Original Article

In vitro Antibiotic Susceptibility of *Aeromonas hydrophila* Isolated From Disease Ornamental Fish

Malinee Jongjareanjai* Nongnut Assawawongkasem Nantarika Chansue

Abstract

Thirty bacterial isolates were derived from ornamental fish patients (n=30) of the Veterinary Medical Aquatic Animal Research Center (VMARC), Faculty of Veterinary Science, Chulalongkorn University. The isolated bacterial strains were identified by using commercial biochemical identification kit (API Kits, bioMerieux® SA France). The antibiotics susceptibility testing was performed by disc diffusion method, using 24 types of antibiotic discs (OXOID, Oxoid Ltd, UK). The result showed that the majority of the isolated bacteria was *Aeromonas hydrophila* (27/30). In the *A. hydrophila* susceptibility study, 66.30% of bacteria were antibiotic resistant, 9.35% were intermediately sensitive and 24.35% were sensitive strains against the tested antibiotics. Chloramphenicol showed the highest efficacy against the bacterial strains tested. Other effective antibiotics included sulphamethoxazole-trimetroprim and amikacin. 100% of the isolated bacteria showed resistance to metronidazole, 92.31 were resistant to penicillin and amoxicillin. It is noticeable that there were large numbers of antibiotic resistance fish pathogenic bacteria in ornamental fish, which indicated that the risk of ineffective antibiotic treatment without laboratory susceptibility test in sick fish would be at least 75.65% in doubt.

Keywords : antibiotic, bacteria, ornamental fishes, sensitivity.

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บทคัดย่อ

ผลทดสอบความไวรับของเชื้อ *Aeromonas hydrophila* ต่อยาปฏิชีวนะที่ได้จากปลาสวยงามป่วย

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การศึกษาเพาะแยกเชื้อแบคทีเรีย 30 ตัวอย่างจากปลาสวยงามป่วย (n=30) ที่ได้จากศูนย์วิจัยโรคสัตว์น้ำ (VMARC) จุฬาลงกรณ์มหาวิทยาลัย โดยวินิจฉัยแยกเชื้อด้วยชุดทดสอบคุณสมบัติของเชื้อทางชีวเคมีสำเร็จรูป API Kits bioMerie® SA France) และทดสอบความไวของเชื้อต่อยาปฏิชีวนะ 24 ชนิดด้วยแผ่นทดสอบสำเร็จรูป (OXOID, Oxoid Ltd, UK) ผลการศึกษาพบว่าเชื้อแบคทีเรียที่พบมากที่สุดในปลาสวยงามคือ *Aeromonas hydrophila* (27/30) เมื่อทดสอบความไวของเชื้อ *A.hydrophila* ต่อยาปฏิชีวนะโดยเทียบเป็นสัดส่วนพบว่าตัวอย่างเชื้อทั้งหมดมีความดื้อร้อยละ 66.30 มีความไวปานกลางร้อยละ 9.35 และมีความไวร้อยละ 24.35 ต่อยาปฏิชีวนะที่ทดสอบ โดยในการศึกษานี้พบว่ายา chloramphenicol มีประสิทธิภาพในการต่อต้านเชื้อแบคทีเรียที่แยก และพิสูจน์ได้มากที่สุด ลำดับถัดมาคือ sulphamethoxazole-trimetroprim และ amikacin ในทางตรงข้าม metronidazole เชื้อมีความดื้อต่อยาร้อยละ 100 และมีความดื้อต่อยา penicillin และ amoxicillin ร้อยละ 92.31 การศึกษานี้แสดงให้เห็นถึงอัตราการดื้อต่อยาปฏิชีวนะที่มีสูงของเชื้อโรคในปลาสวยงาม และมีโอกาสผิดพลาดจากการใช้ยาปฏิชีวนะอย่างน้อยร้อยละ 75.65 หากไม่มีการทดสอบความไวของเชื้อต่อยาก่อนการใช้รักษา

คำสำคัญ : ยาปฏิชีวนะ แบคทีเรีย ปลาสวยงาม ความไวรับ

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Introduction

Bacterial disease is one of the most important diseases in ornamental fishes and a significant cause of high fish morbidity and mortality rates (Barker, 2001). Different pathogenic, especially, gram negative bacteria has been reported to be isolated from naturally-infected fish. Many stress factors could contribute to bacterial infection in ornamental fish, namely, poor water quality, crowding, transportation and inadequate nutrition. (Musa et al., 2008)

