

Antibiotic Resistance of Bacterial Species Isolated from Diseased Goldfish, *Carassius auratus*

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Abstract

The antibiotic resistance test of bacterial species isolated from diseased goldfish, *Carassius auratus* was determined by disk diffusion method. Total 41 strains were identified into 5 species including *Aeromonas hydrophila*, *Erwinia* spp., *Pasteurella pneumotropica*, *Pantoea* spp. and *Proteus panneri*. Twelve antibiotics were investigated namely amoxycillin, ampicillin, chloramphenicol, ciprofloxacin, erythromycin, enrofloxacin, novobiocin, norfloxacin, oxytetracycline, oxolinic acid, doxycycline and tetracycline. Thirty strains of *A. hydrophila* were resistant to amoxycillin and ampicillin (100%), novobiocin (83.33%), erythromycin and oxolinic acid (66.67%), oxytetracycline and tetracycline (50%), chloramphenicol and doxycycline (33.33%), ciprofloxacin and norfloxacin (16.67%), and enrofloxacin (13.33%, respectively). *Erwinia* spp. and *P. panneri* were resistant to all antibiotics. Furthermore, *Pantoea* spp. and *P. pneumotropica* were highly resistant patterns against antibiotics. However, enrofloxacin was an effective drug against *A. hydrophila*, *Erwinia* spp. and *P. panneri*. From this result, it could be concluded that all bacterial strains isolated from diseased goldfish showed multi-resistance to antibiotics.

Keywords : Antibiotic resistance, Bacterial diseases, Goldfish

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การดื้อยาปฏิชีวนะของเชื้อแบคทีเรียที่แยกได้จากปลาทองป่วย

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บทคัดย่อ

การศึกษาการดื้อยาปฏิชีวนะในแบคทีเรียที่แยกจากปลาทองป่วย (*Carassius auratus*) ด้วยวิธี disk diffusion method สามารถจำแนกแบคทีเรียได้ 5 ชนิด จำนวน 41 สายพันธุ์ ได้แก่ *Aeromonas hydrophila*, *Erwinia* spp., *Pasteurella pneumotropica*, *Pantoea* spp. และ *Proteus panneri* การทดสอบความดื้อยาต่อยาปฏิชีวนะจำนวน 12 ชนิด ได้แก่ amoxycillin, ampicillin, chloramphenicol, ciprofloxacin, erythromycin, enrofloxacin, novobiocin, norfloxacin, oxytetracycline, oxolinic acid, doxycycline และ tetracycline เชื้อแบคทีเรีย *A. hydrophila* จำนวน 30 สายพันธุ์ พบรดับต่อต้านยา amoxycillin และ ampicillin (100%), novobiocin (83.33%), erythromycin และ oxolinic acid (66.67%), oxytetracycline (50%), chloramphenicol และ doxycycline (33.33%), ciprofloxacin และ norfloxacin (16.67%), และ enrofloxacin (13.33%) ตามลำดับ ส่วน *Erwinia* spp. และ *P. panneri* พบรดับต่อต้านยาทั้ง 12 ชนิด ถัดมาเชื้อแบคทีเรีย *Pantoea* spp. และ *P. pneumotropica* พบรดับต่อต้านยาปฏิชีวนะที่ค่อนข้างสูง สำหรับยาที่มีประสิทธิภาพในการยับยั้งการเจริญเติบโตของเชื้อแบคทีเรีย *A. hydrophila*, *Erwinia* spp. และ *P. panneri* คือ ยาเอโนโรฟลีซิน จากการทดลองครั้นนี้สรุปได้ว่าการดื้อยาปฏิชีวนะของเชื้อแบคทีเรียมีความแตกต่างกันขึ้นกับชนิดของแบคทีเรีย

คำสำคัญ : การดื้อยาปฏิชีวนะ โรคติดเชื้อแบคทีเรีย ปลาทอง

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Introduction

In the current issue of aquaculture or live stocks, many researches are focused on the anti-microbial resistance in animals, also its meats and environments. This circumstance can affect to humans who consume the food with drug residues accumulation (WHO, 2014). Goldfish is important ornamental fish species which popular culture, usually rearing in aquarium. Moreover, Thailand was ranged in one of ten countries to import ornamental fish species to global trade (Dey, 2016). The common disease occurred in ornamental fish were caused by bacterial agents which classified to gram-negative bacteria (Lewbart, 2001). Bacterial pathogens have been reported to cause a disease in fish including *Aeromonas* sp., *Edwardsiella* sp., *Pseudomonas* sp., *Shewanella* sp., *Citrobacter* sp., *Plesiomonas* sp., (Walczak *et al.*, 2017) *Vibrio* (Saad El-deen and Elkamel, 2015) *Mycobacterium* sp., *Streptococcus* sp., *Flavobacterium* sp. (Yanong, 2013). Infected fish caused by bacteria has been used the antibiotics for curing for a long time until the present time. The problem of misused of antibiotics can induced the resistance bacteria has been emphasized in aquaculture and other fields. Many organizations namely FAO, WHO have concerned for increasing of resistant bacteria might affected to the food safety and human health as well as animal health (Miranda *et al.*, 2013). The common antibiotic have been reported to treat the bacterial infection in fish such as erythromycin, amoxicillin, ampicillin, oxytetracycline, tetracycline, gentamicin (Yanong, 2013), ciprofloxacin (Abraham *et al.*, 2004), erythromycin and kanamycin (Jeeva *et al.*, 2013), norfloxacin and enrofloxacin (El-deen and Rawway, 2014). This research would like to determine the antibiotic resistance of bacterial species isolated from diseased goldfish.

