Changes in the morphology of catfish infected by *Edwardsiella tarda* as indicator of food safety

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Abstract. *Edwardsiella tarda* is a pathogenic bacterium in catfish. The aim of the research was to observe the morphological changes of catfish infected by *Edwardsiella tarda* as catfish safe consumption indicator. Catfish was artificially infected with *Edwardsiella tarda* in aquarium for 7 days. The observation included catfish behavior, organoleptic freshness, meat morphology and the number of *Edwardsiella tarda* found in catfish skin, meat as well as in aquarium water. At the end of culture, it was known that the number of *Edwardsiella tarda* in meat, skin and water were $10^5$ cfu/g, $10^6$cfu/g and $10^2$ cfu/mL. The fishy smell in aquarium could be sensed starting from day 3 after the pathogen introduction which was in concomitant with appearing red spots in the catfish skin. The freshness of catfish based on organoleptic tests was still around 9-7. From the SEM photographs showed the change in the structure of catfish meat. Histology observation showed the myofibril fragility increased. It recommended for consumers to keep on eye in appearance of red the spots color on catfish skin since it was visible indicator of early stage of spoilage caused by pathogen.

Keywords: *Edwardsiella tarda*, catfish meat morphological, catfish consumption

1. Introduction

*Edwardsiella tarda* is pathogen bacterium which can be live with a variety of host included avian, reptilian, piscine and mammalian [1]. It is a Gram-negative bacterial, facultative anaerobe, member of family Enterobacteriaceae having ability to survive and replicate in host phagocytes [2], typically can be isolated from water environments and animals that inhabit water. It has been reported that *E tarda* in human primarily associated with gastrointestinal disease, but there are also associated with extra intestinal disease, such as septicemia, meningitis, cholecystitis, and osteomyelitis but not associated with high rates of death [3]. *E tarda* is also a rare pathogen the peripartum period, but life-threatening condition for mother and the neonate [4]. *E tarda* should be categorized as a severe food waterborne infection, which results in high morality for patients with liver cirrhosis [5], sepsis and necrotizing fasciitis [6].

*E tarda* in catfish caused mass death in catfish rearing, similar to death from infection *Aeromonas hydrophila*. Both of these bacteria are hemolysin. Catfish consumers often do not pay attention to the morphology of catfish that are infected bacterial disease. Research on bacterial disease in catfish is generally related to the immune system and fish vaccination. Not much data about morphological
changes in catfish which infected \textit{E. tarda} as indicator of food safety. The purpose of the research was to observe the morphological changes of catfish infected by \textit{E. tarda} as a food safety indicator.

2. Methods

2.1. Artificial infection of \textit{E. tarda}

The catfish were obtained from local catfish aquaculture at Jalan Tidar Malang East Java with an average total length of 100 cm and total weight around 100 g. All the catfish were culturing in the aquarium that had been cleaned and disinfected. The aquariums were filled with 20 L water. Each aquarium was filled with 10 catfish and acclimated in this aquarium for 2x24 hours before infected with \textit{E. tarda}. Feeding was given 3 times a day. Water quality of the aquarium during the research was examined. The measured parameters were dissolved oxygen, pH and temperature. 

\textit{E. tarda} was obtained from Juanda Airport fish quarantine center. It was streaked in Tryptic soy agar (Merck 105458) and incubated at 37\degree C for 24 hours. One needle-loop of the bacterial colony from TSA was cultured in 100 mL Tryptic Soy Broth (Merck 10549) and incubated at 37\degree C for 24 hours. The bacterial cell density was measured using UV Visible spectrophotometer (Shimadzu) at wavelength of 600 nm and plated on TSA. As much as 20 mL of 10^{11} cfu/mL \textit{E. tarda} was transferred in 20 L aquarium.

Catfish were infected with \textit{E. tarda} by culturing for 7 days. Daily observation included swimming behavior, feeding respond, and blotchy sores on the skin. Every day, one catfish was taken and observed for the freshness organoleptic, total count of \textit{E. tarda} from water, catfish meat and skin as well as meat structure using SEM and histology.

2.2. Organoleptic testing

Fish freshness scoring were done using serve fresh catfish to the 15 panelists. They were asked to score based on attribute testing including mucus, meat texture, smell and meat consistency. Scoring scale used 1 as minimum value and 9 as the highest value.

2.3. SEM preparation

Scanning electron microscope (SEM) type Phenom Pro X (Holland, 2011) was used to analyze catfish meat myofibrils. Preparations were done in Mechanical Engineering Faculty of Brawijaya University. Catfish meat vertically cut 1x1 cm in size, dried in oven of 60\degree C for 30 minutes and then put it in sample holder until scanning process was complete.

