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Identification and Antibiotic Sensitivity Test of the Bacteria Isolated from Tra Catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) Cultured in Pond in Vietnam

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ABSTRACT

Tra catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) cultured in Vietnam is one of the most important products for export to international market such as U.S.A., Japan and Europe. The bacterial isolation was conducted from pond-cultured Tra catfish in the provinces with intensive culture system. There were a total of 97 isolates from 65 diseased fish. Sixty-six isolates from 97 isolates were identified by biochemical tests. They were consisted of *Edwardsiella ictaruli* (58 isolates [87.9%]), *Aeromonas hydrophila* (2 isolates [3.03%]) and 6 unidentified isolates (9.07%). Antibiotic sensitivity test was also conducted to determine the drugs that could be used to control these two bacteria. Effective drugs that showed good sensitivity against *E. ictaluri* were ciprofloxacin, amoxycillin and ampicillin, florfenicol, doxycycline and oxytetracycline (in order of number of isolates that showed sensitive). Two isolates of *Aeromonas hydrophila* were sensitive to sulphamethoxazole, ciprofloxacin, oxytetracycline, enrofloxacin, erythromycin, sulphamethoxazole/trimethoprim, doxycycline and florfenicol.

Key words: *Pangasianodon hypophthalmus*, bacterial identification, antibiotic sensitivity test

INTRODUCTION

Tra catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) is one of the most common fish being cultured in Vietnam with rapid development in the provinces of Cuu Long Delta including the province of An Giang, Dong Thap and Can Tho. The production of this catfish reached 500,000 tons in 2005 which was increased by 150% in 2006. Not only for exports but also the domestic consumption was increased drastically by 30 % during this period.

However, it appears that the higher the increase of fish production, the higher the risk of disease outbreak. The main reason is a high density of cultured fish in the intensive system. The waste from the unconsumed feed, excretion and the poor pond management lead to fish disease occurring.

Among the disease incidence commonly in Tra catfish, the bacterial infection is worth discussed and considered due to the high mortality and the loss of income. For example, the *Edwardsiella ictaluri* infection, firstly reported from channel catfish cultured in U.S.A., could damage 50 % of production every year (Hawke, 1979) and the bacteria can cause 50-90% mortality for Tra catfish in Vietnam (Dung *et al.,* 2004).

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Bacteria always presents in the water environment. It can grow to large number, invade to fish and spread the disease when reaching the suitable condition (Valerie *et al.*, 1993). The investigation to the appearance of bacteria that causes the disease from the organs of the fish is important in terms of providing the guidelines for prevention, control and treatment of disease problems.

The objectives of this study were to investigate the cause of bacterial infection in Tra catfish cultured in the earthen pond of Vietnam. Antibiotic sensitivity test were also conducted with isolated bacteria to determine which drugs were effective to control pathogen.

MATERIALS AND METHODS

Clinical signs of the diseased fish

External examination

The diseased fish may or may not develop the typical symptoms for each specific disease depending on the etiological agent. To diagnose as well as to identify the disease, the external appearance of animal has to be observed by behavior and physical change. There are some diagrammatic representations of possible external disease signs on fish such as sluggish behavior, twirling, spiral movement, faded pigment, darkened pigment, swollen-eye (exophthalmia), hemorrhage in the eye and mouth, erosion of the jaws, hemorrhage in the opercula region, gill damage, white nodules on the gills or skin, white spots on the head, fin rot and hemorrhage at the base of fins (Austin and Austin, 1993).

Internal examination

The symptom of internal organs of diseased fish was observed including gas filled, abnormal color of organ such as discoloration, hemorrhages, swollen kidney, heart, intestine, pale spleen and liver.

Bacterial isolation and identification

The diseased Tra catfish were collected randomly from the farms in 5 provinces namely Vinh Long, Can Tho, Dong Thap, An Giang and Ben Tre with the total of 65 samples. All farms were operated by earthen pond system with the water supply from the branch of Mekong River. The average pond size was about 0.5 ha with stocking density of 40-50 fry/sq.m. Water was pumped in twice a day. Commercial floating feed were applied twice a day. Market size at 0.6-1 kg/fish could be achieved within 6 months.

External and internal clinical signs of infected fish were observed and noted as described. After opening the body wall of fish, the surface of organs (liver, spleen and kidney) was sterilized by swabbing with 70% ethanol before the isolation of the bacteria. Pure bacterial colonies were isolated on the Brain Heart Infusion Agar (BHIA) plates and incubated at 28-30°C (Vinitnantharat, 1987). All isolates were tested and identified by Gram's stain, catalase, oxidase test, motility, O/F test and API 20E (BioMerieux).