Materials and methods**1. Fish sampling**

A total of sixty goldfish (*Carassius auratus*) were randomly collected from local pet shop in Maha Sarakham District during June – August 2017. The fish were average 14.08 g in body weight (BW); 7.54 cm in total length (TL). Fish samples were over-dosed euthanasia for bacterial isolation from internal organs, for example, kidney, liver and spleen.

2. Bacterial identification

The bacterial isolates were cultivated and purified on tryptic soy agar (TSA) and incubated at 30°C for 24 hr. Bacterial identification were performed by gram staining and biochemical characteristics according to Buchanan and Gibbon (1974) and using the commercial kit API 20E (Özoğul *et al.*, 2010).

3. Antibiotic resistance test

Forty-one strains of bacterial isolates were performed by disc diffusion method against twelve antibiotics including amoxycillin (AML, 10 µg); ampicillin (AMP, 10 µg); chloramphenicol (C, 30 µg); ciprofloxacin (CIP, 5 µg); erythromycin (E, 15 µg); enrofloxacin (ENR, 5 µg); novobiocin (NV, 30 µg); norfloxacin (NOR, 10 µg); oxytetracycline (OT, 30 µg); oxolinic acid (OA, 2 µg); doxycycline (DO, 30 µg) and tetracycline (TE, 30 µg). The resistance test was done in Muller hinton agar (MHA) in triplicate and incubated at 30°C for 24 hr. Then, the diameter of inhibition zone was measured and compared with the standard value according to CLSI (2008).

Results and Discussion

Results

1. Gross appearance of fish sampling

Infected goldfish were externally revealed fin hemorrhage, fin rot, imbalance swimming, lethargy, scale protrusion. Some fish showed white spots in the whole body. Necropsy finding were obviously discovered ascites, pale liver and gall bladder enlargement (Fig. 1).

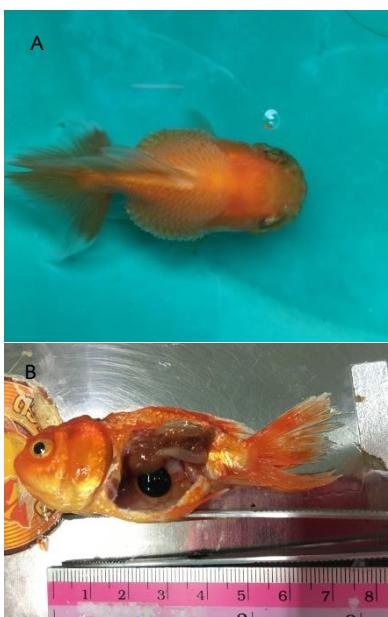


Fig. 1 Infected goldfish with bacterial diseases showed scale protrusion (A), pale liver and gall bladder enlargement (B)

2. Bacterial identification

Forty-one strains of bacterial isolates were successfully identified from the infected goldfish. Five bacterial species were classified namely *Aeromonas hydrophila*, *Erwinia* spp., *Pasteurella pneumotropica*, *Pantoea* spp and *Proteus panneri* as shown in Table 1. Moreover, *A. hydrophila* was a dominant species in the infected goldfish with

prevalence of 73.17%. Furthermore, *P. pneumotropica* (12.20%), *Pantoea* spp. (9.76%), *Erwinia* spp. (2.44%) and *P. panneri* (2.44%), respectively.

3. Antibiotic resistance test

Twelve antibiotics against forty-one bacterial strains were determined for antibiotics resistance (Table 2). Thirty strains of *A. hydrophila* were resistant to amoxycillin and ampicillin (100%), novobiocin (83.33%), erythromycin and oxolinic acid (66.67%), oxytetracycline and tetracycline (50%), chloramphenicol and doxycycline (33.33%), ciprofloxacin and norfloxacin (16.67%), and enrofloxacin (13.33%), respectively. Next, one strain of *Erwinia* spp. and one strain of *P. panneri* were resistant to all antibiotics. Furthermore, four strains of *Pantoea* spp. were all resistant (100%) to amoxycillin, ampicillin, oxytetracycline and novobiocin. Five strains of *P. pneumotropica* were resistant (100%) to chloramphenicol, oxytetracycline, amoxycillin, ampicillin and novobiocin. However, enrofloxacin was an effective drug against *A. hydrophila*, *Erwinia* spp. and *P. panneri* in the present study.