2.4. Histology preparation

Histology examination was done in Anatomy Pathology Laboratory Medical Faculty of Brawijaya University. Histology preparation comprised fixation, tissue selection, dehydration, making block tissue, incision tissue, dying tissue. Dot slide of tissue were used light microscope Olympus XC10 (Germany, 2013) and Olyvvia software.

2.5. Total plate count of \textit{E. tarda}

Water culture, catfish skin as well as catfish meat were suspended and serially diluted in physiological salt solution. Enumeration of \textit{E. tarda} was done after inoculation on blood agar (base, Merck 110886) added with sterile sheep blood.

3. Result and Discussion

Water quality for catfish culture can be seen in Table 1. Catfish have arborescence organ so it can be live normally with low concentration of dissolve oxygen, 3 mg/L. A pond for catfish culture should have dissolve oxygen value minimal 5 mg/L. If it not, it should be helped with aeration [7]. In all of
aquarium in the treatment showed decreasing of dissolve oxygen. In this research, there were no cleaning aquarium activities. In the fifth day of culture, dissolved oxygen in treatment culture reached 2 mg/L. It influenced the swimming habit of catfish as seen in Table 3. Catfish swim close the surface water of the aquarium in search for free oxygen. Temperature and pH water catfish culture still in the normal range. Catfish treatment did not respond of feed were also seen from the fifth day of the culture. *E. tarda* infection in catfish had been known since 1973 causing stomach puffy and smell bad and called as red disease [8]. Body wound and fin crack were seen in the second day of the culture and became getting worse in the seventh day of the culture. The symptom of fish infected by *E. tarda* known as Edwardsiellosis detected by by sign appearing of ascites, exophthalmia, and internal organ injuries [9], especially the sign reddening ulcers [10]. Bacterial adhesion at the host to virulence was related to complex exoprotein-enzyme T1SS to T6SS which released by *E. tarda* [11]. The ability of excretion of these complex enzyme-protein especially T3SS and T4SS can cause gastrointestinal disease in human [12]. [13] said that fish infected by *E. tarda* showed abnormal swimming habit as swerving swims like spiral swimming, come swimming the near water level or floating in the water. Other symptoms that can be seen were loss of pigmentation, exophthalmia, the eyes opacity and hemorrhage on the fin and skin as seen in Table 4. Figure 2 showed catfish loss of its pigment so it no longer looks black. Catfish cutlet also showed it loses blood flow so catfish meat looked pale (Figure 3). Blood flow in fish played a role in the channel of the fish immune system. There was an interaction between immune system proteins with pathogen [14].

### Table 1. Aquarium Water Quality

| Days | Control pH | Treatment pH | Control DO(mg/L) | Treatment DO(mg/L) | Control Temperature (°C) | Treatment Temperature (°C) | Standard Temperature (°C) |
|------|------------|--------------|------------------|-------------------|-------------------------|---------------------------|--------------------------|
| 0    | 7.17       | 6.4          | 6.5-8            | 5.24              | 5.11                    | Minimal                   | 3                        | 23.3                     | 24.7                     | 25-30                   |
| 1    | 7.21       | 6.69         | 4.39             | 3.49              | 4.00                    | 3.20                      | 2.25                     | 24.5                     | 24.3                     |
| 2    | 7.06       | 6.27         | 4.00             | 3.20              | 3.83                    | 3.17                      | 2.28                     | 24.4                     | 24.4                     |
| 3    | 7.09       | 6.50         | 3.83             | 3.17              | 3.97                    | 2.81                      | 2.32                     | 24.3                     | 24.3                     |
| 4    | 7.05       | 6.71         | 3.63             | 2.69              | 3.76                    | 2.16                      | 2.22                     | 24.0                     | 24.0                     |
| 5    | 6.95       | 6.74         | 3.76             | 2.16              | 3.35                    | 2.14                      | 2.15                     | 24.0                     | 24.0                     |
| 6    | 7.00       | 6.27         | 3.35             | 2.14              | 6.50                    | 6.27                      | 6.4                      | 25.0                     | 25.0                     |
| 7    | 6.82       | 6.62         | 6.27             | 6.50              | 6.30                    | 6.4                       | 6.50                     | 24.0                     | 24.0                     |

