Aeromonas hydrophila gene characterization and antimicrobial resistance profile of Aeromonas hydrophila isolated from milkfish (Chanos chanos) in Gresik, Indonesia

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Background and Aim: Motile Aeromonas septicemia is a crucial disease in freshwater fish. Aeromonas hydrophila is a disease agent associated with sporadic fish mortality, food safety, and public health. This study aimed to estimate the prevalence and the presence of the aerolysin gene and antimicrobial resistance profile of A. hydrophila isolated from milkfish in Gresik, Indonesia.

Materials and Methods: A total of 153 milkfish gill samples were collected from 16 locations in Gresik and then cultured and identified using biochemical tests. The aerolysin gene was investigated using a polymerase chain reaction, and antimicrobial resistance profiles of the recovered isolates were investigated.

Results: Of the 153 examined samples, 35 (22.9%) were confirmed positive for A. hydrophila and 22 (62.9%) presented the aerolysin gene. The recovered isolates were resistant to the following antibiotics: Amoxicillin (62.9%), tetracycline (60%), streptomycin (54.3%), cefotaxime (51.4%), gentamycin (31.4%), kanamycin (28.6%), erythromycin (25.7%), chloramphenicol (20%), and trimethoprim (14.3%). Meanwhile, only ciprofloxacin, nalidixic acid, and imipenem were indicated as susceptible.

Conclusion: The presence of the aerolysin gene is vital in determining the virulence of A. hydrophila. The study results indicated a high aerolysin gene prevalence. In addition, this study emphasized antibiotic use monitoring, food safety improvement, and negative impact reduction on human health and the environment.

Keywords: aerolysin gene, Aeromonas hydrophila, antimicrobial resistance, milkfish, public health.

Introduction

Infectious and parasitic disease control in fish is a factor that determines the aquaculture business sustainability levels [1]. Milkfish (Chanos chanos) is a freshwater fish that has a high commodity level in Gresik, Indonesia. Aeromonas hydrophila is one of the Gram-negative bacteria that potentially and massively infect milkfish aquaculture. A. hydrophila infection can occur in high stocking densities, high temperatures, high organic matter, and even in well-maintained ponds. Extreme environments can trigger stress levels in fish and increase the risk of aquaculture-reared fish infections [2].

A. hydrophila optimally grows at a maximum temperature of 38–41°C and a minimum of 0–5°C at a pH of 5.5–9 and reproduces asexually or binary fission with cell elongation followed by nuclear division [3]. A. hydrophila has a habitat in estuarine and freshwater areas, and its presence is related to the content of organic matter or aquatic sediments. In addition, A. hydrophila is found in tropical and subtropical areas [4]. Its infection often occurs in the dry season because of the relatively high organic matter content in the waters. A. hydrophila plays a role in the decomposition of organic matter; thus, it is often observed in reared water [5].

Acute infection can be mediated through wounds, the digestive tract, and gills, then spreads in the blood vessels and causes hemorrhagic septicemia [6]. The study conducted by Rasmussen-Ivey et al. [7] reported...
that *A. hydrophila* has components of hemolysin, cytotoxic enterotoxin, lipase, and aerolysin (*aer-A*) genes that cause acute hemorrhagic septicemia. Inappropriate use of antibiotics has implications for the incidence of antimicrobial resistance, especially multidrug resistance, which is a current issue. Further, the use of antibiotics has the potential to increase *A. hydrophila* resistance in addition to polluting the environment and being expensive [8].

In general, bacteria can resist various antibiotics and enrich their virulence features. Antimicrobial *A. hydrophila* resistance is a global problem due to antibiotic misuse [9]. Thus, this study aimed to estimate the prevalence of *A. hydrophila* isolated from milkfish in Gresik, Indonesia. In addition, the characterization of the *aer-A* gene and antimicrobial resistance profile was emphasized.

**Materials and Methods**

**Ethical approval**

This study was approved by the Ethical Committee of Animal Care and Use, Universitas Airlangga, with reference No.388/HRECC.FO/M/III/2020.

