Isolation and Antibiotic Susceptibility of *Aeromonas* spp. From Freshwater Fish Farm and Farmed Carp (Dam of 16 Tishreen, Lattakia)

N. Daood

Tishreen University, Faculty of Science, Dept of Botany, Lattakia, Syria.

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ABSTRACT

A total of 30 water samples and 45 infected fish (carp, *Cyprinus carpio*) were collected from freshwater fish farm (Dam of 16 Tishreen-Lattakia) and analysed bacteriological. Macroscopic examinations of infected fish had showed the presence of haemorrhagic skin lesions with brown or red spots throughout their skin. A total of 64 *Aeromonas* strains were isolated. The *Aeromonas* isolates were distributed as follows: *Aeromonas hydrophila* (34, 53%), *A. caviae* (16, 25%), *A. sobria* (9, 14%) and (5, 8%) of unidentified aeromonads. Collectively, *Aeromonas* spp. are considered as opportunistic causative agents of human gastroenteritis and other infections. Antibiotic susceptibility tests were carried on all strains of isolated *Aeromonas* spp. using twenty different antibiotics by agar disk diffusion method.

The majority of *Aeromonas* spp. strains was found to be completely resistant to penicillin, ampicillin with high levels of resistance (>75%) to streptomycin, amoxicillin and novobiocin. Approximately 65% of the tested strains showed resistance to oxytetracycline. Around half of the tested strains was resistance to tetracycline (53%), cephalothin (52%) and erythromycin (50%). Variable responses were observed towards the antibiotics nalidixic acid, colistin, and chloramphenicol and the resistance levels were 30, 17 and 12% respectively. Resistances to gentamicin, amikacin and kanamycin appeared in less than 8% of the studied strains. All studied strains of *Aeromonas* spp. were susceptible to the antibiotics ciprofloxacin, trimethoprim- sulphamethoxazole, lincomycin, cefixime and neomycin. No significant difference was found in antibiotic responses among strains isolated from water or fish.

Key words: *Aeromonas* spp., Antibiotic, Susceptibility, Resistance, Fish.

عزل أنواع من الأيروموناس واختبار تحسسها للصادات الحيوية من ماء وأسماك الكارب في مزرعة سمكية مائية عذبة (سد 16 تشرين- اللاذقية)

نـزيـه داؤد

قسم علم النبات– كلية العلوم – جامعة تشرين – اللاذقية – سورية

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الملخص

جمعت 30 عينة ماء و45 عينة أسماك مصابة (نوع كارب أو شبوط) من مزرعة سمكية مائية عذبة (سد 16 تشرين- اللاذقية) وحللت جرثومياً. أظهر الفحص العياني للأسماك المصابة وجود آفات جلديـة نزفية مع بقع بلون بني أو أحمر على كامل جلدها. عزل ما مجموعه 64 سلالة للأيروموناس. توزعـت عزلات الأيروموناس كما يأتي: النـوع *Aeromonas hydrophila* (34, 53%)، النـوع *A. caviae* (16, 25%)، النوع *A. sobria* (9, 14%) وأنواع غير محددة من الأيروموناس (5, 8%). إجمالاً، تعد أفراد الأيروموناس عوامل ممرضة انتهازية تسبب التهاب معدة وأمعاء وعدوات أخرى في البشر. أجريت اختبارات التحسس للصادات الحيوية على كل السلالات المعزولة مـن أنـواع الأيروموناس باستخدام عشرين صاداً حيوياً بوساطة طريقة الانتشار من القرص.

وجد أن القسم الأعظم من سلالات الأيروموناس كان كامل المقاومة للبنـسيللن والأمبيـسيللين مـع مستويات مرتفعة (أكثر من 75%) لمقاومة الستربتومايسين، أموكسيسيللين ونوفوبيوسين. أظهر 65% تقريباً من السلالات المختبرة مقاومة للأوكسي تتراسيكلين. كان نحو نصف السلالات مقاوماً للتتراسيكلين (53%)، للسيفالوتين (52%)، وللإريثرومايسين (50%). لوحظت استجابات متنوعـة تجـاه الـصادات الحيوية: نالديكسيك أسيد، كوليستين، وكلورامفينيكول، وكانت مستويات المقاومـة 30%، 17% و12% على التوالي. ظهرت مقاومة الجنتاميسين، الأميكاسين والكانامايسين في أقل مـن 8% مـن الـسلالات المدروسة. كانت السلالات المدروسة للأيروموناس كلّها حساسة للصادات الحيوية: سيبروفلوكـساسين، تريميتوبريم- سلفاميزوكسازول، لينكومايسين وسيفيكسيم. لم توجد فروقات مهمة إحصائياً في الاستجابة للصادات الحيوية بين السلالات المعزولة من الماء و المعزولة من الأسماك.