### Table 2. Physiological observation

| Days | Feeding habit | Swimming habit |
|------|---------------|----------------|
|      | Control       | Treatment      | Control         | Treatment                  |
| 0    | Normal,       | Normal,        | Normal          | Normal                     |
|      | 15 minutes    | feed out in    | 15 minutes      | swim in the bottom         |
|      |               | 15 minutes     | 15 minutes      | aquarium                  |
| 1    | Normal,       | Some catfish   | Normal          | Normal                     |
|      | 15 minutes    | normal,        | normal          | swim in the bottom         |
|      |               | swim in 15     | 15 minutes      | aquarium                  |
|      |               | minutes        | still leftover  | aquarium                  |
| 2    | Some catfish  | Some catfish   | Normal          | Normal                     |
|      | normal,       | normal,        | normal          | but not moving             |
|      | 15 minutes    | in 15 minutes  | 15 minutes      | in the bottom aquarium     |
|      |               | still leftover | 15 minutes      | aquarium                  |
| 3    | Feed response | Feed response  | Normal,         | Normal                     |
|      | decreased,    | decreased,     | normal,         | but not moving             |
|      | more feed     | even more      | 15 minutes      | moving in the bottom       |
|      | remain        | feed remain    | 15 minutes      | aquarium                  |
| 4    | Feed response | Feed response  | Normal,         | Swim to the water surface  |
|      | decreased,    | decreased,     | normal          |                           |
|      | even more     | even more      | 15 minutes      |                           |
|      | feed remain   | feed remain    | 15 minutes      |                           |
| 6    | Most catfish  | Most catfish   | Swim to the     |                           |
|      | don't have    | don't have     | water surface   |                           |
|      | appetite,     | appetite,      |               |                           |
|      | even more     | even more      |               |                           |
|      | feed remain   | feed remain    |               |                           |
| 7    | Most catfish  | Most catfish   | Hang on to the   |                           |
|      | don't have    | don't have     | water surface   |                           |
|      | appetite,     | appetite,      |               |                           |
|      | even more     | even more      |               |                           |
|      | feed remain   | feed remain    |               |                           |

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Mucus in catfish can be correlated with the amount of bacteria [15]. Table 4 showed catfish more mucus production in 4th day’s infection. It followed by fishy odor which can be smelled from aquarium. Adhesion of pathogenic bacteria in fish mucus is the initial stages of pathogenic invasion to the host [16]. Temperature ambient changed (from 23°C to 35°C) and decreased Mg++ ion concentration causing exo protein-enzyme complex (T3SS and T4SS) activated [17]. [18] said that E. tarda virulence related to the expression complex exo-protein-enzyme T3SS, while hemolysis pathogenicity associated with complex protein-enzyme T6SS. This complex protein-enzyme was activated by hemolysis protein 147kDa. E. tarda infection in fish marked by red spot, abscesses in the skin as seen in Figure 1, Table 3 and Table 4. [19] said that injection catfish with E. tarda 10⁴ cfu/mL via intra peritoneal showed abdominal distention, skin wound, more mucus, nodule in liver, as well as degenerative liver and kidney. E. tarda can still live and replicated in host serum although the real mechanism still not clear enough [20].

| Days | Body injuries       | Fin injuries       |
|------|---------------------|--------------------|
|      | Control             | Treatment          | Control           | Treatment           |
| 0    | No body injuries    | No body injuries   | No fins injury    | No fins injury      |
| 1    | No body injuries    | Body near the tail wounded | No fins injury    |
| 2    | Bloodly wounded in the body and near the tail | Pale fin, more crackers in the fin |
| 3    | More bloody wounded in the body | Pale fin, more crackers in the fin |
| 4    | More bloody wounded in the body | Pale fin, some fins bloody wound |
| 5    | More bloody wounded in the body | Blood vessel in the fin wound |
| 6    | More bloody wounded in the body | Blood vessel in the body wound |
| 7    | More bloody wounded in the body | Blood vessel in the body wound |

![Figure 1. Morphology of catfish infected E. tarda](2nd day, wound on the fin, 3rd day, depigmentation on the skin, 5th day, swelling in the stomach, 6th day, bloody skin)
### Table 4. Catfish body morphology

| Days | Fish color                  | Mucus                        | Smell            | Treatment                        |
|------|-----------------------------|------------------------------|------------------|----------------------------------|
| 0    | Normal, Typical catfish color, black | Pale black color             | Normal           | Odorless                         |
| 1    | Normal, Normal              | Normal                       | Odorless         | Odorless                         |
| 2    | Normal, Normal              | A little mucus in the body   | Odorless         | Odorless                         |
| 3    | Black color in catfish slightly pale | Slimy body                  | Started to smell fishy | Fishy smell getting stronger     |
| 4    | Black color in catfish began turn pale | Slimy body                  | Fishy smell getting stronger | Fishy smell getting stronger     |
| 5    | Black color in catfish began turn pale | Slimy body                  | Pungent odor like ammonia | Pungent odor like ammonia get stronger |
| 6    | Pale black color            | Slimy body                  | Pungent odor like ammonia | Pungent odor like ammonia get stronger |
| 7    | Pale black                  | More mucus in the body       | Pungent odor like ammonia | Pungent odor like ammonia get stronger |

**Figure 2.** Depigmentation of catfish infected by *E. tarda*
Catfish cutlets that were infected by *E. tarda* showed a loss of blood flow so it looked pale (Figure 3) [21] said that *E. tarda* like other pathogen bacteria replicated in macrophages which were strongly associated with the T3SS protein-enzyme complex, while the T6SS protein-enzyme complex played a role in virulence. *E. tarda* is a hemolysin bacterium, red blood cell in the aorta and capillaries be reduced causing catfish flesh become pale as seen in Figure 3. *E. tarda* is an invasive microbe causing hemolysis [22].

