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Short communication

The Prevalence and Antimicrobial Susceptibility of Aeromonas Hydrophila Infection of Marketed Catfish in Besut, Terengganu

Fatin Nabilah Binti Meor Aziz[1], Noor Afiza Badaluddin[2], Norshida Binti Ismail[3]*

Faculty of Bioresources and Food Industry,
Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia.
**Corresponding author E-mail: norshidaismail@unisza.edu.my*

Abstract

The commercial catfish covers about 37% of value of production in aquaculture sectors in Malaysia. *Aeromonas hydrophila* is the most common bacterial infection that occurred in catfish culture, mainly due to poor environment and water quality. The occurrence of bacterial disease affect the economical production of farm and may contribute to the excessive usage of antibiotics which can lead to antibiotic resistance problem. This recent study aimed to investigate the prevalence and antimicrobial susceptibility of *A. hydrophila* infection in commercial hybrid catfish, (*Clarias gariepinus* × *C. batracus*) from several wet markets in Besut, Terengganu, Malaysia. A total of 28 catfish were collected randomly from different wet markets in Besut area. The organs such as heart, liver and spleen were taken out and inoculated on Blood Agar with ampicillin. The suspected colonies were subculture for at least three times to get pure colonies. For the identification of *A.hydrophilla*, the pure colonies were analysed using Gram's staining technique and five biochemical tests namely; Catalase, Oxidase, Methyl Red, Voges Proskauer and Triple Sugar Iron Test. For antimicrobial susceptibility test, the disc diffusion method was conducted by using four types of antibiotic discs, vancomycin, cephazolin, meropenem and colistin sulphate. The prevalence of *A. hydrophila* infection in this study were 18% (n=5). For disc diffusion method, resistance index for all isolates were ranged from 0.25 to 0.50. Therefore, proper management in fish farming area and routine monitoring of drug susceptibility patterns overtime is necessary.

Keywords: *Prevalence, Aeromonas hydrophila, Catfish, Besut, Bacterial infection*

1. Introduction

Primarily, cultured catfish began in the early of 1960's in Malaysia. The catfish production declined at the early 1980's due to disease problem and at mid-year, *Clarias gariepinus* become popular due to faster growth rate, resistant to disease, and readily accepted by natives [1].

According to Department of Fisheries, the commercial catfish (*C. batracus* × *C. gariepinus*) is the important cultured catfish which covers about 37% value of production for fish farming in Malaysia. However, among the challenge awaiting the farmers for export purposes is to get permit and achieve the specified term and criteria of fishes, including free from diseases [2].

Catfish is the main freshwater commodity which is very popular among the people in Terengganu because the price is affordable and it has been assimilated as a local delicacies. People like to buy freshly cut fish, hence, the fishmonger always keep a group of catfish in tanks or small aquarium prior selling. In many cases, the catfish were seen not being kept in optimum captivity condition during this period, which might exposed the fish to stress and diseases especially from bacteria.

The bacterial diseases in cultured fish are considered as main problem in aquaculture system [3]. The *Aeromonas* bacterial species are responsible for wide range of fish and human disease and it has been shown to inflict diseases in warm water fish including catfish. *Aeromonas hydrophila* is a type of bacteria which associate with poor water quality and captivity environment. *A. hydrophila* produced many extracellular proteins associated with pathogenicity and environmental adaptability. Commonly, aerolysin, enterotoxins and hemolysin are the main of virulence factors effected the phatogenicity of *Aeromonas* species and induce the other factors such as adhesin and mucinase production [4]. Shayo et al. [5] reported that the infected fish show clinical abnormalities within four days, resulting in 95% of mortality rate.

A. hydrophila has the ability to grow at refrigerated temperature so it considered as food borne pathogen of emerging importance [6]. The consumption of fish contaminated with *Aeromonas* by exposed human to the risk of foodborne infection [7].

The identification of *A. hydrophila* requires major understanding on the detection of clinical sign shown by infected fish by observing the clinical sign or any abnormality on fish which may be difficult to be performed without proper understanding in disease related knowledge. Hence, this present study is aiming to detect *A. hydrophila* prevalence in marketed catfish in several wet market in Besut and to determine the antibiotic susceptibility patterns of *Aeromonas hydrophila* isolated from marketed catfish. The information gathered in this study will contributed to the baseline data on the *A. hydrophila* infection on catfish from Besut. Which can be used for diseases monitoring and proper management in the future.