الكلمات المفتاحية: الأيروموناس، صاد حيوي، حساسية، مقاومة، أسماك.

I- Introduction

Species of *Aeromonas* are Gram-negative, non-spore-forming, rod-shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in natural habitats such as aquatic environments and soils. (Ku¨hn *et al*., 1997). Members of the genus Aeromonas are divided into motile and non motile aeromonads; motile aeromonads comprise mesophilic group mostly represented by the species *A. hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, and *A. schubertii* whereas *A. salmonicida* is classified as psychrophilic non motile aeromonads. In contrast to motile aeromonads, *A. salmonicida* has not been associated with human infections. Currently, the species *A. hydrophila, A. veronii* and *A. caviae*, are recognized as causative agents of wide range of human infections especially gastroenteritis (Challapalli *et al*., 1988; Kuijper *et al*., 1989; Janda and Abbott, 1996).

Motile aeromonads are worldwide distributed bacteria in freshwater habitats, and considered as frequent causes of disease that infect cultured and feral fishes. *A. hydrophila* is the main causative agent of the ulcerative disease known as "haemorrhagic septicemia", that manifested as red skin sores (Guz and Kozinska, 2004). It is important to note that these motile aeromonads compose part of the normal intestinal microflora of healthy fish (Trust *et al*., 1974). In this context, *A. hydrophila* is generally found in the gastrointestinal tract of fish and is considered an opportunistic pathogen (Swann and White, 1989). Therefore, the presence of these bacteria, by itself, is not indicative of disease. However, occurrence of the disease is usually associated with an extreme change in environmental conditions, such as stress, overcrowding, a sudden change of temperature, transfer of fish, mishandling, poor water quality, high nitrite and carbon dioxide levels (Dixon and Issvoran, 1993; Ko *et al*., 1996; Cipriano, 2001).

On the other hand, the intensive fish farming, in recent years, has lead to a widespread antibiotic use for treating of resulted bacterial diseases in fish. Of these diseases, septicaemia caused by *A. hydrophila* and other aeromonads is considered as serious disease and currently treated with many antimicrobial drugs worldwide.

Consequently, the huge use of antimicrobials in veterinary and consistently aquaculture has been associated with increased levels of antibiotic resistance in aquatic bacteria at all (Ko *et al*., 1996; Schwarz and Noble, 1999; Mirand and Zemelman, 2002). As result, the developing of antimicrobial resistance among aquatic bacterial pathogens will ultimately reduce the efficiency of antimicrobial agents

used for treating. Moreover, the increased antibiotic resistance confer bacterial pathogens additional virulent feature.

The aim of this study was to determine the prevalence and most frequent *Aeromonas* species associated with infections of fish in freshwater fish farm and the antibiotic susceptibility profile of the isolates in order to verify the most effective antibiotics which may be used for treatment and to determine the state of resistance among these local isolates as compared to worldwide related results.

II- Material and Methods.

Sampling. The samples can be divided into two categories: water and fish samples. As total as 30 water samples and 45 infected fish were collected from "Dam of 16 Tishreen" freshwater fish farm located 20 Km east of Lattakia city. Samples were sporadically collected throughout two years of 2007 – 2008. In addition to the dam, infected fish were also collected from local retail markets. Water and fish samples were aseptically collected using sterile bottles and plastic bags respectively. All samples analysed directly in the same day within two hours of collection. Skin lesions and internal subsamples were obtained from infected fish with symptoms of disease on their skin. Internal organs sampled included intestine, liver, gill**,** kidney, and heart of an infected fish.