**Study period and location**

This study was performed for 5 months (March–July 2020). The milkfish samples were collected from Dukun (6°58'44.0"S 112°26'24.5"E) (n = 7), Panceng (6°56'29.8"S 112°27'56.7"E) (n = 8), Ujung Pangkah (6°52'49.9"S 112°33'11.7"E) (n = 11), Sidayu (6°58’18.4"S 112°35’17.6"E) (n = 15), Bungah (7°03’19.1"S 112°34’34.9"E) (n = 14), Manyar (7°07’21.2"S 112°36’14.5"E) (n = 11), Gresik (7°09’01.8"S 112°39’11.8"E) (n = 12), Kebomas (7°09’59.1"S 112°38’17.5"E) (n = 7), Dukud Sampayan (7°07’38.2”S 112°31’49.4”E) (n = 8), Cerme (7°12’24.7”S 112°34’36.9”E) (n = 11), Benjing (7°15’02.9”S 112°29’09.2”E) (n = 10), Balong Panggang (7°15’39.3”S 112°25’54.4”E) (n = 7), Wringinanom (7°21’24.6”S 112°31’12.1”E) (n = 8), Menganti (7°17’34.9”S 112°35’07.9”E) (n = 8), Kedamean (7°19’20.5”S 112°33’57.6”E) (n = 7), and Dryorejo (7°21’11.1”S 112°37’43.9”E) (n = 9) (Figure-1). Identification of *aer-A* gene and antimicrobial resistance of *A. hydrophila* were carried out at the Gamma Scientific BioLab, Malang, and Institute of Tropical Diseases.

**Isolation and identification**

A total of 153 freshly dead milkfish were collected from 16 locations in Gresik based on rearing ponds. All samples were transferred to the laboratory in a polyethylene bag and icebox. Swab gills were pre-enriched in alkaline peptone water (Oxoid CM1028, UK) at 37°C for 24 h, then cultured on blood agar (BA) (Oxoid CM55) at 28°C for 24 h. Growth colonies on BA media with hemolytic characteristics were then purified on trypticase soy agar (Oxoid CM131) at 37°C for 18–24 h. Colonies were also subcultured on Rimler-Shotts (RS) (Thermo Fisher Scientific Pte. Ltd., Australia) media at 37°C for 24 h, and then the yellow-colored colonies were further identified according to biochemical characteristics using potassium cyanide (KCN), catalase, oxidase, lipase, gelatinase, and protease tests [10].

**Aerolysin gene identification**

The *aer-A* gene was identified using the primers forward 5’–CAAGAACAAGTTCAAGTGCCA–3’ and reverse 5’–ACGAAAGGTTGGTCCAGT–3’ (GoTaq® Green DNA polymerase, Promega Corp, USA). A total of 12.5 μL of polymerase chain reaction (PCR) buffer mixture was prepared that consisted of 2.5 μL of magnesium chloride (25 mM), 0.5 μL of deoxynucleoside triphosphate mixture (200 μM), 2.5 μL of the forward primer (12 μM), 2.5 μL of the reverse primer (12 μM), 12.5 mM (GoTaq® Green DNA polymerase, Promega Corp), and 3 μL of DNA template [11].

Furthermore, the DNA amplification process for the *aer-A* gene was initiated by denaturation at 95°C for 5 min, annealing at 59°C for 30 s, elongation at 72°C for 30 s, and final elongation at 72°C for 7 min. All stages were repeated for 50 cycles. The amplification results were then visualized with agarose gel electrophoresis of 0.8% buffer, tris-acetate-ethylenediaminetetraacetic acid, and 100 bp marker (GoTaq® Green DNA polymerase, Promega Corp). The *aer-A* gene-positive samples were revealed at an amplicon length of 309 bp [11].

**Antimicrobial resistance evaluation**

*A. hydrophila* isolates were inoculated in tryptic soy broth, then incubated at 35°C for 24 h. Broth suspension was inoculated in the Mueller-Hinton broth (Oxoid CM0405), then the turbidity level was adjusted to the 0.5 McFarland standard. Thereafter, the isolates were streaked on the Mueller-Hinton agar (Oxoid CM0337) followed by disk (Oxoid) placement and incubated at 37°C for 24 h. The antibiotic disks used were amoxicillin (AML, 25 μg), cefotaxime (CTX, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (CIP, 5 μg), erythromycin (E, 15 μg), gentamicin (CN, 10 μg), imipenem (IPM, 10 μg), kanamycin (K, 30 μg), nalidixic acid (NA, 30 μg), streptomycin (S, 10 μg), tetracycline (TE, 30 μg), and trimethoprim (SXT, 25 μg). The evaluation was performed under the Clinical and Laboratory Standards Institute guidelines for the following antibiotics: AML, CTX, C, E, CN, IPM, S, and TE [12]. Meanwhile, CIP was evaluated as per the guideline of the European Committee on Antimicrobial Susceptibility Testing [13], K was evaluated as per the guideline of the Comite de l’Antibiogramme de la Societe Francaise de Microbiologie [14], NA and SXT were evaluated as per the guideline of the French Society of Microbiology [15].

