Original article DOI: 10.19027/jai.22.1.97-105

Pathogenicity test bacteria from *Oreochromis niloticus* and *Clarias gariepinus* aquaculture ponds

Uji patogenisitas bakteri yang diisolasi dari media budidaya ikan nila *Oreochromis niloticus* dan ikan lele *Clarias gariepinus*

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(Received January 13, 2023; Accepted March 2, 2023)

ABSTRACT

Present research aimed to examine the pathogenicity of some bacteria which were isolated from ponds aquaculture to some freshwater fish. A number of novel pathogenic bacterial strains that infect fish have been identified within the previous five years. Isolating and testing bacteria from culture media for pathogenicity is one method used to stop disease outbreaks in fish farming. Previously, the bacteria were isolated from the water and feces of *Oreochromis niloticus* and *Clarias gariepinus* aquaculture in Samarinda, which were identified as *Escherichia coli*, *Enterobacter cloacae*, and *Enterobacter amnigenus*. An 0.1 mL of each bacterium with 1.23-5.46 ($\times 10^6$ CFU/mL) was intramuscular injection and evaluated the clinical signs, external pathology, and fish mortality. The freshwater fish using in this research were *O. niloticus*, *C. gariepinus*, and *Pangasius pangasius* with size 10-15 g from Loa Kulu, Kutai Kartanegara Regency, East Kalimantan, Indonesia. The result showed that each bacteria caused mortality in fish: *E. coli* bacteria caused fish mortality 23.33-66.67%; *E. cloacae* 10.00 to 90.00%; and *E. amnigenus* by 3.33-56.67%. The average death time of *E. coli* and *E. amnigenus* in all three fish were under 48 hours, while *E. cloacae* caused mortality less than 48 hours in *C. gariepinus* and *P. pangasius*. In conclusion, bacteria Koch postulate test showed that *E. coli*, *E. cloacae*, and *E. amnigenus* are putative pathogenic bacterium in *O. niloticus*, *C. gariepinus*, and *P. pangasius*.

Keywords: *Enterobacter amnigenus*, *Enterobacter cloacae*, *Escherichia coli*, fish pathogen, Postulat Koch

ABSTRAK

Penelitian ini bertujuan untuk mengkaji patogenisitas beberapa bakteri yang diisolasi dari air budidaya dan feses ikan pada beberapa ikan air tawar. Sejumlah strain bakteri patogen baru yang menginfeksi ikan telah diidentifikasi dalam lima tahun belakangan. Upaya isolasi, karakterisasi, dan uji patogenisitas merupakan salah satu metode yang digunakan untuk mencegah dan mengantisipasi pengendalian wabah penyakit baru pada budidaya ikan. Sebelumnya, bakteri dari air dan feses budidaya ikan *O. niloticus* dan *C. gariepinus* di Samarinda berhasil diidentifikasi sebagai *Escherichia coli*, *Enterobacter cloacae*, dan E*nterobacter amnigenus*. Sebanyak 0,1 mL dari setiap bakteri dengan kepadatan berkisar 1,23-5,46 ($\times 10^6$ CFU/mL) disuntikkan secara intramuskular dan dievaluasi tanda-tanda klinis, patologi eksternal, dan mortalitas ikan. Ikan air tawar yang digunakan dalam penelitian ini adalah ikan nila (*O. niloticus*), ikan lele (*C. gariepinus*), dan ikan patin (*P. pangasius*) dengan ukuran 10-15 g dari Desa Loa Kulu, Kabupaten Kutai Kartanegara, Kalimantan Timur, Indonesia. Hasil penelitian menunjukkan bahwa masing-masing bakteri menyebabkan kematian pada ikan sebagai berikut, bakteri *E. coli* menyebabkan kematian ikan 23,33-66,67%; *E. cloacae* 10,00- 90,00%; dan *E. amnigenus* sebesar 3,33-56,67%. Rata-rata waktu kematian *E. coli* dan *E. amnigenus* pada ketiga ikan tersebut di bawah 48 jam, sedangkan *E. cloacae* menyebabkan kematian kurang dari 48 jam pada *C. gariepinus* dan *P. pangasius*. Kesimpulannya, uji postulat koch bakteri menunjukkan bahwa *E. coli*, *E. cloacae*, dan *E. amnigenus* merupakan bakteri yang berpeluang patogen pada ikan nila, lele, dan patin.