Culture media and identification. The samples were cultured by streaking on three kinds of culture media: R-S agar (Shotts and Rimler,1973), defibrinated sheep blood agar plates (Hi media, *Ltd*) and Mac Conkey agar (Hi media, *Ltd*) plates. The cultured media were incubated at 25ºC and 37ºC for 24-48 hr aerobically. After incubation, the suspected colonies to be as motile aeromonads (i.e.: yellow and haemolytic colonies) were isolated. The identification of bacterial isolates as motile aeromonads was based on the colony morphology, Gram-staining, motility, produce of haemolysins, oxidase production, and glucose fermentation (Hi media, *Ltd*), as well as other biochemical tests (Murray *et al*., 1999; Brenner *et al*., 2005).

Antibiotic susceptibility testing. All bacterial isolates were tested for their sensitivity to twenty antibiotics by the disc diffusion method (Bauer *et al*., 1966). The antibiotics, their codes and concentrations were as follows: amikacin (AN, 30µg), amoxicillin (AMX, 25µg), ampicillin (AM, 10µg), cefixime (CFX, 5µg), cephalothin (CF, 30µg), chloramphenicol (C, 30µg), ciprofloxacin (CIP, 5µg), colistin (CL, 50µg), erythromycin (E, 30µg), gentamicin (GM, 10µg), kanamycin

(K, 30µg), lincomycin (L, 15µg), nalidixic acid (NA, 30µg), neomycin (N, 30µg), novobiocin (NOV 30µg), oxytetracycline (OX, 30µg), penicillin G (P,10 UI), streptomycin (S, 30µg), tetracycline (TE, 30µg), and trimethoprim- sulphamethoxazole (ST, 1.25 + 23.75µg). First, pure cultures of tested *Aeromonas* spp. strains were cultivated in Tryptic Soy Broth (Himedia, *Ltd*) and incubated at 25°C for 6–8 h. Subcultures were then streaked onto Mueller Hinton agar plates (Himedia, *Ltd*) using a sterile cotton swab. Results were recorded after incubation at 35°C for 24 h. Tested bacterial strains were classified into three categories: sensitive, intermediate, and resistant and depending on the diameters of inhibition zones and standards supplied by Himedia Laboratories and comparing with other related references (NCCLS. 1999). All tests were carried in the laboratories of the Department of Botany in the Faculty of Science- Tishreen University.

III- Results.

A total of 64 bacterial strains of motile aeromonads were isolated from 75 samples of water and infected fish. The species composition and sources of these strains are presented in Table 1. Of these 64 strains, 18 (28.12%) strains were isolated from water against 46 (71.87%) from infected fish. The isolation frequencies of these 64 strains upon anatomical parts of fish and water samples were as follows: skin lesions 14 (21.87%), intestine 19 (29.69%), heart 7 (10.94%), gill 6 (9.37%) and water 18 (28.12). Neither liver of infected fish nor kidney harboured strains of *Aeromonas* spp.. On the other hand, *Aeromonas* species were distributed as follows: *A. hydrophila* 34 (53.12%), *A. caviae* 16 (25%), *A. sobria* 9 (14.06%) and as well as 5 (7.81%) unidentified *Aeromons* strains (table 1); the identification of these five strains was only performed to genus level. *A. hydrophila* appeared to be the main pathogen of aeromonads frequently associated with infected fish rather than water samples.

Table 1. Distribution of *Aeromonas* species isolated from water and infected fish in Dam of 16 Tishreen freshwater fish farm.

	Distribution (Nu & %) of *Aeromonas* spp. (n=64) according to site of isolation:					**Total**
	Anatomical parts of infected fish				**Water**	
	Skin	**Intestine**	**Heart**	**Gill**		
A. hydrophila	8(12.5)	14(21.87)	6(9.37)	2(3.12)	4(6.25)	34(53.12)
A. caviae	4(6.25)	3(4.69)	1(1.56)	2(3.12)	6(9.37)	16(25)
A. sobria	1(1.56)	2(3.12)	0	1(1.56)	5(7.81)	9(14.06)
unidentified Aeromonas	1(1.56)	0	0	1(1.56)	3(4.69)	5(7.81)
Total	14(21.87)	19(29.69)	7(10.94)	6(9.37)	18(28.12)	64