**Statistical analysis**

The data were descriptively evaluated and presented in tables.
Results

Of the 153 collected milkfish samples, 35 (22.9%) were confirmed positive for A. hydrophila infection based on the biochemical evaluation of KCN and oxidase reactions. The isolates were followed by a PCR test to reveal the aer-A gene with 309 bp amplicon size and 100 bp ladder. A total of 22 (62.9%) of the 35 samples were confirmed positive for the aer-A gene (Table-1).

The antibiotic susceptibility investigation reported that all samples were susceptible to CIP and IPM. Meanwhile, the evidence of the highest antibiotic resistance was reported in AML (62.9%), TE (60%), S (54.3%), and CTX (51.4%). The lowest resistance was reported to CN (31.4%), K (28.6%), E (25.7%), C (20%), and SXT (14.3%) (Table-2).

Discussion

This study evaluated 153 samples and reported a prevalence rate of 22.9%. This prevalence remained in a low category compared to the prevalence rate of 80% in tilapia in Egypt [16]. Another recent study in Egypt reported a lower percentage of A. hydrophila from Nile tilapia (41%) [17], 46.4% in freshwater aquaculture in Vietnam [18], 75.4% from seafood in South Korea [19], 53.3% in fresh Mugil flesh [20], 90.16% in Sukabumi, 90.05% in Surabaya, and 88.31% in Jepara isolate [21]. A. hydrophila infection is one of the main focuses and has a crucial impact on the aquaculture sector. This bacterium is pathogenic and causes Motile Aeromonas Septicemia (MAS) disease in freshwater fish culture in tropical areas [22]. In general, the clinical symptoms in the organs as a hemorrhagic septicemia manifestation include ulceration in the eyes, gills, fins, scales, and muscles in the abdominal area. Moreover, hemorrhage is often found in the liver, spleen, intestines, and kidneys when necropsy is performed [23].

We revealed the characteristics of cream-colored bacteria in colonies, rod-shaped cell morphology, Gram-negative, producing catalase enzymes, oxidases, fermenting lactose, and several proteases based on the biochemical A. hydrophila identification (Table-1). A previous study reported the same findings that A. hydrophila is also motile, catalyzes D-mannitol, forms a hemolysis zone on BA media, and properly grows on RS and KCN media [24].

A. hydrophila contains exoenzymes that are encoded by lipase, nuclease, and serine protease genes and contains exotoxin derivatives called aerolysin. Our study identified aer-A genes in 22 of 35 isolates (62.9%) (Table-1). The previous studies indicated aer-A gene in 96% of isolates from catfish [25], 37.5% of clinical isolates in Canada [26], 62.7% of isolates from zebrafish [27], 28.8% of clinical isolates in Tokyo and Kanagawa Prefecture, Japan [28], and 33.3% of isolates from Nile tilapia in Egypt [17]. Aerolysin is known to be highly virulent and increases the pathogenicity of A. hydrophila. An in vitro study revealed that epithelial integrity disorders may occur due to aerolysin lyses tight junction protein as a gastrointestinal mucosal barrier [29]. However, studies on the molecular mechanisms of aerolysin-induced cell lysis remained limited. Some evidence of aerolysin-related pathogenesis is limited to reporting the possibility of...
Table 1: Prevalence of *A. hydrophila* in the examined samples.