Antibiotic sensitivity test

Bacteria was scrapped off the agar media and diluted in 0.85% sodium chloride and matched with the McFarland number corresponding for 3×10^8 cells/ml. Then, by using the cotton swab, the bacterial suspension was spread on Mueller Hinton agar (MHA) plate or on BHIA in case of poor growth of bacteria (Hawke,1979). Four to five antibiotic discs were placed on the prepared media plate and incubated at 30°C for 24 hours. Diameter of developed clear zone of each disc was measured and compared to the standard value and classified each drug as R for Resistant, I for Intermediate and S for Sensitive (Susceptible). The antibiotic discs included sulphamethoxazole RL/25μg, ciprofloxacin CIP/5 μg, amoxycillin AMOX/25 μg, ampicillin AMP/10 μg, oxytetracycline OT/30 μg, enrofloxacin ENR/5 μg, erythromycin E/15 μg, sulphamethoxazole/

trimethoprim SXT/25 μg, florfenicol FFC/30 μg and doxycyline DO/30 μg.

RESULTS

Clinical signs

The infected Tra catfish showed similar behavior, external and internal clinical signs, such as loss of appetite, loss of pigmentation and lethargic swimming behavior. The external clinical signs included hemorrhage on the anus, fin and scattering on their skin and in some serious cases with the hole on its skull. There were many small spots of lesion with the diameter 1-3 mm developed in the internal organs including liver, kidney and spleen. These organs were swollen and quite soft when touched.

Isolation and identification of bacteria samples by biochemical characteristics

There were a total of 97 bacterial isolates from 65 fish samples in which 66 isolates were identified by biochemical tests with API 20 E and some additional tests. The results showed 58 isolates identified as *Edwardsiella ictaruli* (87.9%), 2 isolates as *Aeromonas hydrophila* (3.03%) and 6 unidentified species (9.07%).

The results of biochemical tests for 58 *E. ictaruli* isolates are presented in Table 1. Two of these test results were different from the tests firstly described by Hawke (1979) including Citrate (Simmons') (41 isolates as positive) and Gelatin (4 isolates as positive). Hawke (1979) reported the Citrate (Simmons') and Gelatin test to be negative. For *Aeromonas hydrophila*, by checking from the database system of the API 20E identification, the percent of identification was 99% reliable.

Antibiotic sensitivity test

From 58 isolates of *E. ictaruli* tested for antibiotic sensitivity with 10 antibiotics, it was found that the most effective antibiotic was ciprofloxacin (91.4% isolates showed sensitive), followed by ampicillin and amoxycillin (84.5%), florfenicol (74.1%), doxycycline (68.9%) and oxytetracycline (63.7%). All isolates were resistant to sulphamethoxazole, erythromycin and sulphamethoxazole/trimethoprim. In addition, there were 39 isolates that were intermediate to enrofloxacin (70.6%) and only 5 and 4 isolates of *E.ictaruli* showing intermediate to ciprofloxacin (8.6%) and doxycycline (6.8%) respectively. Resistance was also found in oxytetracycline (36.2%), florfenicol (25.9%), ampicillin and amoxycillin (15.5%) and enrofloxacin (12.1%) (Figure 1).

Two isolates of *Aeromonas hydrophila* were sensitive to sulphamethoxazole, ciprofloxacin, oxytetracycline, enrofloxacin, erythromycin, sulphamethoxazole/trimethoprim, doxycycline and florfenicol. Both isolates were resistant to amoxycillin and ampicillin.

DISCUSSION

The study on bacterial diseases of Tra catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) cultured in earthen pond of Vietnam clearly showed that the majority of infection was caused by *Edwardsiella ictaruli* counting for 87.9% of all isolates. *E. ictaluri* was first reported by Hawke (1979) who had isolated and described this bacteria from pond-cultured channel catfish (*Ictalurus punctatus)* in U.S.A and named this disease "Enteric Septicemia of Catfish" (ESC) (Hawke *et al.*, 1981). ESC was believed to be infectious only to ictalurids. Sanchez (1981) studied the susceptibility of five species of fish to *E. ictaluri* and found that only channel catfish was highly susceptible. Tilapia (*Tilapia aurea*) was slightly susceptible while largemouth bass (*Micropterus salmoides*), golden shiner (*Notemigonus crysoleucas*) and bighead carp (*Aristichthys nobilis*) were not susceptible to *E. ictaluri*. The results from this study clearly indicated that

