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Full Length Research Paper

Antibiotic susceptibility and resistance of *Aeromonas* spp. isolated from fish

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The sensitivity of 24 isolates of *Aeromonas* spp. isolated from the muscle of different species of fish was compared using commonly used antibiotics. The *in vitro* susceptibility of the isolates was studied by disk diffusion method using discs (Oxoid) containing the following antibiotics were used: penicillin G (10 U), tetracycline (30 µg), gentamycin (30 µg), azitromycin (15 µg), trimethoprim-sulfamethoxazole (25 µg), naladixic acid (30 µg) and norfloxacin (10 µg). All bacterial strains studied in this research showed high degree of resistance to antibiotics. According to the results, 91.6% of studied isolates were resistant to antibiotics. Resistance rate to tetracycline and penicillin was more than other antibiotics. Multiple drug resistance was also observed in all *Aeromonas hydrophila* isolates.

Key words: *Aeromonas* spp., antibiotic susceptibility, seafood.

INTRODUCTION

Aeromonas spp. are Gram-negative, rod shaped, facultative anaerobic bacteria. Members of this genus are found wide spread in natural habitats such as soil, fresh and brackish water, sewage and wastewater (Araoju et al., 1991). Over the past two decades, the number of recognized species in this genus has expanded from four species (*Aeromonas hydrophila*, *A. sobria*, *A. cavia*, and *A. salmonicida*) to 16 recognized hybridization groups (Demarta et al., 1999; Janda, 1996), and nine of these taxa have been recovered from clinical and environ-mental samples and therefore could be pathogenic for humans (Moyer, 1987; Janda, 1996; Janda and Abbott, 1996).

Motile *Aeromonas* species are widely distributed in nature and have been recognized as normal microflora of aquatic and terrestrial organisms. The most common, *A. hydrophila*, causes disease in fish, frogs, and several other animals (Farmer et al., 1992; Hazen et al., 1978; Janda and Abbott, 1996). Seafood is an important source of transmission of *Aeromonas* spp. infections to humans (Lee et al., 2000). Members of the genus *Aeromonas* can also be transmitted to human by ingestion of other foodstuffs such as vegetables (Callister and Agger, 1987), meat (Okrend et al., 1987), chicken (Hanninen, 1993). Some *Aeromonas* species have been frequently recognized as responsible for several diseases, both in human and animals including fish (Cahil, 1990; Krovacek et al., 1995). Of the currently described species of *Aeromonas*, the *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas jandaei* and *Aeromonas sobria* have been linked to human diseases including wound infections, gastroenteritis and septicemia (Janda and Abbott, 1998). A greater risk of infection is reported in young children, elderly people, and immunocompromised patients (Janda, 1991). The pathogenesis of *Aeromonas* spp. involves a wide group of virulence determinants and endotoxins, their roles not being conclusively established for any human infections. As for other bacteria, it appears that only certain intraspecific subsets of strains act as human pathogens (Von Graevenitz, 2007).

Antibiotic multi-resistance bacteria in seafood have great importance all over the world (Ansari and Raissy, 2010; Schmidt et al., 2000; Farmer et al., 2003). Since pathogen bacteria including *Aeromonas* species are

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Table 1. Antibiotic susceptibility and resistance of *Aeromonas* spp.

Species	Tetracycline		Gentamycin		Azitromycin		Penicillin		SXT		Nalidixic acid		Norfloxacin	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
A. hydrophila (6)	1	5	3	3	4	2	1	5	3	3	3	3	3	3
Other *Aeromonas* species (18)	3	15	6	12	8	10	4	14	11	7	12	6	7	11
Total (24)	4	20	9	15	12	12	5	19	14	10	15	9	10	14

responsible of several diseases, many studies have been focused on these bacteria (Messi et al., 2005; Lobova et al., 2006) although, no previous study on the antimicrobial sensitivity of *Aeromonas* species was found in Iran.

The present study was undertaken to determine the degree of antibacterial resistance of motile *Aeromonas* species isolated from fish from the Persian Gulf and Oman Sea.

MATERIALS AND METHODS

Samples

This study was conducted from March to July 2011. Different species of fish (n = 54) were collected from the local fish markets, Shahrekord, Iran. Straight after collection the samples were transferred into cool boxes with an internal temperature of 2 to 4°C. During th e transport to the laboratory the temperature was continually recorded with a logger (Testo 174, Testo GmbH and Co., Lenzkirch, Germany). All samples were processed within a short time after arrival.

