, B and C were infected intra-peritoneally with $1.02x10^6$ cfu of E tarda, while group D was not infected. Following observation of clinical signs, group A was treated with ciprofloxacin by 1hour immersion at 25mg/litre of water and group B orally at 100mg/kg body weight of fish for five days. Fish in group (C) were not treated with ciprofloxacin. Fish were observed for mortality daily, and cumulative mortality was compared between groups using ANOVA and Duncan Multiple Range Test. Cumulative mortality of 56.66% observed in group C was significantly higher ($p < 0.05$) than in groups A and B treated via oral (3.33%) and immersion (3.33%) route respectively. Infected fish were effectively treated by both routes of ciprofloxacin application, but the oral route is considered less stressful for the sick fish.*
**Keywords:** Pathogenicity, Ciprofloxacin, *Edwardsiella tarda*, edwardsiellosis, *Clarias gariepinus*.

## INTRODUCTION

The African catfish species, especially *Clarias gariepinus* remains the predominantly farmed species in Nigeria (Salami *et al.* 1993; Adeyemo *et al.* 1994 and Atanda, 2009), and the massive adoption of intensive rearing systems including the water recirculating and the water flow-through production systems has further enhanced productivity, promoting its culture to a full fledge industry in the country (Jamu and Ayinla, 2004). However, catfish farmers are faced with the challenges of high mortality due to epizootics which devastate stocks with little or no respite, since health-care is not readily available (Oladosu *et al.*, 2011). In many cases, epizootics are rarely investigated and farmers are quick to apply 'medication' ranging from topical bath with common salt and formalin, to antibiotic application in ponds with no regard for proper diagnosis, antibiotic sensitivity or dosage (unpublished). All these are in the bid to salvage the huge investment already committed to production. Studies have shown that epizootics in farmed catfish in Nigeria are due to high parasitic infestation, especially with monogenetic trematodes and *Trichodina spp*. (Obiekezie and Taege, 1991; Ekanem and Obiekezie, 1994; Oladosu *et al.*, 2011), and infection due to bacterial and fungal organisms (Olufemi, 1985; Ibiwoye *et al.*, 1989; Olufemi *et al.*, 1991; Oladosu *et al.*, 1994a,b).

Edwardsiellosis, a septicaemic disease characterized by severe lesions in the skin, muscles and internal organs of several commercially important species (El-Jakee *et al.*, 2008; Noga, 2010) is yet to be officially reported in Nigeria but the causal agent, *Edwardsiella tarda* was isolated in pond water (unreported) in a recent survey of pathogenic diseases of cultured catfish in Nigeria (Competitive Agriculture Research Grant Scheme of the Agriculture Research Council of Nigeria; RFA 2, 29). There is therefore the possibility that there had been unnoticed incidences of edwardsiellosis in cultured fish in Nigeria, especially when disease incidence in cultured fish is rarely investigated. The fact that *Edwardsiella tarda* infection has been observed in human cases of gastroenteritis, meningitis, nephritis and liver and skin abscesses

(Pastor, 1981; Nettles and Sexton, 1997; Pavanelli *et al.*, 1998; Manchanda *et al.*, 2006; Noga, 2010) necessitated the study on the characterization and pathogenicity of the isolate, as well as its possible treatment option in cultured fish.

## MATERIALS AND METHODS

### Characterization and identification of isolate

A stocked culture of *Edwarsiella tarda* originally isolated from pond water was sub-cultured on trypticase soy agar (TSA) and incubated at 37° C for 48 hrs. The size, colour and shape of colonies as well as the morphology and Gram staining reaction of the cells were observed. Biochemical tests including catalase, oxidase, urease, indole and methyl red as well as sugar fermentation tests were also carried out on the isolate, using the 24hr culture in trypticase soy broth (TSB). Motility of the bacterial cells was observed via the hanging drop method, using the 24hr broth culture.

### Experimental infection of *Clarias gariepinus*

One hundred and twenty apparently healthy *Clarias gariepinus* juveniles were purchased from a commercial farm in Ibadan Nigeria. They were acclimatized for two weeks in four groups (A, B, C and D) of 15 fish each, replicated once. Each group was held in forty liters (40 L) of water contained in 50 L plastic troughs arranged in a flow-through system. Three replicate groups (A, B and C) were thereafter infected intra-peritoneally (under the pectoral fin) with 0.25 ml of bacterial suspension in normal saline ($1.02 \times 10^6$ CFU of organism; McFarland standard No. 0.5). The fourth replicate group (positive control) was not infected. The fish were then daily observed for clinical signs, pathological lesions and mortality.