2. Methodology

2.1. Laboratory Preparation

Firstly, the glass materials such as beakers, glass rods and broth tubes are washed with detergent, and dry at 70°C in drying oven and sterilized at 170°C for 1 hour. Secondly, all the plastics wares,

such as Falcon tubes and plates are washed, dry at 70°C in drying oven and autoclave at 121°C for overnight before use.

2.2. Sample Collection

The total number of 28 catfish (*Clarias gariepinus*) are collected at wet markets randomly in Besut, Terengganu. Some of fishes had ulcerations on body skin and show the abnormalities in appearance whereas most of fishes appear healthy. Fish infected with *A. hydrophila* will show skin ulceration, abdominal bloating, pale gills, lack of appetite and swimming anomaly [8]. The fishes then are kept in tanks with aeration and transported to the laboratory.

2.3. Streaking from Lesion on BA Plates

Any external and internal lesion samples such as haemorrhage and ulceration on fishes were directly swabbed and inoculated to the BA plates by using sterile cotton swab. Heart, liver and spleen were taken out and swabbed for the same inoculation process. The plates then are incubated at 25°C for 36-48 hours. Individual colonies are separated from plates on the basis of colour, shape and size and further cultured onto relevant media to obtain pure culture.

2.4. Morphological Characterization and Biochemical Identification

The bacteria were sub-cultured on to TSA plates to obtain fresh 24 hours culture. The *Aeromonas hydrophila* grown in yellow colonies. Colonies were sub-cultured again onto TSA plates. Gram's staining was conducted to observe the shape, size and colour of colonies and flagellation. Solution need for Gram's staining are crystal violet, Gram's iodine, 95%.ethyl alcohol and safranin. The separate colonies, subject to undergo 5 types of biochemical identification by using Oxidase, Catalase Test, Triple Sugar Iron, Voges Proskauer, and Methyl Red test.

2.5. Antibiotic Susceptibility Test (AST)

The fresh sub-culture is obtained, and the cultures were inoculated into the Muller-Hinton Broth (MHB) by using loop. Then, the inoculation is waited about 10 to 15 minutes to observe the color changes of MHB. By using the cotton swab, immersed the cotton swab to the MHB and inoculated to MH agar plates gently from side to side. The plates were rotated and continued swabbing for 5 times. The four quadrant were marked, with 4 types of antibiotics, which are Vancomycin, Colistin sulphate, Meropenem and Cephazoline. The antibiotic discs were placed on the agar by using forcep. Then, the plates were incubated at 37°C for 24 hours. The presence of inhibition zone were measured.

3. Results

3.1. Prevalence of *Aeromonas hydrophila* in samples

From 28 samples, five samples gave positive result for the presence of *A. hydrophila.* They were S3, S6, S8, S16 and S22.

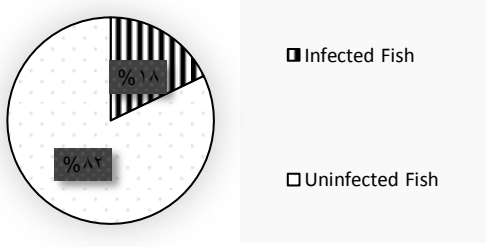

Figure 3.1: The prevalence of *Aeromonas hydrophila* infection on marketed catfish from wet market in Besut, Terengganu

3.2. Antibiotic Susceptibility Test (AST) Result

Table 3.1: Percentage (%) of antibiotic resistance of present isolates and MAR Index for *Aeromonas hydrophila*

Sample Isolated	VA	KZ	MEM	CT	MAR
S3	−−	++	++	++	0.25
S6	−−	+−	++	−−	0.50
S8	−−	++	++	−−	0.50
S16	−−	+−	++	++	0.25
S22	−−	−−	++	++	0.50
% Resistance	**100%**	**20%**	**0%**	**40%**	

VA = Vancomycin, KZ = Cephazolin, CT = Colistin Sulphate, MEM = Meropenem

−− indicate resistance
+− indicate intermediate
++ indicate susceptible

From the table, isolated *A. hydrophila* were resistant toward vancomycin (5 µg) that shown 100% of resistance percentage (5 out of 5). For cephazolin (30 µg) antibiotic, shown 20% of resistancy (1 out of 5 samples) while for colistin sulphate (10 µg) shown 40% of resistancy (2 out of 5). They were no antibiotic resistance for meropenem (10 µg).