Kata kunci: *Enterobacter amnigenus, Enterobacter cloacae*, *Escherichia coli*, pathogen, postulat koch

INTRODUCTION

In recent decades, bacterial infections affect aquaculture productivity, causing high mortality (Karvonen *et al*., 2019; Thongkao & Sudjaroen, 2019; Nugroho *et al*., 2017). Bacteria of *Escherichia coli, Enterobacter cloacae,* and *E. amnigenus* are commonly found in humans, animals, water, soils, plants, insects, and processed products, and can even be used as an indicator of pollutant waste in waters (Dong *et al*., 2020; Onmaz *et al*., 2020; Heiman *et al*., 2015). Many types of *Enterobacter* sp. bacteria have been identified and there are several species that are pathogenic not only in humans (Davin-Regli & Pagès, 2015), but also in fish. However, some of them are not causing mortality in cultured fish. In Aquaculture, almost every years founded the new bacteria which are pathogenic in fish (Rajasekaran, 2008; Schubiger *et al*., 2015).

Recent research showed that some bacteria exist in fish farming ponds and have been found that infected *O. niloticus* i.e *Escherichia coli*, *Salmonella arizonae, Citrobacter braakii, C. freundii, Enterobacter sakazakii, E. cloacae, Raoultella ornithinolytica, Klebsiella ozaenae* (Oliveira *et al*., 2017). In addition, isolated the *E. cloacae* which caused mortality in *Mugil cephalus* in Muttukadu lagoon (Sekar *et al*., 2008), while (Hardi *et al*., 2018) successfully found 13 isolates of bacteria from *O. niloticus* and *C. gariepinus* ponds in Samarinda which caused mortality up to 90%, in *O. niloticus*. Past research also determined that LD_{50} of *Enterobacter cloacae*, and *E. amnigenus* bacteria in *O. niloticus* fish ranged from $10^{6.4}$ CFU/mL. Tilapia fish injected with *Escherichia coli, Enterobacter cloacae*, and *E. amnigenus* bacteria showed pale signs of the liver and kidney, anemia, intestinal congestion, ulceration of the anus accompanied by mucus and bleeding in their infected external organs (Monir *et al*., 2020). This research evaluated characteristics and pathogenicity *Escherichia coli, Enterobacter cloacae*, and *E. amnigenus* bacteria in some freshwater fish *O. niloticus, C. gariepinus,* and *P. pangasius*.

MATERIALS AND METHODS

Aquarium test preparation

The study was performed using an aquarium (41×27×30 cm, length × width × height). Before being used, the aquarium was washed using chlorine, dried, and filled with 30 L of water. Aeration for each aquarium was provided for 24 hours before the fish were distributed into the aquarium. Water pH for cultivation was maintained in a range 7-8, while temperature about 28 °C, and DO 6-8 mg/L.

Fish preparation

The fish that were used in the pathogenicity test were tilapia (*O. niloticus*), African catfish (*C. gariepinus*), and pangasius fish (*P. pangasius*). All fish had average initial body weight about 10-15 g which was obtained from Loa Kulu Village, Loa Kulu, Kutai Kartanegara, East Kalimantan, Indonesia. Ten fish was used in each aquarium with three replications for each bacterial test. Firstly, fish were acclimatized in the laboratory for seven days and followed by immersion in 3% formalin solution for five minutes for sterilization from bacteria in fish body (Francis-Floyd, 1996). The immersion was repeated if bacteria growth was found. The examination was continued by isolating the gills and kidneys in the BHIA media (OXOID®) at a temperature of 35 °C for 24-48 hours.