The isolated *Aeromonas* strains were tested against twenty antibiotics and the results of their sensitivity are presented in table 2 and figure 1. The results of the antibiotic sensitivity testing revealed perfect resistance among *Aeromonas* spp. strains towards penicillin, ampicillin and high levels of resistance (>75%) to streptomycin, amoxicillin and novobiocin. Of these three latter, the highest resistance level was found to be against streptomycin (83%). Approximately, 65% of the tested *Aeromonas* spp. strains showed resistance to oxytetracycline. Around half of tested *Aeromonas* spp. strains was resistant to tetracycline (53%), cephalothin (52%) and erythromycin (50%). The tested strains showed variable responses towards the antibiotics nalidixic acid, colistin, and chloramphenicol and the resistance levels were 30, 17 and 12% respectively. Resistances to gentamicin, amikacin and kanamycin appeared in less than 8% of the tested strains. In the present study, all studied strains of *Aeromonas* spp. were susceptible to the antibiotics ciprofloxacin, trimethoprim- sulphamethoxazole, lincomycin, cefixime and neomycin. No significant difference was found as related to responses to antibiotics by strains isolated from water or fish. On the basis of species level and by comparison of antibiotic susceptibility profiles, no significant differences (with some exceptions) in individual response to antibiotics were observed. However, some of the antibiotics such as cephalothin, colistin and oxytetracycline appeared to be more effective against *A. caviae* and A. *sobria*. as compared with *A. hydrophila*. Intermediate susceptibility were observed among small proportion of the tested strains toward some antibiotics including amoxicillin, colistin, nalidixic acid, oxytetracycline and streptomycine (table 2).

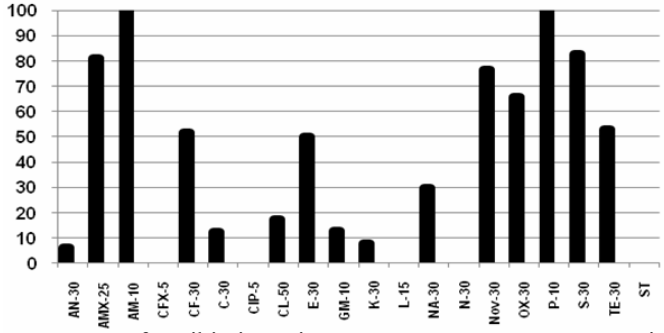

Figure 1. Percentage of antibiotic resistance among *Aeromonas* spp. isolated from water and infected fish in freshwater fish farm (Dam 16 Tishreen, Lattakia). Amikacin (AN,30µg), amoxicillin (AMX, 25µg), ampicillin (AM,10µg), cefixime (CFX,5µg), cephalothin (CF,30µg), chloramphenicol (C, 30µg), ciprofloxacin (CIP, 5µg), colistin (CL, 50µg), erythromycin (E, 30µg), gentamicin (GM,10µg), kanamycin (K, 30µg), lincomycin (L, 15µg)**,** nalidixic acid (NA, 30µg), neomycin (N, 30µg), novobiocin (NOV 30µg), oxytetracycline (OX, 30µg), penicillin G (P,10 UI), streptomycin (S, 30µg), tetracycline (TE, 30µg), and trimethoprim- sulphamethoxazole (ST, 1.25 + 23.75µg).

Table 2. Results of antibiotic susceptibility testing carried on *Aeromonas* spp. strains isolated from water and infected fish in fresh water fish farm Dam of 16 Tishreen- Lattakia.