| Distributions     | Suspected isolates (%) | BA (n = 7) | TSA (n = 8) | RS (n = 11) | KCN (n = 14) | Catalase | Oxidase | Lipase | Gelatinase | Protease | Confirmed Aeromonas aer-A gene positive (%)
|-------------------|------------------------|-----------|-------------|-------------|--------------|----------|---------|--------|------------|----------|----------------------------------------
| Dukun             | 4 (2.6)                | 4 (2.6)   | 4 (2.6)     | 3 (2.9)     | 3 (2.9)      | 0        | 0       | 0      | 2 (5.7)    | 3 (2)    | 2 (5.7)                                
| Pancen            | 4 (2.6)                | 4 (2.6)   | 4 (2.6)     | 3 (2.9)     | 3 (2.9)      | 0        | 0       | 0      | 2 (5.7)    | 3 (2)    | 2 (5.7)                                
| Ujung Pangkah     | 8 (5.2)                | 7 (6.5)   | 6 (5.5)     | 5 (5.5)     | 5 (5.5)      | 0        | 0       | 2      | 5 (2.6)    | 4 (2.6)  | 2 (5.7)                                
| Sidayu            | 7 (4.6)                | 7 (6.5)   | 5 (5.5)     | 5 (5.5)     | 5 (5.5)      | 0        | 0       | 2      | 5 (2.6)    | 4 (2.6)  | 2 (5.7)                                
| Bungah            | 7 (4.6)                | 7 (6.5)   | 5 (5.5)     | 5 (5.5)     | 5 (5.5)      | 0        | 1       | 1      | 5 (1.3)    | 3 (2)    | 2 (5.7)                                
| Manyar            | 3 (2)                  | 3 (2)     | 3 (2)       | 3 (2)       | 3 (2)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Gesah             | 4 (2.6)                | 2 (2)     | 2 (2)       | 2 (2)       | 2 (2)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Kebomas           | 0 (0)                  | 0 (0)     | 0 (0)       | 0 (0)       | 0 (0)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Duduk Sampeyan    | 0 (0)                  | 0 (0)     | 0 (0)       | 0 (0)       | 0 (0)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Cerme             | 0 (0)                  | 0 (0)     | 0 (0)       | 0 (0)       | 0 (0)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Benjeng           | 0 (0)                  | 0 (0)     | 0 (0)       | 0 (0)       | 0 (0)        | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Balong Panggang   | 1 (0.7)                | 1 (0.7)   | 1 (0.7)     | 1 (0.7)     | 1 (0.7)      | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Wrininganom       | 2 (1.3)                | 2 (1.3)   | 2 (1.3)     | 2 (1.3)     | 1 (0.7)      | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Menganti          | 1 (0.7)                | 1 (0.7)   | 1 (0.7)     | 1 (0.7)     | 1 (0.7)      | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Kedamean          | 4 (2.6)                | 4 (2.6)   | 4 (2.6)     | 4 (2.6)     | 4 (2.6)      | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Driyorejo         | 2 (1.3)                | 2 (1.3)   | 2 (1.3)     | 2 (1.3)     | 2 (1.3)      | 0        | 0       | 1      | 1 (0.7)    | 1 (0.7)  | 1 (0.7)                                
| Total             | 53 (34.6)              | 47 (30.7) | 39 (25.5)   | 35 (22.9)   | 34 (22.2)    | 35 (22.9)| 30 (19.6)| 35 (22.9)| 22 (62.9) | 12 (7.7) | 12 (7.7)                                

BA=Blood agar, TSA=Trypticase soy agar, RS=Rimler-Shotts, KCN=Potassium cyanide. Aerolysin (aer-A) gene investigation at 309 bp in confirmed samples.
transitions that produce new genotypes through transposons or transformations that produce new genotypes through genetic recombination. Resistant genes, through these various mechanisms, can move from one bacterium to another and lead to the rapid spread of resistance [43, 44].

Conclusion

The prevalence of *A. hydrophila* from milkfish in Gresik, Indonesia, is 22.9% and 62.9% of positive isolates confirmed the aer-A gene. Meanwhile, antimicrobial resistance was reported to be expressed in AML, TE, S, CTRX, CN, K, E, C, and SXT. In addition, only CIP and IPM were susceptible to all isolates. However, identifying the pathological lesions in the liver, spleen, and intestine of milkfish in MAS need to be further disclosed. Therefore, the collaboration of veterinarians and aquaculture managers is necessary to mitigate the risk of spreading the disease, particularly, the importance of epidemiological studies to control diseases, curative actions taken, and their impact on public health.

Authors’ Contributions

MTEP, FF, and DKW: Conceptualized and designed the study. MTEP, FF, DKW, and AP: Collected the samples. FF and DKW: Performed sample identification. SK: Data analysis. AP and SK: Visualization and validation of tables and figures. FF, SK, and SC: Drafted the manuscript. MTEP and SC: Revised and submitted the manuscript. All authors have read and approved the final manuscript.