### Medication Trial

Commercial antibiotics sensitivity discs were used to determine the antibiogram of the test isolate. Following the observation of clinical signs and mortality in infected fish, treatment with the antibiotic to which the isolate was best sensitive commenced via oral and immersion application in two separate groups. In the orally medicated group (B), fish biomass was calculated and used to determine the daily feed ration (3% biomass). Ciprofloxacin (Ciproplan$^{R}$; oral solution, Agraphan-Farma Ca BV) was sprayed evenly on the feed at a dosage of 100 mg/kg body weight (Gang-Joon and Jeong-Joo, 1997), using vegetable oil as the carrier (at a rate of 15 ml oil per kg of feed). The medicated feed was allowed to dry and fed to the fish twice daily for five days. Treatment by immersion was done in 40 L of water containing 25 mg/L of ciprofloxacin (Oladele *et al.*, 2011). Fish group treated by immersion (A) was transferred into the antibiotics solution for one hour exposure period, after which they were returned to antibiotic-free water and fed with non-medicated diet.

The positive control group (D) was neither infected nor medicated, but fed on non-medicated diet twice daily, while the negative control group (C) was infected but not medicated, receiving daily diet of non-medicated feed, twice also. The cumulative mortality was statistically compared between treatments using ANOVA and Duncan Multiple Range test.

## RESULTS

The isolate produced small, grey, circular, entire edged and transparent colonies on TSA. Microscopic examination of Gram stained smear of the isolate revealed Gram negative short rods. As shown in Table 1, the organism was observed to be motile, indole, catalase and methyl red positive but urease, oxidase and Voger-Proskauer negative. There was fermentation of glucose with gas formation but the organism is lactose, mannitol and sucrose negative, while hydrogen sulphide is produced in triple sugar iron agar.

**Table 1: Some morphological and biochemical characteristics of *E tarda* isolated from pond water**

| Characteristics | Observations |
|---|---|
| **Colonial morphology** | Small, grey coloured, circular, entire edged and transparent. |
| **Cellular morphology** | Short rods |
| **Gram staining** | -ve |
| **Motility** | +ve |
| **Production of $H_2S$** | +ve |
| **Indole** | +ve |
| **Urease** | -ve |
| **Catalase** | +ve |
| **Oxidase** | -ve |
| **Gas in Glucose** | +ve |
| **Lactose** | -ve |
| **Mannitol** | -ve |
| **Sucrose** | -ve |
| **Methyl red** | +ve |
| **Voger-Proskauer** | -ve |

+ve = Positive; -ve = Negative

Results of antibiogram (Table 2) showed that the *E. tarda* isolate was sensitive to ciprofloxacin, ofloxacin, norfloxacin and gentamycin. Sensitivity was highest with ciprofloxacin, while the isolate was resistant to tetracycline, ampicillin and cefuroxime.

**Table 2: Sensitivity of *E. tarda* isolate to antibiotics**

| Replicates | Antibiotics | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NB 10mg | AX 20mg | OF 5mg | C 10mg | CF 30mg | AM 25mg | G 10mg | N 100mg | CIP 5mg | TE 50mg |
| 1 | 24 | 15 | 28 | 5 | R | R | 10 | 15 | 31 | 2 |
| 2 | 22 | 12 | 26 | 2 | R | R | 18 | 12 | 30 | R |
| 3 | 24 | 10 | 28 | 4 | R | R | 22 | 12 | 29 | R |
| Mean | 23.33 | 12.33 | 27.33 | 3.66 | R | R | 16.66 | 13 | 30 | R |

All values in all replicates are in millimeter.

NB =Norfloxacin; AX = Amoxycilin; OF =Ofloxacin; C = Chloramphenicol; CF = Cefuroxime; AM =Ampiclox; G = Gentamycin; N =Nitrofurantoin; CIP = Ciprofloxacin; TE = Tetracycline.

Clinical disease was observed in fish experimentally infected with the isolate two days post infection, and clinical signs including abdominal dropsy, generalized skin necrosis, with fish hanging vertically in water column (Fig. 1) were prominent. Postmortem examination revealed haemorrhagic enteritis, malodorous liquifactive necrosis of the viscera, with the accumulation of blood tinged fluid (Fig. 2).