A. hydrophila is also considered a normal flora as well as a primary and secondary fish pathogen, including ornamental fish (Austin and Austin, 1999). Hettiarachchi and Cheong (1994) described *A. hydrophila* as the major cause of disease in freshwater ornamental fish. The clinical signs are presence of eroded fins, hemorrhages on the skin and at the base of the caudal fin, sloughing scales and hemorrhaging intestinal wall. Also, Musa et al. (2008) reported that bacterial isolated from sick freshwater ornamental fish from aquarium shops in Malaysia was mostly *A. hydrophila* (60%).

Antibiotics have been used for treatment and prevention of bacterial diseases, but the success of treatment depends on antibiotic susceptibility of etiologic bacteria (Yanong, 2006). Bacterial resistance is an important issue that needs to be considered when choosing an appropriate antibiotic. While it may be necessary to start treatment course based on personal experience, it is also prudential to have bacterial identification and antibiotic sensitivity tests carried out at the same time. However, field trials and antibiotics susceptibility profiles are essential for effective treatment.

In this study, an *in vitro* screening of a wide range of antibiotics was carried out to investigate their potential efficiency against bacteria isolated from diseased ornamental fish. The objectives of this study were to investigate the prevalence of pathogenic bacterial species and to evaluate the antibiotic susceptibility profile of

major bacterial pathogens causing ornamental fish diseases in Thailand.

Materials and Methods

Bacteria isolation: The bacterial isolates used in this study were derived from 30 ornamental fish patients (1 isolate was taken from 1 fish) brought by owner into the Veterinary Medical Aquatic Animal Research Center (VMARC), Faculty of Veterinary Science, Chulalongkorn University, Thailand for diagnosis and treatment during 2008. The samples were collected from the lesions and isolated by standard biological methods using blood agar and Müeller-Hinton agar. The isolated bacterial strains were identified using an appropriate type of commercial biochemical identification kit (API20E or API20Strep or API20NE kits, bioMerieux® SA France).

Antibiotic susceptibility: The antibiotic test discs (OXOID, Oxoid Ltd, UK) with their concentrations shown were used to detect antibiotic susceptibility of bacterial isolates as follow: penicillin (P) 10 U, erythromycin (E) 15μg, kanamycin (K) 30 μg, streptomycin (S) 10 μg, chloramphenicol (C) 30 μg, nalidixic acid (NA) 30 μg, nitrofurantoin (F) 300 μg, sulphamethoxazole-trimetroprim (SXT) 25 μg, gentamicin (CN) 10 μg, neomycin (N) 10 μg, oxolinic acid (OA) 2 μg, tetracycline (TE) 30 μg, furazolidone (FR) 15 μg, oxytetracycline (OT) 30 μg, novobiocin (NV) 30 μg, ampicillin (AMP) 10 μg, colistin (CT) 10 μg, cephalothin (KF) 30 μg, norfloxacin (NOR) 10 μg, ciprofloxacin (CIP) 5 μg, enrofloxacin (ENR) 5 μg, amoxycillin (AML) 10 μg, amikacin (AK) 30 μg and metronidazole (MT) 2 μg.

Antibiotic susceptibility test was performed by disc diffusion method in Müeller-Hinton agar plates. Then, they were inoculated with 0.1 ml isolated bacteria which had been cultured in glucose-supplemented (1%) nutrient broth for 18 hrs. Antibiotic-impregnated discs were placed on the solid medium and incubated at 30°C for 24 hrs. Then zones of inhibition formed around the disc were measured. Antibiotic susceptibility was determined by the diameter size of the clear zone. The zone radius was scaled from the center of the antibiotic disc to the end of the clear inhibition zone. Zone diameters were interpreted as sensitive, intermediate and resistant according to the clinical and laboratory standard Institute (CLSI, 2005)

Results

From this study, the result showed that the majority of the isolated bacteria was *Aeromonas hydrophila* (27/30). One strain of *Enterococcus durans, Flavobacterium* sp. and *Serratia marcescens* were also isolated from sick ornamental fish.