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Table-2: Antimicrobial resistance profile of *Aeromonas hydrophila* isolates (n = 35).

| Antimicrobials | Resistant (%) | Intermediate (%) | Sensitive (%) |
|----------------|--------------|-----------------|--------------|
| AML 25         | 22 (62.9)    | 2 (5.7)         | 11 (31.4)    |
| CTRX 30        | 18 (51.4)    | n/a             | 17 (48.6)    |
| C 30           | 7 (20)       | n/a             | 28 (80)      |
| CIP 5          | n/a          | n/a             | 35 (100)     |
| E 15           | 9 (25.7)     | 4 (11.4)        | 22 (62.9)    |
| CN 10          | 11 (31.4)    | 3 (8.6)         | 21 (60)      |
| IPM 10         | n/a          | n/a             | 35 (100)     |
| K 30           | 10 (28.6)    | 4 (11.4)        | 21 (60)      |
| NA 30          | n/a          | 5 (14.3)        | 30 (85.7)    |
| S 10           | 19 (54.3)    | 5 (14.3)        | 11 (31.4)    |
| TE 30          | 21 (60)      | n/a             | 14 (40)      |
| SXT 25         | 5 (14.3)     | n/a             | 30 (85.7)    |

AML=Amoxicillin, CTRX=Cefotaxime, C=Chloramphenicol, CIP=Ciprofloxacin, E=Erythromycin, CN=Gentamycin, IPM=Imipenem, K=Kanamycin, NA=Nalidixic acid, S=Streptomycin, TE=Tetracycline, SXT=Trimethoprim

Competing Interests

The authors declare that they have no competing interests.

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References

1. Bui, S., Oppedal, F., Sievers, M. and Dempster, T. (2019) Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. *Rev. Aquac.*, 11(1): 168–186.
2. Bebak, J., Wagner, B., Burns, B. and Hanson, T. (2015) Farm size, seining practices, and salt use: Risk factors for *Aeromonas hydrophila* outbreaks in farm-raised catfish, Alabama, USA. *Prev. Vet. Med.*, 119(1): 161–168.
3. Farfán Sellarés, M., Albarán Avila, V., Sanglas Baulenas, A., Loren Egea, J.G. and Fusté Munné, M.C. (2013) The effect of recombination in *Aeromonas*. In: Torrero, D.M., Cortés, A. and Mariño, E.L., editors. Recent Advances in Pharmaceutical Sciences III. Ch. 11. Transworld Research Network, India. p179–193.
4. Hochzedez, P., Hope-Rapp, E., Olive, C., Nicolas, M., Beaucaire, G. and Cabié, A. (2010) Bacteremia caused by *Aeromonas hydrophila* complex in the Caribbean Islands of Martinique and Guadeloupe. *Am. J. Trop. Med. Hyg.*, 83(5): 1123.
5. Erova, T.E., Kosykh, V.G., Fadl, A.A., Sha, J., Homeman, A.J. and Chopra, A.K. (2008) Cold shock exoribonuclease R (VacB) is involved in *Aeromonas hydrophila* pathogenesis. *J. Bacteriol.*, 190(10): 3467–3474.
6. Austin, B. (2010) Vibrios as causal agents of zoonoses. *Vet. Microbiol.*, 140(3–4): 310–317.
7. Rasmussen-Ivey, C.R., Figueras, M.J., McGarvey, D. and Liles, M.R. (2016) Virulence factors of *Aeromonas hydrophila* in the wake of reclassification. *Front. Microbiol.*, 7(8): 1337.
8. Mansour, A., Mahfouz, N.B., Husien, M.M. and El-Magd, M.A. (2019) Molecular identification of *Aeromonas hydrophila* strains recovered from Kafrelsheikh fish farms. *Slov. Vet. Res.*, 56(22): 201–208.
9. Sonkol, R.A., Torky, H.A. and Khalil, S.A. (2020) Molecular characterization of some virulence genes and antibiotic susceptibility of *Aeromonas hydrophila* isolated from fish and water. *Afr. J. Vet. Sci.*, 64(2): 34–42.
10. Pridegon, J.W. and Klesius, P.H. (2011) Molecular identification and virulence of three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009. *Dis. Aquat. Org.*, 94(3): 249–253.
11. Christy, G., Kusdwartati, R. and Handijatno, D. (2019) Determination of the aerolysin gene in *Aeromonas hydrophila* using the polymerase chain reaction (PCR) technique. *IOP Conf. Ser. Earth Environ. Sci.*, 236(1): 012097.
12. Clinical and Laboratory Standards Institute. (2017) M100 Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, United States. p296.
13. CASFM/EUCAST. (2018) *Aeromonas* spp. In: French Society of Microbiology American Society for Microbiology, Washington, DC. p117.
14. CASFM. (2013) Comité de l’Antibiogramme de la Socie’te’ Francaise de Microbiologie; Proposer les Recommandations. United States: CASFM.
15. Gajdács, M. (2019) Resistance trends and epidemiology of *Aeromonas Plesiomonas* infections (RETEPAPI): A 10-year retrospective survey. *Infect. Dis.*, 51(9): 710–713.
16. Abdelsalam, M., Ewiss, M.Z., Khaleda, H.S.,
Mahmoud, M.A., Elgendy, M.Y. and Abdel-Moneam, D.A. (2021) Coinfections of Aeromonas spp., Enterococcus faecalis, and Vibrio alginolyticus isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. *Microb. Pathogen.*, 160(11): 105213.

