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ORIGINAL ARTICLE

Antibiotic resistance profiling and phenotyping of *Aeromonas* species isolated from aquatic sources

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Abstract This study aimed to investigate antibiotics resistance pattern and phenotyping of *Aeromonas* species isolated from different aquatic sources in Melaka, Malaysia. A total of 53 *Aeromonas* species were isolated from the following sources: sediment (n = 13), bivalve (n = 10), sea cucumber (n = 16) and sea water (n = 14) and resistance to 12 antibiotics – Tetracycline (30 μg), Kanamycin (30 μg), Oxytetracycline (30 μg), Ampicillin (10 μg), Streptomycin (10 μg), Gentamicin (10 μg), Sulphamethoxazole (25 μg), Nalixidic acid (30 μg), Trimethoprim (1.25 μg), Novobiocin (5 μg), Penicilin (10 μg) and Chloramphenicol (10 μg) was tested. The results obtained from this study reveal multi drug resistance pattern among the isolates. All the isolates were completely resistant to Ampicillin, Novobiocin, Sulphamethoxazole and Trimethoprim, respectively but susceptible to Tetracycline (100%), Kanamycin (5.7%), Gentamicin (5.7%) and Oxytetracycline (24.5%). Antibiotics phenotyping of the bacteria revealed 21 different phenotypes among the isolates.

1. Introduction

Aeromonadaceae is an ubiquitous bacterial family due to the fact that members of this family have been isolated from diverse sources such as seafood, river water, bottled water, chlorinated and unchlorinated water, vegetables, pasteurized and unpasteurized milk, individuals with compromised immunity, fresh water (Abulhamd 2010), drinking water (Pablos et al., 2011), (Odeyemi et al., 2012), catfish and tilapia fish (Ashiru et al., 2011; Suhet et al., 2011), cold and warm blooded animals such as birds (Roh et al., 2011), meat products (Dallal et al., 2012), crocodiles (Tel and Keskïn, 2012) and chicken (Kaskhedikar and Chhabra 2009; Papadakis et al., 2012).

Most common biochemical characteristics of members of this family are Gram negative, oxidase positive, facultatively anaerobes, and catalase positive and prevalent in aquatic environment. Due to the ubiquitous nature of members of Aeromonadaceae, humans easily come in contact and become infected with the pathogenic species. However, infections are mostly determined by type of strain and type of virulence factors (Odeyemi and Ahmad 2014). Among virulence factors

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Table 1 Antibiogram profile.

Antibiotics	Number of resistance of isolates				
	Bivalve (n = 10)	Sea Cucumber (n = 16)	Sea water (n = 14)	Sediment (n = 13)	Total
Tetracycline	–	–	–	–	–
Kanamycin	–	3	–	–	3 (5.7%)
Oxytetracycline	–	1	–	12	13 (24.5%)
Ampicillin	10	16	14	13	53 (100%)
Streptomycin	4	5	8	8	25 (47.2%)
Gentamicin	–	3	–	–	3 (5.7%)
Sulphamethoxazole	10	16	14	13	53 (100%)
Nalixidic acid	1	2	9	12	24 (45.3%)
Trimethoprim	10	16	14	13	53 (100%)
Novobiocin	10	16	14	13	53 (100%)
Penicilin	9	13	14	13	49 (92.5%)
Chloramphenicol	2	3	4	2	11 (20.8%)

associated with *Aeromonas* species are aerolysin, lipase, protease, DNase, heamolysin and amylase (Aberoum and Jooyandeh 2010; Sharma et al., 2010). Infections such as bacteremia (Hochedez et al., 2010), respiratory tract infections (Issa and Napolitano 2011), gastroenteristrics (Igbinosa et al., 2012), septicemia (Papadakis et al., 2012), urinary tract infection and diarrhea (Senderovich et al., 2012) have been associated with Aeromonads. According to Odeyemi and Ahmad (2014), *Aeromonas* species have also been linked to both food and water-borne diseases in different parts of the world especially developing countries due to poor personal hygiene and lack of quality water. These bacteria are either motile or non-motile species. Currently, more than 26 species and 8 sub species of Aeromonads have been identified (Figueras et al., 2011).

The discovery of antibiotics decades ago, has helped to overcome microbial infections and diseases affecting humans and animals. However, bacteria that are initially susceptible to commonly used antibiotics are becoming resistant. Among these antibiotic resistant bacteria are some species of *Aeromonas* especially clinical and environmental isolates. According to Maria José (2012), there is an emerging public health related problem due to antibiotic resistance of *Aeromonas* spp. to commercial antibiotics. Water bodies receive human and animal waste especially wastewater discharges mostly believed to contain antimicrobial agents that can make natural flora in these water bodies to become resistant (Goñi-Urriza et al., 2000). Antibiotic resistance has been observed in environmental *Aeromonas* isolates isolated from heavily polluted waters (Huddleston et al., 2006; Aravena-Román et al., 2012).

In Malaysia, most of the studies on antibiotic resistance of *Aeromonas* species have been focused mainly on clinical, seawater and sediment isolates. There is a dearth of information on other aquatic sources as potential reservoir. This study therefore aimed to investigate antibiotics resistance pattern and phenotyping of *Aeromonas* species isolated from different aquatic sources in Malaysia.

2. Materials and methods

*2.1. Sample collection and isolation of **Aeromonas** spp. from seawater*

Water samples were obtained from Melaka using sterile 500 mL, stored in ice box and transported to the laboratory for microbiological analysis. 1 mL of both seaweed and sea grass that was cut into pieces using sterile scissors was weighed aseptically into sterile 9 mL normal saline water for subsequent serial dilution up to seven replicas. 0.1 mL of samples labeled 10^1, 10^3 and 10^5 was plated on modified Rimler Shott (mRS) agar (Odeyemi et al., 2012) for isolation of presumptive *Aeromonas* spp. Master plates and stock culture of selected presumptive isolates were prepared. The isolates were then phenotypically characterized.

2.2. Sample collection and isolation of Aeromonas spp. from sediment, rinsed sample water from bivalve and sea cucumber

Samples of sediment, bivalve and sea cucumber were collected from Mussel farm Sebatu, Melaka. The samples were transported in ice box to the laboratory for processing. Serial dilution of the sediment sample was carried out using sterile universal bottles. One gram (1 g) of the sediment was introduced into sterile 9 mL 0.65% saline water and further diluted up to seven replicas. 0.1 mL of samples labeled 10^1, 10^3 and 10^5 was seeded on modified RimlerShott (mRS) agar for isolation of presumptive *Aeromonas* spp.

Bivalves and sea cucumber were rinsed with 100 mL of sterile seawater (SSW). The first rinse was kept for further processing while the bivalves (11 samples) and sea cucumber (2 samples) were further washed twice to keep them free of any adherent particles. 1 mL of rinsed sample water (RSW) was serially diluted from 10^1 to 10^5 using SSW as diluents. Thereafter, 10 µL of dilutions 10^1 10^3 and 10^5 was then inoculated on mRS medium.

2.3. Isolation of Aeromonas spp. from bivalves and sea cucumber

Bivalves were dissected after removing the shell. 5 g of bivalve muscle was weighed into sterile universal bottle containing SSW and vortexed for 2 min to dislodge the bacteria. The sample was further processed as above. Sea cucumber was also dissected while 10 g of the intestine and 10 g of the body tissue were weighed into sterile universal bottle, vortexed for 2 min to dislodge the bacteria and then processed as described above.

2.4. Biochemical characterization of presumptive isolates

All presumptive *Aeromonas* isolates were inoculated on Aeromonas medium with the following composition L-lysine 5 g/L,