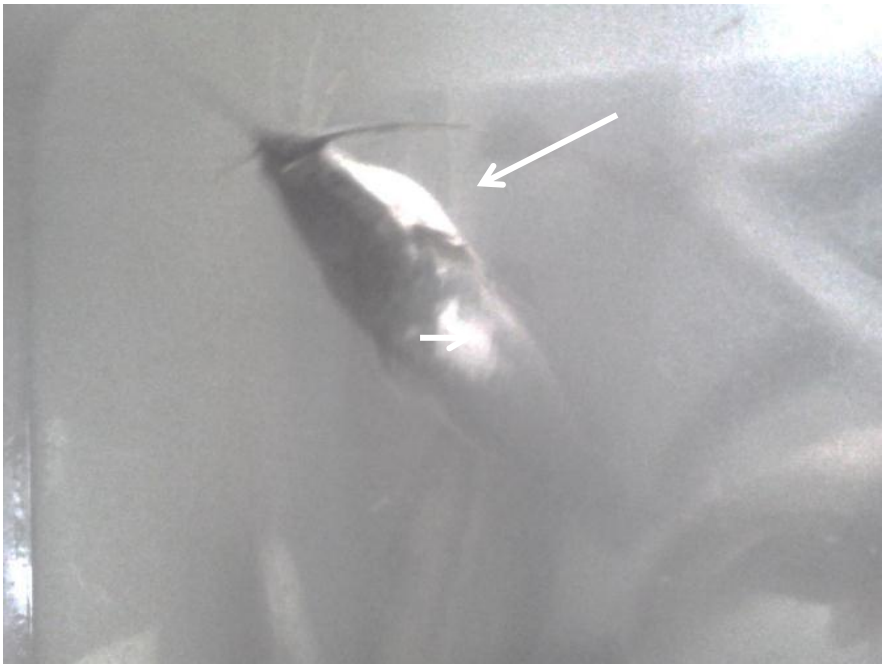

**Fig.1: Experimentally infected, sick catfish hanging vertically in water with dermal lesion (short arrow) and ascites (long arrow).**

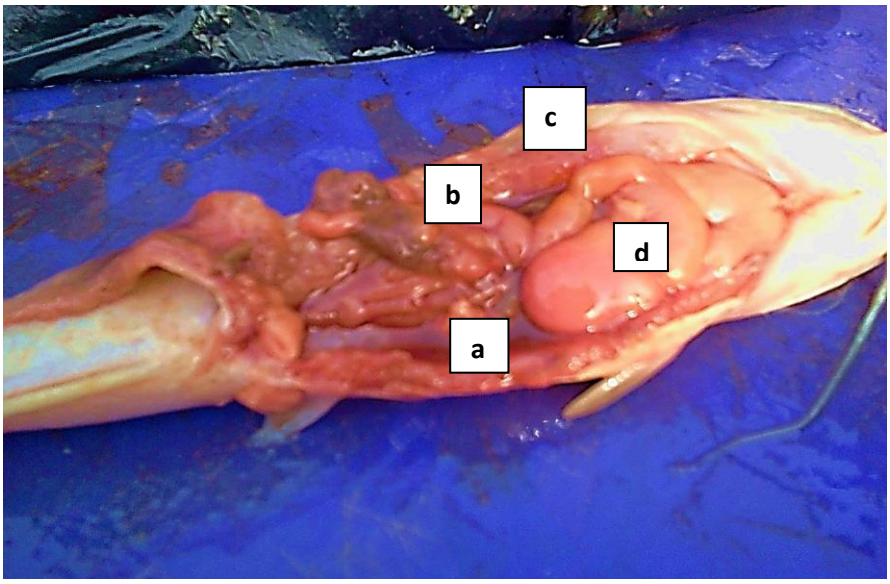


**Fig. 2: Accumulation of blood tinged fluid in the peritoneal cavity (a) with liquifactive necrosis of the liver (b), haemorrhages in the musculature (c) and hyperaemia of the wall of the stomach (d).**

Sick fish were however observed to feed actively, showing no sign of anorexia. As shown in Table 3, cumulative mortality was significantly highest ($p < 0.05$) in fish group that were infected but not treated with ciprofloxacin, reaching an average of 56.66% within seven days post infection. The cumulative mortality in the groups treated with ciprofloxacin by oral and bath application was observed to be 3.33% for both, and hence were not significantly different ($p > 0.05$). However, no mortality was recorded in the group that was neither infected nor medicated (negative control).

**Table 3: Cumulative mortality observed in experimentally challenged fish**

| Groups | Replicates | Number of fish | Mortality | % Mortality |
|---|---|---|---|---|
| **A (Immersion Ciprofloxacin)** | 1 | 15 | 1.0 | 6.66 |
| | 2 | 15 | 0 | 0 |
| | Mean | 15 | 0.5 | $3.33_a$ |
| **B (Oral Ciprofloxacin)** | 1 | 15 | 1.0 | 6.66 |
| | 2 | 15 | 0 | 0 |
| | Mean | 15 | 0.5 | $3.33_a$ |
| **C (Negative control)** | 1 | 15 | 9 | 60.0 |
| | 2 | 15 | 8 | 53.33 |
| | Mean | 15 | 8.5 | $56.66_b$ |
| **D (Positive control)** | 1 | 15 | 0 | 0 |
| | 2 | 15 | 0 | 0 |
| | Mean | 15 | 0 | $0_a$ |

*Values in the same column and with the same subscript are not significantly different ($p>0.05$).