Enterobacter bacteria preparation

The three species bacteria (*Escherichia coli, Enterobacter cloacae*, and *E. amnigenus*) for the pathogenicity test were isolated from the water and feces in *O. niloticus* and *C. gariepinus* aquaculture ponds in Samarinda City (Hardi *et al*., 2018). In total of eight isolates were grown in tryptic soy agar (TSA) media at a temperature of 30 °C for 24-48 hours, followed by the reidentification and sensitivity test to antibiotics using API 20E. The obtained isolates were cultured in tryptic soy broth (TSB) media and incubated at 30 °C for 24 hours. The isolates bacteria obtained and density (CFU/mL) at $LD_{50\text{-}96}$ for fish injection were list in Table 1 based on the previous research by Hardi *et al*. (2018).

Identification bacteria using API 20 E

This bacterium is prone to contamination; therefore, a new identification is required to confirm that the bacteria utilized in this investigation are the right bacteria. The reidentify and characterize bacteria, API 20 E kit was used (BioMerieux, Inc). Respectively, each isolate was stabbed into tubes, containing semi-solid nutrients and incubated at 35-37 °C for 24 h. The incubated tubes were examined for detecting motility of inoculated isolates followed by preservation in the refrigerator at 4 °C.

Sensitivity test to antibiotic

The fact that certain pathogenic bacteria in fish are currently resistant to antibiotics is a grave warning that improper chemical drug use hastens the pathogenicity of microorganisms. As part of bacterial identification and timely warning against disease outbreaks, bacterial sensitivity testing is necessary. Antibiotics bacterial sensitivity test (Inhibition zone method) was completed using seven commercial antibiotics, namely: nitrofurantoin (Nitro), ciprofloxacin (Cipro), oxytetracycline (Oxy), chloramphenicol (Chlo), nalidixic acid (Na), gentamicin (Gent), and norfloxacin (Nor). Each bacteria was cultured in BHIA and after 5 min, the antibiotic disc was placed on the medium and incubated at 35 °C for 24 h. The diameter of inhibition zone (mm) was measured to determine antibiotic resistance.

Pathogenicity test of bacteria in freshwater fish

Each fish was intramuscularly injected using each isolate bacteria (0.1 mL) with densities, as in Table 1. The density of bacteria was calculated using the TPC method as previously performed by Hardi *et al*. (2018) and Hardi *et al*. (2016). After being injected, the fish were kept for 96 h. Observation of macroscopic anatomic pathology of external organs, the number of mortalities, and the average main time death, were measured at 96 h after injection.

Water Quality Control

Water quality measurement during the research was carried out to control the condition of aquaculture water. Temperature, dissolved oxygen, and pH were checked every week using the U-50 Series Multi-Parameter Water Quality Checker Tool.

Observed Parameters

Characteristic of bacteria was observed, bacterial identification using API 20E and sensitivity to various antibiotics nitrofurantoin; ciprofloxacin; oxytetracycline; chloramphenicol; nalidixic acid; gentamicin; and norfloxacin.

Pathology of external organs anatomy

Observations were done to detect any abnormalities in the external organs anatomy of fish at 96 h after injection with bacteria. The observation of pathology was focused in the eye of fish (opacity/O, exophthalmia/ E, lysis/L); skin (slimy/B, pale/P, loose scales/Sl); and fin (fin rot/ Sg).

Mortality and mean time death

The cumulative mortality of *O. niloticus, C. gariepinus*, and *P. pangasius* were calculated every day or 24, 48, 72, and 96 h after injection. Mean time death (MTD) was measured by calculating the average time needed by each isolate bacteria to cause the mortality in *O. niloticus, C. gariepinus*, and *P. pangasius*, using the MTD calculation method (Fitri *et al*., 2019).

RESULTS AND DISCUSSION

Results

Characterization of the Enterobacter bacteria

The eight isolates of bacteria were tested for identification using API 20 E and sensitivity tests for antibiotics, and the results are shown in Tables 2 and 3. *E. coli* bacteria (01, 02, 03) were able to hydrolyze 2-nitrophenyl-ßD-galactopyranoside, but the three of *E. coli* bacteria have diverse ability to hydrolyze sugar. The isolate of *E. coli*-01 and *E. cloacae*-02 had almost the same sugar activity, but the difference was only in

Table 1. The bacteria isolate density at LD_{50-96} for fish injection.