Discussion

In the present study, the dominant bacterial species was *Aeromonas hydrophila* (73.17%) from the infected goldfish which obviously showed hemorrhage, scale protrusion, ascites, pale liver and gall bladder enlargement. These findings were similar to Carevia *et al.* (2013) and Jongjareanai *et al.* (2009) who described *A. hydrophila* was a major bacterial pathogen for rearing goldfish. However, *A. hydrophila* was a normal flora commonly found in all water resources (Ashiru *et al.*, 2011) but it can developed as an opportunistic bacterial pathogen which caused a disease in aquatic animals when their immune system were decreased (Harikrish-

man and Balasundaram, 2008). In case of *P. pneumotropica* and *P. panneri* were reported in guppy, Siamese fighting fish and fancy carp (Chansue and Assawawongkasem, 2008), whereas, *Pantoea* spp. was revealed in natural freshwater fish (Kluga *et al.*, 2017). Moreover, *Erwinia* spp. was reported from rearing water of salmon farm in Ireland (Moore *et al.*, 2014).

The antibiotics resistance test has mainly been studied in *A. hydrophila* from the previous reports. In the present study, *A. hydrophila* showed highly pattern of the antibiotics resistance

which corresponded with Jongjareanjai *et al.* (2009) and Dias *et al.* (2012). Total forty-one strains of bacteria were resistant 100% to amoxycillin and ampicillin. All bacterial species showed multi-resistance to antibiotics. However, the quinolone antibiotic such as ciprofloxacin, enrofloxacin and norfloxacin was an effective drug against *A. hydrophila*, *Erwinia* spp. and *P. panneri* in this present study which similarly reported by El-Deen and Rawway (2014) who described these three antibiotics against *A. hydrophila*.

Table 1. Bacterial strains isolated from infected goldfish

Bacterial strain	isolates	Source
<i>Aeromonas hydrophila</i>	30	liver (n = 15), kidney (n = 5), spleen (n = 10)
<i>Erwinia</i> spp.	1	liver (n = 1)
<i>Pantoea</i> spp.	4	liver (n = 1), kidney (n = 2), spleen (n = 1)
<i>Pasteurella pneumotropica</i>	5	liver (n = 1), kidney (n = 3), spleen (n = 1)
<i>Proteus panneri</i>	1	spleen (n = 1)

Conclusion

Infected goldfish showed hemorrhage, fin rot, imbalance swimming, lethargy, scale protrusion, ascites, pale liver and gall bladder enlargement were observed in this study. Total 41 strains were identified into 5 species including *Aeromonas hydrophila*, *Erwinia* spp., *Pasteurella pneumotropica*, *Pantoea* spp. and *Proteus panneri*. The predominant species was *A. hydrophila*. All bacterial strains isolated from diseased goldfish showed multi-resistance to antibiotics. In addition, enrofloxacin was an effective drug for inhibiting the bacteria viz. *A. hydrophila*, *Erwinia* spp. and *P. panneri*. Moreover, enrofloxacin was permitted drug to use in fish diseases treatment in aquaculture.

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Table 2. Prevalence of antibiotic resistance of bacterial strains isolated from infected goldfish

Antibiotic	Disc concentration (μg)	<i>A. hydrophila</i> (N = 30)	<i>Erwinia</i> spp. (N = 1)	<i>Pantoea</i> spp. (N = 4)	<i>P. pneumotropica</i> (N = 5)	<i>P. panneri</i> (N = 1)
AML	10	R = 100	R = 100	R = 100	R = 100	R = 100
AMP	10	R = 100	R = 100	R = 100	R = 100	R = 100
	30	R = 33.33	R = 100	R = 50	R = 100	R = 100
		I = 0		I = 0		
		S = 20		S = 50		
CIP	5	R = 16.67	R = 100	R = 50	R = 60	R = 100
		I = 0		I = 0	I = 0	
		S = 83.33		S = 50	S = 40	
E	15	R = 66.67	R = 100	R = 75	R = 80	R = 100
		I = 0		I = 0	I = 0	
		S = 33.33		S = 25	S = 20	
ENR	5	R = 13.33	R = 100	R = 50	R = 60	R = 100
		I = 13.33		I = 0	I = 0	
		S = 73.33		S = 50	S = 40	
NV	30	R = 83.33	R = 100	R = 100	R = 100	R = 100
		I = 0				
		S = 16.67				
NOR	10	R = 16.67	R = 100	R = 50	R = 60	R = 100
		I = 16.67		I = 0	I = 0	
		S = 66.67		S = 50	S = 40	
OT	30	R = 50	R = 100	R = 100	R = 100	R = 100
		I = 16.67				
		S = 33.33				
OA	2	R = 66.67	R = 100	R = 50	R = 60	R = 100
		I = 20		I = 0	I = 40	
		S = 13.33		S = 50	S = 0	
DO	30	R = 33.33	R = 100	R = 75	R = 80	R = 100
		I = 16.67		I = 0	I = 0	
		S = 50		S = 25	S = 20	
TE	30	R = 50	R = 100	R = 75	R = 80	R = 100
		I = 16.67		I = 0	I = 0	
		S = 33.33		S = 25	S = 20	

Remark : R = resistant, I = Intermediate, S = sensitive

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