![Figure 3. Flesh of catfish infected by *E. tarda*](image)

The freshness value of catfish infected by *E. tarda* showed decrease during the seventh day of culture, but all of them still included fresh fish categories. The panelist only assessed the freshness of catfish compared to catfish control which did not much differ. They did not pay attention to the presence of wound in the catfish body and in the fins as well as the paleness of catfish color. Catfish infected with *E. tarda* showed myofibrils began to become brittle and myosepta began to stretchable (Figure 4). From the histology observation (Figure 5) it showed the same phenomena.

### Table 5. Freshness testing using organoleptic test

| Days | Meat Texture | Smell | Meat Consistency | Mucus |
|------|--------------|-------|------------------|-------|
|      | Control      | Treatment | Control | Treatment | Control | Treatment | Control | Treatment | Control | Treatment |
| 0    | 9            | 9       | 8.5            | 8.4    | 9        | 9        | 9        | 9        |
| 1    | 9            | 8.7     | 8.4            | 8.6    | 9        | 9        | 9        | 9        |
| 2    | 8.9          | 8.6     | 8.4            | 8.2    | 9        | 9        | 9        | 9        |
| 3    | 8.8          | 8.3     | 8.3            | 7.9    | 9        | 8.8      | 9        | 8.7      |
| 4    | 8.8          | 8.2     | 8.3            | 7.7    | 9        | 8.6      | 9        | 8.5      |
| 5    | 8.8          | 7.7     | 8.2            | 7.6    | 9        | 8        | 8.8      | 7.7      |
| 6    | 8.8          | 7.5     | 8.1            | 7.3    | 8.8      | 7.7      | 8.8      | 7.3      |
| 7    | 8.8          | 7.3     | 8.1            | 7.2    | 8.8      | 7.7      | 8.6      | 6.4      |

Table description: meat texture 9: solid, elastic, difficult to tear from the spine; texture 7: rather dense, somewhat elastic, difficult to tear from the spine. Smell 9::fresh specific types, smell 7: neutral. Meat consistency 9:
specific types, whole belly meat; meat consistency 7: less bright, whole belly meat. Mucus 9: clear transparent; mucus 6: mucus become turbid

Figure 4. SEM photograph catfish meat infected by *E. tarda*. Arrow lines showed myosepta fragile
(a: control, b: 1st day, c: 2nd day, d: 3rd day, e: 4th day, f: 5th day, g: 6th day, h: 7th day *E. tarda* infection)

Myofibril fragility (Figure 5) was associated with the amount of *E. tarda* (Figure 6). The initial amount of *E. tarda* infected in water was $10^{11}$ cfu/mL. In our research showed the total amount of *E. tarda* in water was around $10^2$cfu/mL. *E. tarda* in catfish meat was around $10^6$-$10^9$ cfu/mL and the highest *E. tarda* in the catfish skin, $10^7$-$10^8$ cfu/mL. This amount was enough to make catfish skin paler and myofibril fragile. These bacteria initially attach themselves to the mucus, bound and follow bloodstream penetrate to the skin and the flesh. *E. tarda* need protein-enzyme complex T3SS and T6SS to invade the host. The presence of this bacteria invasion caused myosepta of catfish meat to be damaged and myofibril to be brittle, even though the consistency of the catfish meat cutting still intact. [23] said that T3SS and T6SS play important role in the pathogenicity of *E. tarda*. Lateral evpP transfer genes in the T6SS gene play an important role in the invasion of *E. tarda* in the host [24].
Figure 5. Histology catfish meat infected *E. tarda*. Arrows showed myosepta fragile.
Figure 6. Total plate count of *E. tarda* in water, catfish skin and meat

4. Conclusion

*E. tarda* in meat was around $10^4$-$10^5$ cfu/mL caused the characteristic black color of the catfish on the skin to turn pale, wound on the skin and fin and myofibril fragility although the freshness value was still good enough. Catfish consumers should be aware of the presence of pale skin color, wound in the skin and fins as indicator of catfish food safety.

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