Figure 1 showed the antibiogram of the *A. hydrophila* isolates (n=27) against 24 types of antibiotics. In the present study, there were 66.30% cases of antibiotic resistance, 9.35 % of intermediate sensitivity and 24.35% cases of sensitive bacterial strains against the tested antibiotics. The data indicated that 100% of the bacteria tested were resistant to metronidazole, 92.31% were resistant to penicillin and amoxicillin, 88.89% were resistant to colistin, 84.62% were resistant to oxytretacycline, 83.33% were resistant to ampicillin, 81.82% were resistant to tetracycline, 80.95% were resistant to neomycin, and 80.0% were resistant to novobiocin.

Chloramphenicol showed the highest efficacy against the bacterial strains tested (59.09% sensitive and 31.82% resistant). Other effective antibiotics were sulphamethoxazole-trimetroprim (58.33% susceptibility and 41.67% resistant), amikacin (50.00% sensitive and 50.00% resistant).

Discussion

Aeromonas hydrophila was the dominant specie (89.66%) found as the cause of bacterial disease in ornamental fish investigated in this study. *A. hydrophila* has been the most common bacteria associated with aquatic animal disease (Barker, 2001). In Malaysia aquarium shop, 60% of *A. hydrophila* were isolated from sick freshwater ornamental fish (Musa et al, 2008). and