Water quality parameters observed are within acceptable limits (Table 4), as fish were reared under the continuous water flow through rearing system.

**Table 4: Physico-chemical parameters of water in experimental facility**

| Experimental Groups | Replicates | Water quality parameters Temp. $^oC$ | D.O mg/L | pH | $NO_{2\ mg/L}$ | $NH_{3\ mg/L}$ |
|---|---|---|---|---|---|---|
| A (Immersion Ciprofloxacin) | 1 | 29-31 | 6.0-6.1 | 7.0-7.2 | 0 | 0.52 |
| | 2 | 29-31 | 5.6-5.8 | 6.8-7.0 | 0 | 0.5 |
| B (Oral Ciprofloxacin) | 1 | 29-30 | 6.0-6.2 | 6.8-6.9 | 0 | 0.5 |
| | 2 | 30-31 | 6.2-6.5 | 6.9-7.1 | 0 | 0.5 |
| C (Negative control) | 1 | 29-30.1 | 6.1-6.5 | 7.2-7.4 | 0 | 0.5 |
| | 2 | 29-30 | 5.8-6.0 | 6.8-7.0 | 0 | 0.5 |
| D (Positive control) | 1 | 30-31.5 | 5.6-6.9 | 6.9-7.2 | 0 | 0.5 |
| | 2 | 30-31 | 6.2-6.5 | 7.1-7.4 | 0 | 0.5 |

## DISCUSSION

In recent times, Nigeria has witnessed an increase in the adoption of intensive fish production systems associated with high stocking densities, with the attendant stressful culture conditions that predisposes farmed fish to diseases (Oladosu *et al.*, 2011). However, there has been no commensurate improvement in fish health management strategies, which is preposterous for the fledging aquaculture industry. Effective disease control requires proper diagnosis, which is hinged on good knowledge of the

pathogenesis of disease conditions. This makes the identification and characterization of causal agents very important for the purpose of definitive diagnosis and effective control of diseases.

The colony and cellular morphological features as well as biochemical characteristics observed for the isolate used in this study agreed with the observations made for *E tarda* by various researchers including Waltman *et al.* (1986) and Noga (2010). Although the organism used in this study was not isolated from a clinical case, it was however observed to be highly pathogenic to *Clarias gariepinus* as demonstrated in the experimental infection. The clinical manifestations and the lesions recorded in this study are consistent with those reported in natural edwardsiellosis in cultured fish (Miwa and Mana, 2000; El-Jakee *et al.,* 2008; Noga, 2010).

The use of ciprofloxacin in the treatment of experimentally infected fish in this study was based on antibiogram, which indicated ciprofloxacin as the drug of choice. This is in agreement with the observation of Aoki *et al.* (2008) who reported efficacious treatment of edwardsiellosis by oral administration of ciprofloxacin.

Mortality was controlled within twenty four hours of commencement of oral and immersion application of ciprofloxacin showing that both route of drug administration were efficacious. However, the oral route of medication appeared to be less stressful as already observed by Johnson and Amend (1983), as there was no need for fish handling. Also, drug was applied via medicated food in routine feeding as the affected fish still fed actively, hence the process was devoid of drudgery and will be less laborious for the farmer. The uptake and distribution of ciprofloxacin in the tissue of catfish had earlier been observed to be fast, attaining high tissue concentration in one hour (Oladele *et al.,* 2011). The immersion method of ciprofloxacin administration has the appeal of shorter application period (one hour) that is once and for all. It may however require stressful fish handling, use of large quantity of drug and possible contamination of the environment when treated water is discharged. This may result in the development of drug resistance by the exposed microbial biota, which may portend grave danger for both fish and public health.

In the light of the findings of this study, especially regarding the pathogenicity of the *E. tarda* isolate and its multi-drug resistance, the organism may assume serious fish and public health significance as earlier observed by Greenlees *et al* (1998). It is therefore important that commercial fish farms should guard against drug mis-use and ensures efficient biosecurity while fish handlers should practice good personal hygiene.

## CONCLUSION

This study is a pointer to the possibility of unnoticed incidences of Edwardsiellosis in farmed *Clarias gariepinus* in Nigeria. The pathogenicity of *Edwardsiella tarda* isolated from pond water, and the efficacy of ciprofloxacin chemotherapy via oral and immersion route against edwardsiellosis in *Clarias gariepinus* was verified. The oral route of drug administration was found to be less stressful for the already sick fish, especially that the appetite of the fish is not affected by the disease condition. Fish farmers and handlers should however be educated on the public health significance of these findings. This study is probably the first report on the pathogenicity of *Edwardsiella tarda* in an African catfish species, to the best of our knowledge.

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