Code of Bacteria	Isolate	Bacteria concentration (CFU/mL)
A1	*Escherichia coli-01*	2.22×10^6
A2	*Escherichia coli -02*	4.69×10^6
A3	*Escherichia coli -03*	5.04×10^6
B1	*Enterobacter cloacae-01*	5.46×10^6
B2	*Enterobacter cloacae-02*	1.24×10^6
B3	*Enterobacter cloacae-03*	1.23×10^6
C1	*Enterobacter amnigenus-01*	4.08×10^5
C2	*Enterobacter amnigenus-02*	3.75×10^5

the hydrolysis of Sodium pyruvate. Meanwhile, only *E. coli*-03 was able to hydrolyze gelatin and had positive Oxidase. Bacteria *E. cloacae*-01, 02, 03 were able to hydrolyze 2-nitrophenyl-ßD-galactopyranoside, L-ornithine, D-glucose, D-mannitol, D-sorbitol, D-sucrose, D-melibiose, and L-arabinose, while *E. amnigenus*-01 and 02 bacteria were able to hydrolyze D-melibiose and Amygdalin.

Tests with antibiotic sensitivity showed that each bacteria have different sensitivity to commercial antibiotics (Nitro, Cipro, Oxy, Chlor, Na, Gent, and Nor). All bacteria except *E. amnigenus*-02 was only sensitive to antibiotics with inhibitory zones of >15 mm ie Cipro, Chlor, Na, and Nor (Table 3).

Pathogenicity of isolate bacteria in tilapia, catfish, and pangasius

Injection with bacteria was carried out with densities ranging from 10^5-10^6 CFU/mL (Table 1). After the injection, *O. niloticus, C. gariepinus,*

Table 2. Overview of bacteria characteristics using API 20E.

	A1	*A2*	*A3*	*B1*	*B2*	*B3*	*C1*	*C2*
2-nitrophenyl-ßD-galactopyranoside	+	+	+	+	+	+	+	-
L-arginine	-	-	-	+	+	-	+	-
L-lysine	+	+	-	-	-	+	-	-
L-ornithine	+	+	-	+	+	+	+	-
Trisodium citrate	-	-	-	+	+	-	+	-
Sodium thiosulfate	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-
L-tryptophan	-	-	-	-	-	-	-	-
L-tryptophan	+	+	-	-	-	+	-	-
Sodium pyruvate	-	+	-	+	+	-	-	+
Gelatin	-	-	+	-	-	-	-	-
D-glucose	+	+	-	+	+	+	+	-
D-mannitol	+	+	-	+	+	+	+	-
Inositol	-	-	-	+	+	-	-	-
D-sorbitol	+	+	-	+	+	+	-	-
L-rhamnose	+	+	-	-	-	+	+	-
D-sucrose	+	+	-	+	+	+	+	-
D-melibiose	+	+	-	+	+	+	+	+
Amygdalin	-	-	-	+	+	-	+	+
L-arabinose	+	+	-	+	+	+	+	-
Oxidase	-	-	+	-	+	-	-	-

Table 3. Bacterial sensitivity to various antibiotics (mm).