Antibiotics	No. and (%) of *Aeromonas* spp. strains (*n*= 64):		
	Sensitive	Intermediate	Resistant
Amikacin (30µg)	60 (93.75)	0 (0)	4 (6.25)
Amoxicillin (25µg)	10 (15.62)	2 (3.12)	52 (81.25)
Ampicillin (10µg)	0 (0)	0 (0)	64 (100)
Cefixime (5µg)	64 (100)	0 (0)	0 (0)
Cephalothin (30µg)	31 (48.44)	(0)	33 (51.56)
Chloramphenicol(30µg)	56 (87.5)	0 (0)	8 (12.5)
Ciprofloxacin (5µg)	64 (100)	0 (0)	0 (0)
Colistin (50µg)	50 (78.12)	3 (4.69)	11 (17.18)
Erythromycin (30µg)	32 (50)	0 (0)	32 (50)
Gentamicin (10µg)	61 (95.31)	0 (0)	3 (4.69)
Kanamycin (30µg)	59 (92.19)	0 (0)	5 (7.81)
Lincomycin (15µg)	64 (100)	0 (0)	0 (0)
Nalidixic acid (30µg)	41 (64.06)	6 (9.37)	19 (29.68)
Neomycin (30µg)	64 (100)	0 (0)	0 (0)
Novobiocin (30µg)	15 (23.44)	0 (0)	49 (76.56)
Oxytetracycline (30µg)	18 (28.12)	4 (6.25)	44 (65.62)
Penicillin G (10 UI)	0 (0)	0 (0)	64 (100)
Streptomycin (30µg)	9 (14. 06)	2 (3.12)	53 (82.81)
Tetracycline (30µg)	30(46.87)	0 (0)	34 (53.12)
Trimeth/ sulpha (25µg)	64 (100)	0 (0)	0 (0)

IV - Discussion

Bacterial isolates: The present research was designed after many trials and comparative studies carried on bacterial content of both healthy and infected fish as well as antibiotic susceptibility patterns for their isolates (data not published). The isolation of those three species of *Aeromonas* is agreed with consideration of *A. hydrophila, A. sobria, and A. caviae* as the most bacterial causative agents typically associated with septicemia in fish (Austin *et al*., 1989). However, the high frequency of isolation for *A. hydrophila* (53.12%) as compared with that of the other two species *A. caviae* (25%) and *A. sobria* of (14.06%) can be attributable to the main role of pathogenicity played by *A. hydrophila* resulting from its increased virulence (Boulanger *et al*., 1977; Paniagua *et al*., 1990). It was clear from our results that these pathogenic bacteria were concentrated in the infected fish rather than water body or what is called free living bacteria (table 1). This can be explained by the fact of chemotactic response of motile aeromonads (certainly these three above species) to skin mucus of fish (and even mucus receptors of animal cell lines) as feature of their virulence (Ascencio *et al*., 1998). Furthermore, that result is in full agreement with pathological findings that the free

living aeromonads (in water) have lesser chemotactic response to skin mucus than isolates that were obtained from lesions on diseased fish (Trust, 1986). On the other hand, the largest number of isolates obtained from intestine of infected fish may be partly due to style of aeromonads existence as part of intestinal microflora of healthy fish; however, these bacteria as other opportunistic pathogens, become pathogenic under stress condition. Actually, the presence of these opportunistic pathogens in the large numbers in both of intestine and skin of infected fish is in full agreement with consideration of that two sites as portal of entry (Cipriano, 2001). For treatment purposes, this result may be with great importance (i.e. the manner by which the therapeutic antibiotic will be administered).

Antibiotic resistance: It is a common practice, in many countries, to use of antimicrobial agents in the aquaculture systems and notably fish farms to control the bacterial diseases and for disinfection purposes. The most frequently antimicrobials used in these situations include: oxytetracycline, ciprofloxacin, nitrofurantoin, furazolidone, trimithoprim / sulfadiazine, oxolonic acid, and chloramphenicol (Amir *et al*., 2008; Abraham *et al*., 1997). Other antibiotics such as, penicillin, streptomycin, and tetracycline have latter been suggested and used (Cipriano, 2001). The frequently used oxytetracycline and streptomycine in aquaculture either as prophylactic or treating agents may contribute to the developing of high degree of resistance towards these two antibiotics among variety of bacteria isolated from such aquatic environments and animals (Spanggaard *et al*., 1993; Hatha and Lakshmanaperunmalsamy, 1995; DePaola *et al*., 1995). However although it was high, the resistance to that two antibiotics was not absolute in the present study. Nevertheless, levels of oxytetracycline and tetracycline resistance (65% and 54% respectively) were higher than that reported by other authors. For instance, these levels decreased to 44% in study of Stojanov *et al*. (2010) and to 38% for each in study of Guz. and Kozińska (2004) but still lower than absolute resistance observed by Ramazan Adanir and Turutoglu (2007). The perfect resistance to penicillin, ampicillin is similar to that observed by other authors (Guz. and Kozińska, 2004; Ramazan Adanir and Turutoglu, 2007; Kaskhedikar and Chhabra, 2010). It is noteworthy that clinical isolates of *Aeromonas* species from humans are also resistant to ampicillin (Carnahan *et al*., 1991). The high levels of resistant against streptomycin, oxytetracycline and somewhat tetracycline may partially due to their uses as both prophylactic and therapeutic agents in aquaculture systems. This is not true, however, for the completely effective ciprofloxacin, trimethoprim- sulphamethoxazole and somewhat chloramephenicol in the present study. Consequently, these