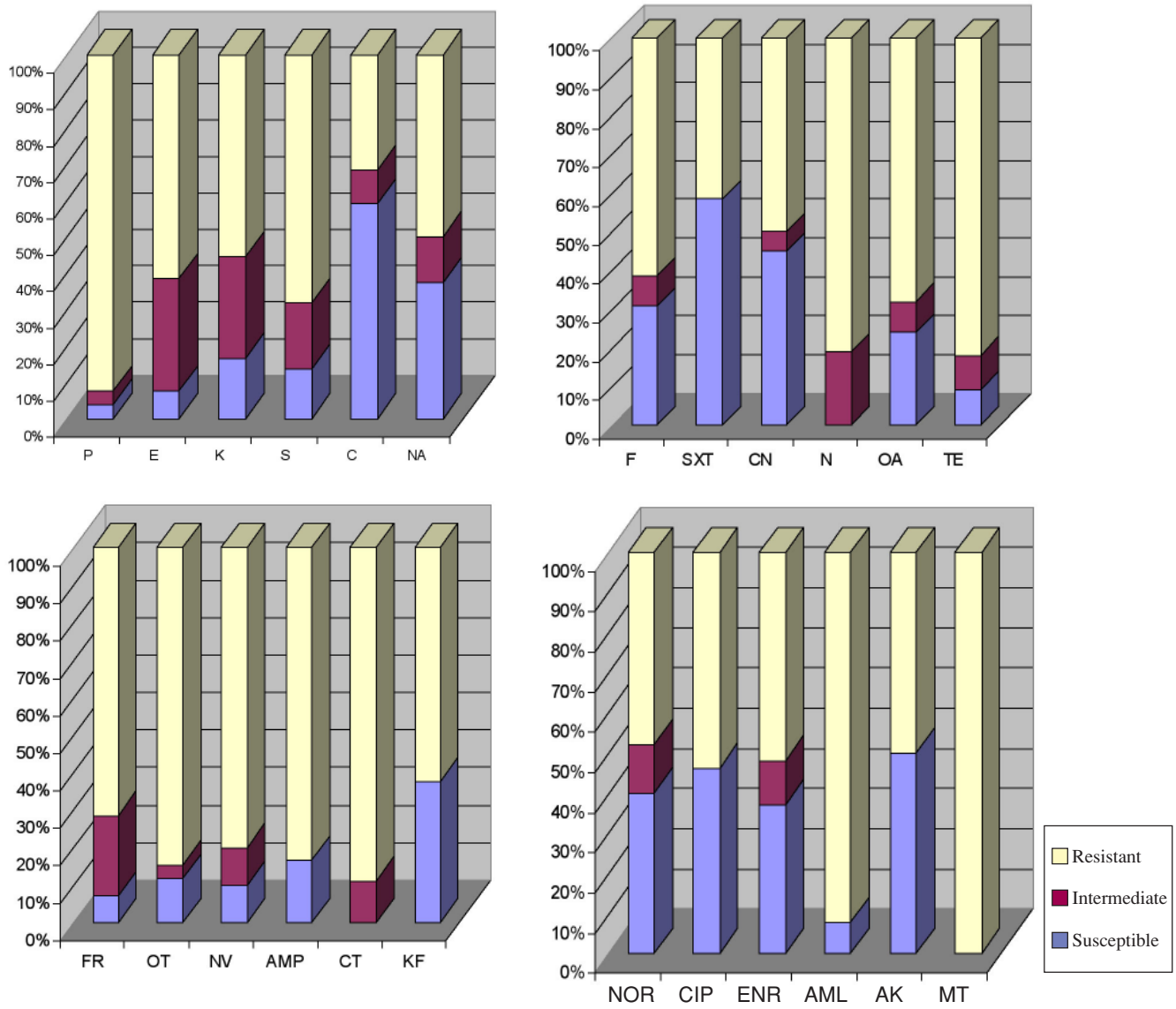

Figure 1 Distribution of bacterial susceptibility to antibiotics (%). P: penicillin, E; erythromycin, K: kanamycin, S: streptomycin, C: chloramphenicol, NA; nalidixic acid, F: nitrofurantoin, SXT: sulphamethoxazole-trimetroprim, CN: gentamicin, N: neomycin, OA: oxolinic acid, TE: tetracycline, FR: furazolidone, OT: oxytetracycline, NV: novobiocin, AMP: ampicillin, CT: colistin, KF: cephalothin, NOR: norfloxacin, CIP: ciprofloxacin, ENR: enrofloxacin, AML: amoxycillin, AK: amikacin, MT: metronidazole.

in the prevalence study of fish and prawn from south India market, 33.5% and 17.6% of *A. hydrophila* were isolated, respectively (Vivekananandhan et al., 2002).

From the present study, Chloramphenicol was found to be more effective in inhibiting growth of the *A. hydrophila* isolates than other drugs therefore, it would be the first drug of choice in application except for the adverse effects and unsuitabilities for food fish. The second drugs of choice were sulphamethoxazole-trimetroprim and amikacin, respectively. On the contrary, metronidazole and colistin would not be recommended for use as antibacterial drugs of choice (100% resistant) while penicillin and amoxicillin were similar (92.31% resistant). Normally, enrofloxacin and oxytretacyclin were widely used for treatment of bacterial infection in aquatic animals as the drug of choice, but this study showed that enrofloxacin (37.04% susceptibility and 51.85% resistant), and oxytretacyclin (11.54% susceptibility and 84.62% resistant) had poor efficacy to eliminate the bacteria.

Our studies also revealed that all bacterial strains had some level of antibiotic resistance. This might indicate antibiotic misuse in aquatic animals. The results showed that none of the tested drugs yielded over 59.09% sensitivity. The minimum resistance of the drugs was as high as 31.82%. Also, the average antibiotic resistance in this study was as high as 66.30% % and only 24.35% were sensitive against the tested antibiotics. This indicated that the risk of ineffective antibiotic treatment without laboratory susceptibility test in sick fish would be at least 75.65% in doubt. This would be considered as a high level of resistance compared to previous reports. Musa et al. (2008) showed average antibiotic resistance in ornamental fish to be 41.85% with 23% intermediary and 34.5% sensitive cases. The report on drug resistance of motile *Aeromonas sp*. of fresh water fish farm by Hatha et al. (2005) showed that 100% of the bacteria tested were resistant fish bacterial pathogens to ampicillin, 94.5% were resistant to novobiocin, 52.7% were resistant to amoxicillin and 40% were resistant to oxytetracycline. However, resistance to chloramphenicol, gentamicin and nalidixic acid (5.5, 7.3 and 1.8% resistance, respectively) were lower in their studies compared to the present study. It is noticeable that the high level of antibiotic resistance fish bacterial pathogens in Thailand may be due to the ineffective control on the use of fish medicine. Norvick (1981) stated that the subtherapeutic use of antibiotics in animal husbandry practice has promoted the emergence and maintenance of multiple antibiotic resistant (MAR) pathogenic bacteria. There was overall increase in drug resistance, fish-pathogens in parallel with the extensive use of chemotherapeutic agent in Japan (Aoki, 1992). The development of high antibiotic resistance trend create a great deal of difficulty of the treatment of bacterial infections in fish. Both high antibiotic resistant bacterial strains and antibiotic residues may contaminate natural environments, thus potentially integrated into human and other animal food chains which should not be overlooked in all parties concerned.

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