Isolate bacteria	Nitro	Cipro	Oxy	Chlor	Na	Gent	Nor
Escherichia coli-01	12 ± 0.02	25 ± 0.00	17 ± 0.00	16 ± 0.02	18 ± 0.00	13 ± 0.01	26 ± 0.00
Escherichia coli -02	13 ± 0.00	27 ± 0.00	22 ± 0.01	20 ± 0.02	16 ± 0.00	13 ± 0.01	25 ± 0.00
Escherichia coli -03	17 ± 0.01	17 ± 0.00	20 ± 0.01	19 ± 0.01	15 ± 0.00	10 ± 0.01	22 ± 0.00
Enterobacter cloacae-01	12 ± 0.02	26 ± 0.00	17 ± 0.01	10 ± 0.02	20 ± 0.00	11 ± 0.01	16 ± 0.01
Enterobacter cloacae-02	10 ± 0.01	16 ± 0.00	13 ± 0.01	16 ± 0.02	17 ± 0.00	12 ± 0.01	20 ± 0.01
Enterobacter cloacae-03	10 ± 0.01	24 ± 0.00	19 ± 0.01	16 ± 0.01	16 ± 0.00	12 ± 0.01	19 ± 0.01
Enterobacter amnigenus-01	13 ± 0.00	21 ± 0.00	12 ± 0.00	17 ± 0.02	17 ± 0.00	12 ± 0.01	23 ± 0.01
Enterobacter amnigenus-02	14 ± 0.02	12 ± 0.00	11 ± 0.00	13 ± 0.02	12 ± 0.00	9.0 ± 0.00	8.0 ± 0.01

Note: Nitro = nitrofurantoin; Cipro = ciprofloxacin; Oxy = oxytetracycline; Chlor = chloramphenicol; Na = nalidixic acid; Gent = gentamicin; Nor = norfloxacin.

and *P. pangasius* showed pathology signs in the organs of the eye, skin and fins (Table 4). Until the 96 hours of post-injection in *O. niloticus*, the fish appeared to have opacity and eye lysis (Figure 1A); the changes in the eyes of *P. pangasius* were also found to have opacity and exophthalmia (Figure 1B), whereas *C. gariepinus* showed that no changes were seen in the eye. Meanwhile, in skin organs, the most common symptom was the pale of fish body both *O. niloticus*, *C. gariepinus* (Figure 1C), and *P. pangasius*. In addition, loose scales was also found in *O. niloticus* and the fin organ of all fish also showed the presence of fin rot.

The mortality of fish after injected was shown in Table 5. The highest mortality, occurred in

Figure 1. Fish eye and body observation *Oreochromis niloticus* (A); *Pangasius pangasius* (B); *Clarias gariepinus* (C) eye lysis.

Table 4. Anatomy pathology observation of *Oreochromis niloticus* (O.n), *Clarias gariepinus* (C.g), and *Pangasius pangasius* (P.p) post-injection with bacteria.

Isolate Bacteria	Eye			Skin			Fin		
	O.n	C.g	P.p	O.n	C.g	P.p	O.n	C.g	P.p
Escherichia coli-01	O	N	O	B,P	B,P, B	B,P, B	Sg	Sg	Sg
Escherichia coli -02	O	N	N	B,P	B,P, B	B,P	Sg	Sg	Sg
Escherichia coli -03	N	N	N	N	N	N	N	N	N
Enterobacter cloacae-01	O	N	N	B, P, Sl	B,P, B	B,P	Sg	Sg	Sg
Enterobacter. cloacae-02	O	N	N	B ; P, Sl	N	B,P	Sg	N	N
Enterobacter cloacae-03	N	N	E	B ; P	N	B,P	N	N	N
Enterobacter amnigenus-01	L	N	E	B, P, Sl	B, P	B,P, B	Sg	Sg	Sg
Enterobacter amnigenus-02	N	N	O	B, P, Sl	N	B	Sg	N	Sg

Note: (O) Opacity; (L) Lysis; (E) Exophthalmia; (B) slimy skin; (P) pale skin; (Sg) Root fins; (Sl) loose fin; (N) normal.

Table 5. The number of dead fish observation of *Oreochromis niloticus*, *Clarias gariepinus*, and *Pangasius pangasius* post-injection with bacteria using $LD_{50\text{-}96}$ doses.