P. hypophthalmus was highly susceptible to this enteric bacteria. This disease was firstly reported in *Pangasius* sp. in Vietnam in 2001 by Ferguson *et al*. (2001) who had found several species of bacteria from affected fish including *Bacillus* and named this disease "bacillary necrosis". Crumlish *et al*. (2002) studied the same disease in Vietnam in *Pangasius hypophthalmus* and confirmed the pathogen as *Edwardsiella ictaluri* with slightly different biochemical characteristics from two type strains from UK. Both reports indicated the same clinical signs of affected fish as found in this study including multifocal irregular white lesions in liver, spleen and kidney. In additions to *E. ictaruli*, *Aeromonas hydrophila* was also another pathogenic bacteria that could cause disease in Tra catfish, but the incidence of this Aeromonad septicemia was very low (3.03%) when compared

Table 1 Biochemical tests for *Edwardsiella ictaruli* isolates compared to type strain of Hawke (1979).

Biochemical tests	Type strain of Hawke (1979)	Number of *E. ictaruli* isolates		
		Number of isolates with +ve reaction	Number of isolates with -ve reaction	Percents of isolates with +ve reaction
Gram's stain	-	0	58	0
Oxidase	-	0	58	0
Catalase	+	58	0	100
Motility at 25 and 30°C	-	0	58	0
O/F	+/+	58	0	100
TSI	K/A	58 (K/A)	0	100
ONPG	-	0	58	0
Arginine dihydrolase	-	0	58	0
Lysine decarboxylase	+	58	0	100
Ornithine decarboxylase	-	0	58	0
Citrate (Simmons')	-	41	17	70.6
Hydrogen sulfide	-	0	58	0
Urease	-	0	58	0
Tryptophane Deaminase	-	0	58	0
Indole production	-	0	58	0
Voges-Proskauer	-	0	58	0
Gelatin	-	4	54	6.8
Glucose	+	58	0	100
D-Mannitol	-	0	58	0
Inositol	-	0	58	0
Sorbitol	-	0	58	0
Rhamnose	-	0	58	0
Sucrose	-	0	58	0
Melibiose	-	0	58	0
Amygdaline	-	0	58	0
Arabinose	-	0	58	0
Nitrate reduction	+	58	0	100
Antibiotic sensitivity test				