Tartor, Y.H., El-Naeeby, E.S.Y., Abdallah, H.M., Samir, M., Yassen, M.M. and Abdelwahab, A.M. (2021) Virulotyping and genomic diversity of *Aeromonas hydrophila* isolated from Nile tilapia (*Oreochromis niloticus*) in aquaculture farms in Egypt. *Aquaculture*, 541(8): 736781.

Ninh, D.T., Le, D.V., Van, K.V., Huang Giang, N.T., Dang, I.T. and Hoai, T.D. (2021) Prevalence, virulence gene distribution and alarming the multidrug resistance of *Aeromonas hydrophila* associated with disease outbreaks in freshwater aquaculture. *Antibiotics*, 10(5): 532.

Park, S.M., Kim, H.W., Choi, C. and Rhee, M.S. (2021) Pathogenicity and seasonal variation of *Aeromonas hydrophila* isolated from seafood and ready-to-eat sushi in South Korea. *Food Res. Int.*, 147(9): 110484.

Ahmed, H.A., Mohamed, M.E., Rezk, M.M., Gharieb, R.M. and Abdel-Maksoud, S.A. (2018) *Aeromonas hydrophila* in fish and humans; prevalence, virulotyping, and antimicrobial resistance. *Slov. Vet. Res.*, 55(20): 113–124.

Kusdarwardi, R., Dinda, N.D. and Nurjanah, I. (2018) Antimicrobial resistance prevalence of *Aeromonas hydrophila* isolates from mottle *Aeromonas* septicemia disease. *IOP Conf. Ser. Earth Environ. Sci.*, 137(1): 012076.

Stratev, D. and Odeyemi, O.A. (2017) An overview of motile *Aeromonas* septicemia management. *Aquac. Int.*, 25(3): 1095–1105.

Rodrigues, M.V., Dias, M.F.F., Francisco, C.J., David, G.S., da Silva, R.J. and Araújo, J.P. Jr. (2019) *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) from Brazilian aquaculture: A public health problem. *Emerg. Life Sci. Res.*, 5(1): 48–55.

Singh, V., Somvanshi, P., Rathore, G., Kapoor, D. and Mishra, B.N. (2010) Gene cloning, expression, and characterization of recombinant aerolysin from *Aeromonas hydrophila*. *Appl. Biochem. Biotechnol.*, 160(7): 1985–1991.

Nawaz, M., Khan, S.A., Khan, A.A., Sung, K., Tran, Q., Kerdahi, K. and Steele, R. (2010) Detection and characterization of virulence genes and integrins in *Aeromonas veronii* isolated from catfish. *Food Microbiol.*, 27(3): 327–331.

Wang, G., Clark, C.G., Liu, C., Pucknell, C., Munro, C.K., Kruk, T.M., Caldeira, R., Woodward, D.L. and Rodgers, F.G. (2003) Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. *J. Clin. Microbiol.*, 41(3): 1048–1054.

Li, J., Ni, X.B., Liu, Y.J. and Lu, C.P. (2011) Detection of three virulence genes *ahl*, *ahp* and *aer* in *Aeromonas hydrophila* and their relationship with actual virulence to zebrafish. *J. Appl. Microbiol.*, 110(3): 823–830.

Watanabe, N. and Morita, K. (2020) Diversity in gene arrangement in a DNA region lacking *aer* in clinical and environmental *Aeromonas hydrophila* isolates. *Antonie van Leeuwenhoek*, 113(1): 71–81.