antibiotics are still effective although their wide therapeutic and sub therapeutic uses in clinical and animal husbandry respectively. As such, this result is likely to be important at least locally. It is worth noting, that a complete sensitivity to ciprofloxacin and trimethoprim-sulphamethoxazole among *A. hydrophila* isolated from farmed fish was also observed by Ramazan Adanir and Turutoglu, (2007) in Turkey. As related to streptomycin, variable results obtained by other authors; consequently, in contrast to Son *et al*. (1997) and Vivekanandhan *et al*. (2002) who separately reported absolute resistance to streptomycin among *Aeromonas* spp., a complete sensitivity to it was before reported by Rahim *et al*. (1984) and latter by Hatha *et al*. (2005) and Ramazan Adanir and Turutoglu (2007). The prevalence of resistance among *Aeromonas* spp. strain to amoxicillin and novobiocin is in agreement with observations of other authors; in this context, Belém-Costa and Cyrino (2006) reported that all strains of *A. hydrophila* isolated from fish were resistant to amoxicillin and novobiocin. Likewise, Xia *et al*. (2004) reported the presence of novobiocin resistance in 90% of *Aeromonas* spp. strains. The resistances to cephalothin (51%), erythromycin (53%) can be compared with that reported by the others (Rahim *et al*., 1984; Son *et al*., 1997; Guz. and Kozińska, 2004). Guz. and Kozińska (2004) found that nalidixic acid was effective against 95% of *Aeromonas* strains isolated from farmed fish; in other related studies, around 10% of tested *Aeromonas* strains were resistant to nalidixic acid (Vivekanandhan *et al*., 2002; Hatha *et al*., 2005). In the present study, the relatively high resistance toward nalidixic acid (30%) seems to be high as compared with that previously recorded levels. The resistance level against colistin (18%) is lower than that reported by Guz. and Kozińska (2004) but seems to be extremely low when compared with complete resistance recorded Kaskhedikar and Chhabra (2010) among strains of *A. hydrophila* isolated from fish. The low resistance level observed against chloramphenicol can compared with that recorded by Hatha *et al*., (2005) who found that less than 20% of *Aeromonas* spp. were resistant to it. Similar result was obtained before by Rahim *et al*., (1984). However, complete sensitivity to chloramphenicol was recorded by other authors (Guz. and Kozińska, 2004; Belém-Costa and Cyrino, 2006; Kaskhedikar and Chhabra, 2010). The very low resistance levels observed towards gentamicin, amikacin and kanamycin (< 8%) is similar to that obtained by Hatha *et al*. (2005) who found 10% of *Aeromonas* strains to be resistant to gentamicin. Moreover, complete susceptibility to these antibiotics was recorded in *Aeromonas* spp. by other authors (Rahim *et al*., 1984; Son *et al*., 1997; Guz. and Kozińska, 2004; Ramazan Adanir and Turutoglu, 2007). Our result about complete sensitivity to neomycine is agreed

with that of Rahim *et al*. (1984) and Ramazan Adanir and Turutoglu (2007). The resistances to kanamycin is lower than that recorded by Guz. and Kozińska (2004) who reported value of 29%. However, complete sensitivity to kanamycin was reported by other authors (Belém-Costa and Cyrino, 2006; Kaskhedikar and Chhabra, 2010).

Overall and as shown above, our antibiotic sensitivity results are still (with some exceptions) within the ranges reported by other authors worldwide.