Isolate Bacteria	*Oreochromis niloticus*				*Clarias gariepinus*				*Pangasius pangasius*			
	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
Escherichia coli-01	0	0	0	1	3	7	16	17	3	0	4	4
Escherichia coli -02	3	4	6	7	11	6	0	2	6	10	4	0
Escherichia coli -03	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae-01	1	1	2	4	10	13	16	18	7	17	2	1
Enterobacter cloacae-02	0	0	0	1	0	0	0	0	1	2	0	0
Enterobacter cloacae-03	1	1	1	1	0	0	0	0	20	0	0	0
Enterobacter amnigenus-01	2	3	8	13	7	12	14	17	1	1	10	1
Enterobacter amnigenus-02	2	6	10	11	0	0	0	0	0	0	1	0

P. pangasius, which was injected with the *E. cloacae*-01 bacteria, reaching 90% of mortality, while the *E. cloacae*-02 bacteria did not cause the death of *O. niloticus* and *C. gariepinus* and only 10% (Table 6). However, the *E. coli*-03 bacteria did not cause mortality in all fish. Further, the *E. coli*-01, *E. coli*-02, *E. cloacae*-01, and *E. amnigenus*-01 caused mortality in *C. gariepinus* with the percentage of mortality 56-63%. In the MTD of *O. niloticus, C. gariepinus* and *P. pangasius* which were infected by bacteria, it can be seen that the *E. cloacae*-03 bacteria caused mortality in *O. niloticus* and *P. pangasius* less than12 hours after infection. Only four bacteria which were *E. coli*-01 and 02, *E. cloacae*-01, and *E. amnigenus*-01can cause mortality in all test fish.

Discussion

The *Oreochromis niloticus, C. gariepinus*, and *P. pangasius* are freshwater fish species that are widely cultivated by farmers. Many cultivation technologies developed can be a source of problems if it is not accompanied by good environmental management (Austin & Austin, 2016). The emergence of pathogenic bacteria from the cultivation environment needs to be considered as a step to maintain the safety of aquaculture. There are many studies on zoonotic bacteria from fish that are reported to be potentially pathogenic not only in humans but also in cultured fish (Austin & Austin, 2016; Sekar *et al*., 2008).

According to Buller (2014), Bacteria *Escherichia, Citrobacter, Edwardsiella, Enterobacter, Erwinia, Ewingella, Hafnia, Klebsiella, Morganella, Pantoea, Plesiomonas, Proteus, Providencia, Rahnella, Salmonella, Serratia, Shigella* and *Yersinia*, all of which have been reported as pathogens in fish. The bacteria such as *E. coli, E. cloacae, E. amnigenus* itself have been widely reported in zoonoses in humans. However, some zoonotic bacterial that are isolated from fish are not pathogenic in fish (Ziarati *et al*., 2022; Haenen *et al*., 2014; Suardana *et al*., 2011; Sekar *et al*., 2008). Current results found that *E. coli* and *E. cloacae* were both pathogenic in fish and caused mortality in *O. niloticus, C. gariepinus*, and *P. pangasius*.

The *E. cloacae* bacteria even caused mortality up to 90% in *P. pangasius*, while *E. coli* reached 66.67% in *P. pangasius*. Meanwhile, the *E. amnigenus* bacteria caused mortality of up to 56.67% in *C. gariepinus*. From the three of fish, *P. pangasius* was susceptive to *E. coli*, *E. cloacae,* and *E. amnigenus*. The fish mortality post-injection with bacteria was caused by the presence of bacterial virulence factors such Siderophores like anguibactin or piscibactin, have been described in Vibrio and Photobacterium pathogens (Lemos & Balado, 2020).

The previous result findings that the *Streptococcus agalactiae* bacteria which produces hemolysin causes death to *O. niloticus* faster and more than the *S. agacatiae* bacteria that does not produce the hemolysin protein (Hardi *et al*., 2011). Li *et al*. (2020); Mahendra (2016); Moreira *et al*. (2021), Abalaka *et al*. (2015) explained that enteric bacteria produce extracellular proteins that are virulent in fish in the form of hemolysin and leukotoxins protein, which cause damage to the fish red blood cells, resulting hemorrhage, hyperplasia, and necrosis in cells and tissues. In addition, hemolysin is a protein that cause virulence factor in bacteria. Austin and Austin (2016), Park *et al*. (2012) and Susanti *et al*. (2016) stated that fish infected with *Edwardsiella tarda* shows some clinical signs of

Table 6. Percentage of fish mortality (%) post-injection with isolate bacteria.