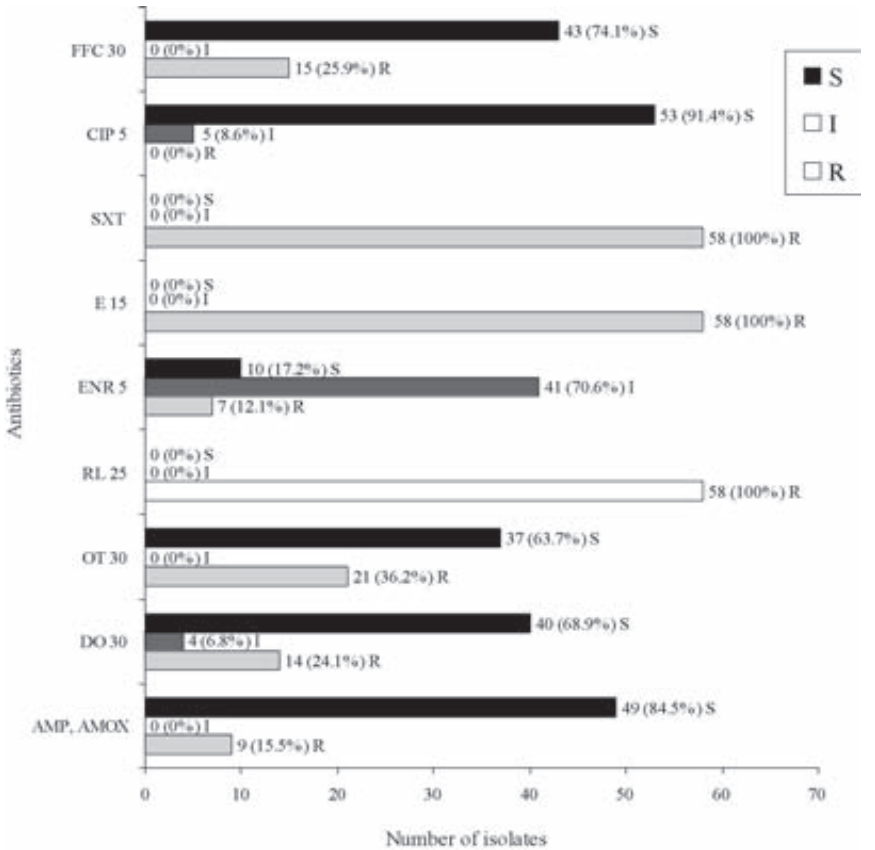

Figure 1 The number of *E.ictaruli* isolates showing sensitive (S), resistant (R) or intermediate (I) to 10 antibiotics (sulphamethoxazole RL/25μg; ciprofloxacin CIP/5 μg; amoxycillin AMOX/25 μg; ampicillin AMP/10 μg; oxytetracycline OT/30 μg; enrofloxacin ENR/5 μg; erythromycin E/15 μg; sulphamethoxazole/trimethoprim SXT/25 μg; doxycycline DO/30 μg and florfenicol FFC/30 μg).

with *E. ictaruli* cases. The external and internal clinical signs of these two diseases were quite similar including hemorrhage in the infected organs, and swollen eyes (Angka *et al*., 1995) (Figure 2). And to distinguish the difference between these two pathogens, only *E. ictaruli* had the specific internal clinical sign including severe necrosis in the trunk kidney and many small white spots of lesion with the 1-3 mm diameter developing in the severe infected organ including liver, kidney and spleen (Dung *et al*., 2004).

From 58 isolates identified as *Edwardsiella ictaluri* from this study, there were 2 from 27 biochemical tests that were slightly

Figure 2 Hemorrhagic and swollen eyes of Tra catfish infected by *Edwardsiella ictaruli*.

different from the type strain reported by Hawke (1979). For gelatin there were 4 isolates with positive reaction instead of negative as reported by Hawke (1979). However, there were 41 isolates that gave the positive reaction on Simmons' citrate while Hawke (1979) reported negative reaction. These differences were not surprising considering the differences of fish species and environment.

In the antibiotic sensitivity test, most isolates (over 60%) of *E. ictaruli* were sensitive to 6 antibiotics where ciprofloxacin showed the highest antimicrobial activity (54 isolates, 91.4%) followed by ampicillin and amoxycillin, florfenicol, doxycycline, and oxytetracycline. On the other hand, all 58 isolates showed resistant to sulphamethoxazole, erythromycin and potentiated sulphonamide (sulphamethoxazole + trimethoprim). Some isolates showed resistant to oxytetracycline (36.2%), florfenicol (25.9%) and ampicillin and amoxycillin (15.5%). This resistance might suggest the status of non-control use of drugs to treat the bacterial disease that lead to the high resistance of bacteria especially with the drug that had been used for a long period of time (Kha and Hung, 2007). For *Aeromonas hydrophila*, both isolates showed sensitive response to oxytetracycline, potentiated sulphonamide, sulphamethoxazole, ciprofloxacin, erythromycin, enrofloxacin, florfenicol and doxycycline but they were resistant to ampicillin and amoxycillin.

Vinitnantharat (1987) reported that oxytetracyline was the choice to treat *E. ictaluri* infection because all of 40 tested isolates showed sensitive result, while erythromycin also showed good inhibition of *E. ictaruli*. The study in Vietnam by Crumlish *et al*. (2002) showed that all *E. ictaluri* isolates showed either partial or full resistance to oxytetracycline, sulphamethoxazole + trimethoprim and oxolinic acid while drugs that showed sensitive reaction to all isolates were furazolidone, ciprofloxacin, nitrofurantoin, norfloxacin, gentamycin, enrofloxacin, florfenicol and amoxycillin.

Oxytetracycline and florfenicol are licensed drugs that are permitted to be used in Vietnamese aquaculture. However, the finding from this study indicated some degree of bacterial resistance to these two drugs especially with oxytetracycline which had been used for quite a long time in aquaculture all around the world.

CONCLUSION

The production of Tra catfish (*Pangasianodon hypophthalmus* [Sauvage, 1898]) has been retarded by bacterial disease outbreak throughout the intensive culture area. *Edwardsiella ictaluri* was the major cause of bacterial disease of this species. The effective antibiotics for the bacteria were ciprofloxacin, amoxycillin, ampicillin, florfenicol, doxycycline and oxytetracycline. However, fish farmers should avoid antibiotic application due to the negative consequences such as drug residue which is quite important issue for product quality. Disease prevention should be carried out by means of good culture and health management to ensure the optimum yields and the best quality of the products.

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