V- Conclusion

Obviously, the current study revealed that *A. hydrophila, A. sobria, and A. caviae* are likely to be the causative agents of septicemia in infected fish. Basically, this result was concluded because of strong association of more resistant *Aeromonas* spp. with infected fish.

In the present study, the completely effective antibiotics were: ciprofloxacin, trimethoprim - sulphamethoxazole, lincomycin, cefixime and neomycin. In addition, it was found that other antibiotics such as amikacin, chloramephenicol, gentamicin and kanamycin to be relatively effective with resistance levels lower than 13% of the tested strains.

Depending on the results of antibiotic susceptibility testing and because of the fish disease caused by *Aeromonas* spp. is a problem with economical effects for farmed carp, there is need to rational and therapeutically use of the more effective antibiotics. Such treatments based on susceptibility tests are recommended.

Although the levels of antibiotic resistance recorded in the present study are not so high by comparison with other related studies, they still high and clearly indicate to responsibility of extensive and arbitrary use of antimicrobials in fish farming. This will lead to selection of more and wide spectrum of resistant bacteria irrespective of the antibiotics that are used in defined farm or site. This appears to be clear in this study and the resistance was observed towards antibiotics other than that used in the studied farm that were mainly represented by tetracyclines and oxytetracyclines. The selecting of more resistant bacterial strains in aquaculture can have large impact on human health. Human bodies can be effected by these bacteria both directly by consumption of raw and undercooked infected fish products or indirectly by contact with these environments. Actually these environments act as reservoir of multi resistant bacteria. Irrespective of the way the resistant bacteria access human bodies by which, these bacteria serve as source of resistance genes to indigenous organisms.

REFERENCES

Abraham, T. J., Manley, R., Palaniappan, R. and Devendran, K. (1997). Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. *J. Aqua*c. *Trop*. 12 (1), 1 -8.

Amir, S., Amy, S. R., Margaret, K., Janelle, B., Shawn, Mc., Polly, W. and Robert, L. (2008). Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environment international*, 34(8):1215-26.

Ascencio, F., Martinez-Arias, W., Romero, M. J. and Wadstrom, T. (1998). Analysis of the interaction of *Aeromonas caviae*, *A. hydrophila* and *A. sobria* with mucins. *FEMS Immunology and Medical Microbiology.* 20, 219-229.

Austin, D. A.; McIntosh, D. and Austin, B. (1989). Taxonomy of fish associated *Aeromonas* spp., with the description of *Aeromonas salmonicida* subsp. smithia subsp. nov. *Systematic and Applied Microbiology*. 11, 277 - 290.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standard single disk method. *Am. J. Clin. Pathol.* 36, 493-496.

Belém-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*. (Piracicaba, Braz.), 63, No 3, 281-284.

Boulanger, Y., Lallier, R. and Cousineau, G. (1977). Isolation of enterotoxigenic *Aeromonas* from fish. *Canadian Journal of Microbiology*. 23: 1161 - 1164.

Brenner, D. J., Krieg, N. R. and Staley, J. R. (2005). Bergey's manual of systematic bacteriology, Springer, USA, vol. 2, part B, pp.557-578.

Carnahan, A. M., Behram, S. and Joseph, S. W. (1991). Aerokey II: a flexible key for identifying clinical *Aeromonas* species. *Journal of Clinical Microbiology*. 29, 2843 -2849.

Cipriano, R. C. (2001). *Aeromonas hydrophila* and motile Aeromonad septicemias of fish. Fish Dis Leaflet 68, United States Department of the Interior fish and wildlife service, Division of Fishery research. Washington, DC, pp.1-25.

Challapalli, M., Tess, B. R., Cunningham, D. E., Chopra, A. K. and Houston, C. W. (1988). *Aeromonas*-associated diarrhea in children. *Pediatric Infectious DiseaseJournal.* 7, 693-8.

DePaola, A., Peeler, J. T. and Rodick, J. E. (1995). Effect of oxytetracycline-medicated feed on antibiotic resistance of Gram negative bacteria in catfish ponds. *Appl. Environ. Microbiol*. 61 (9), 3513-3519.