Table 3.2: The standard deviation of AST of each present isolate *Aeromonas hydrophila*

Sample	Type of Antibiotics			
	VA	KZ	MEM	CT
S3	0	0	0.707 ± 0.5	0.707 ± 0.5
S6	0	1.414 ± 1.0	0.707 ± 0.5	0.707 ± 0.5
S8	0	0.707 ± 0.5	0.707 ± 0.5	0.707 ± 0.5
S16	0	2.828 ± 2.0	2.727 ± 9.0	0.707 ± 0.5
S22	0.707 ± 0.5	0.707 ±3.5	4.950 ± 3.5	0.707 0.5

4. Discussion

Basically, disease outbreaks occur in many factors, including stress, significant change of environmental condition, transportation, handling equipment, poor of quality water and overpopulation. In this study, five isolates of *A. hydrophila* were derived from 28 samples of catfish, resulting in 18% of the sample shown positive bacterial infection of *A. hydrophila.* Considering that the fish collection was made randomly without focusing on diseased or without selecting only fish showing clinical symptom of *A. hydrophila*, the prevalence called for attention especially on the husbandry and welfare of catfish prior to selling. In comparison to the study by Laith & Najiah [9] which collecting diseased catfish from a farm in Marang River, the percentage of disease cause by *A.hydrophila* was 73.3%. Another study on the diseased ornamental fishes by Musa et al. [10] shows that 60% of fish samples were infected by *A. hydrophila*.

The usage of veterinary drug are often applied in agriculture and aquaculture sectors to protect health and increase the value of aquaculture products which finally may bioaccumulate to the in the edible tissue or muscle of fish and seafood. The consumption of the veterinary drug may result in the exposure of consumers to the potentially harmful residues [11]. Four antibiotic namely; vancomycin, colistin sulphate, cephazolin and meropenem were used in this study due to commonly usage of these antibiotic as prohylactic and therapeutic purposes in fish farming area. Among

these four antibiotic, revealed that all isolated *A. hydrophila* was resistance to vancomycin which relatively similar to previous studies of Awan et al. [12] that stated all isolates *A. hydrophila* get from food including fish, were resistance to vancomycin. This due to the continuous usage of the same type of antibiotic with high dosage in the farming area that encouraged the transferring resistant plasmid and intergons in microorganism [13].

Apart from that, all isolated *A. hydrophila*, showed sensitive to meropenem with average inhibition zone 31.7 mm. These finding is compatible with the previous report from Aravenaroman et al. [14] that stated all *A. hydrophila* isolation were sensitive to meropenem drugs.

Multiple Antibiotic Resistance (MAR) index exposes the spread of bacteria resistance in a given population. In this study, three isolates showed 0.5 of MAR index. MAR index with greater than 0.2 indicated the bacterial derives from environment where numerous antibiotic is used [15]. MAR index in the present study ranged from 0.1 to 0.5 and this finding are accordance to previous study [16] which stated that when MAR index is at range 0.2 to 0.3, it indicated the source of origin and could be from commercial industry.

5. Conclusion

All plant parts of *Garcinia prainiana* tested showed different level of antioxidant and antimicrobial properties. The leaves extract showed moderate potential of antioxidant activity with the inhibition percentage of 70.43%. The IC_{50} value of leaves extract was recorded at 0.26 mg/ml with no IC_{50} value recorded for other plant parts. Antimicrobial assay showed zone of inhibition against *Salmonella typhimurium* ATCC 14028 for leaves and twig extracts at 9 ± 0.73 mm and 7 ± 0.5 mm respectively. For quantitative phytochemical analysis, results showed that there is a correlation between total phenolic content to the inhibition percentage. While, no close correlation was observed between total flavonoid content and antioxidant inhibition percentage, but more in-depth study is required to identify the active compounds presence. As overall, *Garcinia prainiana* can be a potential plant for a source of natural antioxidant. Details of this information are very important in order to provide awareness to others about this endangered plant.

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