Bücker, R., Krug, S.M., Rosenthal, R., Günzel, D., Fromm, M., Zeitz, M., Chakraborty, T., Fromm, M., Epple, H.J. and Schulzke, J.D. (2011) Aerolysin from *Aeromonas hydrophila* perturbs tight junction integrity and cell lesion repair in intestinal epithelial HT-29/B6 cells. *J. Infect. Dis.*, 204(8): 1283–1292.

Kong, W., Huang, C., Tang, Y., Zhang, D., Wu, Z. and Chen, X. (2017) Effect of Bacillus subtilis on *Aeromonas hydrophila*-induced intestinal mucosal barrier function damage and inflammation in grass carp (*Ctenopharyngodon idella*). *Sci. Rep.*, 7(1): 1–11.

McEwen, S.A. and Collignon, P.J. (2018) Antimicrobial resistance: A one health perspective. *Microbiol. Spectr.*, 6(2): 9.

Juhas, M. (2015) Horizontal gene transfer in human pathogens. *Crit. Rev. Microbiol.*, 41(1): 101–108.

Ho, T.T., Areechon, N., Srisapooame, P. and Mahasawasde, S. (2008) Identification and antibiotic sensitivity test of the bacteria isolated from catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]) cultured in the pond in Vietnam. *Agric. Nat. Res.*, 42(5): 54–60.

Lijon, M.B., Khatun, M.M., Islam, A., Khatun, M.M. and Islam, M.A. (2015) Detection of multidrug resistance *Aeromonas hydrophila* in farm-raised freshwater prawns. *J. Adv. Vet. Anim. Res.*, 2(4): 469–474.

Belem-Costa, A. and Cyrino, J.E.P. (2006) Antibiotic resistance of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). *Sci. Agric.*, 63(3): 281–284.

Montero Llopis, P., Jackson, A.F., Slusarenko, O., Surowtsev, I., Heinritz, J., Emonet, T. and Jacobs-Wagner, C. (2010) Spatial organization of the flow of genetic information in bacteria. *Nature*, 466(7302): 77–81.

Roy, R.N., Lomakin, I.B., Gagnon, M.G. and Steitz, T.A. (2015) The mechanism of inhibition of protein synthesis by the proline-rich peptide Oncocin. *Nat. Struct. Mol. Biol.*, 22(6): 462–469.

Anju, C.P., Subbramanian, S., Sizochezko, N., Melge, A.R., Leszcynski, J. and Mohan, C.G. (2019) Multiple e-Pharmacophore modeling to identify a single molecule that could target both streptomycin and paromomycin binding sites for 30S ribosomal subunit inhibition. *J. Biomol. Struct. Dyn.*, 37(6): 1582–1596.

Gagnon, M.G., Roy, R.N., Lomakin, I.B., Florin, T., Mankin, A.S. and Steitz, T.A. (2016) Structures of proline-rich peptides bound to the ribosome reveal a common mechanism of protein synthesis inhibition. *Nucleic Acids Res.*, 44(5): 2439–2450.

Chukwudi, C.U. (2016) rRNA binding sites and the molecular mechanism of action of the tetracyclines. *Antimicrob. Agents Chemother.*, 60(8): 4433–4441.

Atif, M., Asghar, S., Mushtaq, I., Malik, I., Amin, A., Babar, Z.U.D. and Scahill, S. (2019) What drives inappropriate use of antibiotics? A mixed-methods study from Bahawalpur, Pakistan. *Infect. Drug Resist.*, 12(3): 687.

Bello-López, J.M., Cabrero-Martínez, O.A., Ibáñez-Cervantes, G., Hernández-Cortez, C., Pelcastre-Rodriguez, L.I., González-Avila, L.U. and Castro-Escarpulli, G. (2019) Horizontal gene transfer and its association with antibiotic resistance in the genus *Aeromonas* spp. *Microorganisms*, 7(9): 363.

Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A.P. and Nautiyal, A.R. (2017) Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials-a review. *Plants*, 6(2): 16.

Wang, C.H., Hsieh, Y.H., Powers, Z.M. and Kao, C.Y. (2020) Defeating antibiotic-resistant bacteria: Exploring alternative therapies for a post-antibiotic era. *Int. J. Mol. Sci.*, 21(3): 1061.

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