Bacteriological analysis

Homogenized fish muscle were added in alkaline peptone water and incubated at 37°C for 16 to 24 h. After i ncubi-tion, one loop full of the enriched culture was streaked on starch ampicillin agar plates and incubated at 37°C for 18 to 24 h. Yellow to honey-coloured, oxidase-positive colonies were taken and tested in Kaper's multi test medium (Thayumanavan et al., 2003). Appearance of an alkaline surface and acid butt on the bottom of the tube after 24 h at 37°C demonstrated the presence of *Aeromonas* spp. *A. hydrophila* was identified by standard physiological and biochemical tests according to Austin and Austin (1999).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed using the disc diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (CLSI, 2008). Discs (Oxoid) containing the following antibiotics were used: penicillin G (10 U), tetracycline (30 µg), gentamycin (30 µg), azitromycin (15 µg), trimethoprim-sulfamethoxazole (25 µg), naladixic acid (30 µg) and norfloxacin (10 µg). The results were recorded as resistant or susceptible by measurement of the inhibition zone diameter according to the interpretive standard of CLSI (2008).

RESULTS

A total of 24 *Aeromonas* isolates including 6 *A. hydrophila* were examined. According to the results *Aeromonas* isolates demonstrated strong resistance to 7 commonly used antibiotics including Tetracycline, Gentamycin, Azitromycin, Penicillin, SXT, Nalidixic acid, Norfloxacin. The results of antibiotic susceptibility test show that 22/24 (91.6%) of the examined isolates were resistant to antibiotics. Resistance rate to tetracycline and penicillin was more than other antibiotics (Table 1). Resistance profile of anti-biotics observed in decreasing order of resistance of the isolates was tetracycline (83.3%), penicillin (62.5%), gentamycin (62.5%), norfloxacin (58.3%), azitromycin (50%), trimethoprim-sulfamethoxazole (41.6%), and nalidixic acid (37.5%). Multiple drug resistance was observed in all *A. hydrophila* isolates. Out of total 6 isolates of *A. hydrophila*, one isolate was resistant to 6 drugs, 1 to 5 drugs, 1 to 4 drugs, 1 to 2 drugs and 2 to 3 drugs.

DISCUSSION

The development of resistant or even multidrug resistant pathogens in recent years has become a major problem in Iran and many countries. Antimicrobial resistance of *Aeromonas* has been studied by many authors (Thayumanavan et al., 2003). Some of the authors Poobalane et al. (2008) indicated that *A. hydrophila* isolated from water, food and clinical samples was not sus-ceptible to many antimicrobial agents. However, antibiotics resistance was found in 91.6% of studied isolates and in all *A. hydrophila* strains. The results also show that resistance to penicillin and tetracycline was observed in 79 and 83% of the isolates. In addition, all *A. hydrophila* strains were multiresistant, what may be the result of the spread of resistance genes among the isolated

bacteria.

Castro-Escarpulli et al. (2003) reported that the best antimicrobial effect on *Aeromonas* is obtained by applying the first-generation quinolone and the second and third generation cephalosprins. Although, Stojanov et al. (2010) indicated that a high percentage of the *Aeromonas* strains were resistant to Flumequine (over 35%) and Olaquindox (around 20%), as a representative of Quinolone.

In this study 22/24 (91.6%) of the examined isolates were resistant to antibiotics. Resistance profile of antibiotics observed in decreasing order of resistance of the isolates was tetracycline, penicillin, gentamycin, norfloxacin, azitromycin, trimethoprim-sulfamethoxazole and nalidixic acid.

Multiple drug resistance was observed in all *A. hydrophila* isolates.

Multiple resistances to antibiotics has been reported by some authors before (Xia et al., 2004) and it was reported that 100% of *A*. *hydrophila* isolates were resistant to methicilline and rifampicin and 90% of strains were resistant to bacitracin and novobiocin.

Resistance of pathogenic bacteria to commonly used antibiotics has been reported before throughout the world (Chowdhury et al., 1989; Schmidt et al., 2000). Persistence of Oxytetracycline and oxalic acid in sediments for several months (Hansen et al., 1992) gives rise to a significant increase in bacterial resistance as well as the risk of having residues of antibiotic substances in fish meat used for human consumption. The antibiotic resistance is transferable to other bacteria through R-plasmids. Potential consequences of excessive antibiotic use in animal culture are the development of drug-resistant bacteria and reduced efficacy of treatment for animal and even human diseases (Rhodes et al., 2000). Furthermore, inappropriate use of antibiotics is likely to cause an unnecessary impact on the environment. The incidence of high antibiotic resistance in fish or other aquatic animals with no recent history of antibiotic usage is related to increased or excessive and prophylactic use of antibiotics in aquaculture farms especially the majority of the fish farms along the river, release their effluents after passage of sedimentation ponds and without further treatment.

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