Isolate Bacteria	*Oreochromis niloticus*	*Clarias gariepinus*	*Pangasius pangasius*
Escherichia coli-01	0.00 ± 0.00^{a}	56.67 ± 11.54^{bcde}	36.67 ± 11.54^{abcd}
Escherichia coli -02	23.33 ± 6.67^{abcd}	63.33 ± 15.27^{def}	66.67 ± 49.32^{def}
Escherichia coli -03	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Enterobacter cloacae-01	13.33 ± 8.81^{abc}	60.00 ± 20.81^{cde}	90.00 ± 10.00^{ef}
Enterobacter cloacae-02	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.00 ± 11.54^{a}
Enterobacter cloacae-03	3.33 ± 3.33^{a}	0.00 ± 0.00^{a}	66.67 ± 57.73^{def}
Enterobacter amnigenus-01	36.67 ± 26.03^{abcd}	56.67 ± 40.41^{cde}	43.33 ± 41.63^{abcd}
Enterobacter amnigenus-02	43.33 ± 26.67^{abcd}	0.00 ± 0.00^{a}	3.33 ± 5.77^{a}

Note: Mean±Standard deviation followed by different superscript (^{abcdef}) on the same column showed no significance different at P<0.05.

hemorrhage in organs, exophthalmia (protruding eyes), opacity of the eyes, distended abdomen, bloody ascites, and enlarged spleen and kidneys. Similar to current findings, fish injected with *S. agactiae* bacteria (Hardi *et al*., 2011) the *Vibrio alginolyticus* bacteria (Yanuhar *et al*., 2022); and *E. cloacae* (Sekar *et al*., 2008) revealed the same results that the same damages also occurred in fish kidneys.

Meanwhile, (Witeskaa *et al*., 2022) found that fish infected with *E. vulneris* bacteria at 10^6 cells /L density has some clinical signs such as hemorrhagic lesions on the skin, exophthalmia, bloody exudate in the intestinal tract or empty digestive tract and abnormal liver. In addition, *E. coli* bacterial infection which has a high level of pathogenicity in fish and humans also has the same pathogenicity homologous with *Edwardsiella tarda* (Nakamura *et al*., 2013). The presence of bacteria in the brain organs will cause fish to swim abnormally like gasping swimming, sideways swimming and even whirling (Hardi *et al*., 2011). Whereas the presence of bacteria in fish kidneys usually causes discolouration in the body of both fish to turn pale or blackened, this is seen histopathologically in fish kidneys that appear to have an increase in the amount of blood in the vessels, which is indicated by dilation of blood capillaries full of erythrocytes in cranial vessels (Dalum *et al*., 2022).

The pathogenicity of bacteria in fish is determined by bacterial strains, fish species, bacterial entrances, and environmental conditions. Based on the different type of bacteria from *E. coli, E. cloacae*, and *E. amnigenus*, all of those bacteria can cause abnormality and mortality in fish. However, based on the strains of each type of *E. coli*, only *E. coli*-02; *Ent. cloacae*-01 and 02 which cause death in tilapia, in fact, *E. coli*-03 did not cause mortality in catfish and pangasius. This finding revealed that each strain has different characteristics that affected the level of pathogenicity. Moreover, according to API 20 E test results, bacteria which has the ability to hydrolyze more sugar has higher pathogenicity compared to enterobacteria that hydrolyze less sugar. This ability may relate to bacterial adhesion properties and colonize in cells of host tissues.

CONCLUSION

The *E. coli, E. cloacae, E. amnigenus* which come from the results of the postulat Koch test indicated the possibility of pathogens in freshwater aquaculture (*O. niloticus, C. gariepinus* and *P. pangasius*), which may be seen from the presence of macroscopic anatomical pathologies in eye, skin and fins organs; and cause the death of aquaculture fish, as well as the fish meantime death (MTD) after bacterial infection which is less than 48 hours.

ACKNOWLEDGMENTS

This research is conducted with funding assistance from the Ministry of Education and Culture through National Competitive Research in 2020 and 2021 and Indonesia Endowment Fund for Education Research.

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