Dixon, B.A. and Issvoran G. (1993). Antibacterial drug resistance in *Aeromonas* spp. isolated from domestic goldfish and koi from California. *J World Aqua Soc*, 24, 102-104.

Guz, L. and Kozinska, E. (2004). Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L). *Bull Vet Inst Pulawy*, 48, 391-395.

Hatha, A.A.M. and Lakshmanaperumalsamy, P. (1995). Antibiotic resistance of *Salmonella* strains isolated from fish and crustaceans. *Lett. Appl. Microbiol.* 21, 47- 49.

Hatha, M., A. A. Vivekanandhan, A.A., Joice, J. G. and Christol. (2005). Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. *International Journal of Food Microbiology* 98, 131– 134.

Janda JM, Abbott SL (1996). Human pathogens. In: Austin B *et al*., eds. *The genus Aeromonas*. London, Wiley: 151–173.

Karunasagar, I., Pai, R. and Malathi, G. R. (1994). Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquaculture*. 128, 203- 209.

Kaskhedikar, M. and Chhabra, D. (2010). Multiple drug resistance in *Aeromonas hydrophila* isolates of fish. *Veterinary World*. 3(2), 76-77

Ko, C. W., Yu K. W., Liu, C.Y., Huang, C.T., Leu, S. H. and Chuang, Y. C. (1996). Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. *Antimicrob Agents Chemother*, 40, 1260-1262.

Ku¨ hn, I., Allestam, G., Huys, G., Janssen, P., Kersters, K., Krovacek, K. and Stensrtro¨m, T.A. (1997). Diversity, persistence, and virulence of *Aeromonas* strains isolated from drinking water distribution systems in *Sweden. Applied and Environmental Microbiology* 63 (7), 2708-2715.

Kuijper, E. J., Steigerwalt, A. G., Shoenmakers, B. S. C. I. M., Peeters, M. F., Zanen, H. C. and Brenner, D. J. (1989). Clinical and epidemiologic aspects of members of *Aeromonas* DNA hybridization groups isolated from human feces. *Journal of Clinical Microbiology* 27, 1531-1537.

Mirand, C.D. and Zemelman, R. (2002). Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. *Sci Total Environ*, 293, 207- 218.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolken, R. H. (1999). Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.

NCCLS (National Committee for Clinical Laboratory Standards). (1999). Performance standard for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard M 31A19 (11). NCCLS, Wayne, Pennsylvania.

Paniagua, C., Rivero, O., Anguita, J. and Naharro, G. (1990). Pathogenicity factors and virulence for rainbow trout (*Salmo gairdneri*) or motile *Aeromonas* spp. isolated from a river. *Journal of Clinical Microbiology*. 28: 350 - 355.

Rahim, Z., Sanyal, S. C., Aziz, K. M. S., Huq, M. I. and Chowdhury, A. A. (1984). Isolation of enterotoxigenic, hemolytic and antibiotic resistant *Aeromonas hydrophila* strains from infected fish in Bangladesh. *Appl. Environ. Microbiol*. 48, 865- 867.

Ramazan Adanir, D. O. and Turutoglu, Hulya. (2007). Isolation And Antibiotic Susceptibility Of *Aeromonas Hydrophila* In A Carp (Cyprinus Carpio) Hatchery Farm. *Bull Vet Inst Pulawy* 51, 361-364.

Schwarz, S. and Noble, W. C. (1999). Aspects of bacterial resistance to antimicrobials used in veterinary dermatological practice. *Vet Dermatol*, 10, 163-176

Shotts, E. B. and R. Rimler. (1973). Medium for the isolation of *Aeromonas hydrophila.* Journal of Applied Microbiology. 26, 550 - 553.

Son, R., Rusul, G., Sahilah, A. M., Zainuri, A., Raha, A. R. and Salmah, I. (1997). Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Tilapia (Tilapia mossambica). *Lett. Appl. Microbiol*. 24, 479-482.

Spanggaard, B., Jorgensen, F., Gram, L. and Huss, H. H. (1993). Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. *Aquaculture* 115, 195-207.

Stojanov, I., Plavša, N, Stojanović, D., Ratajac, R., Radulović, J. P., Pušić, I. and Kapetanov, M. (2010). Susceptibility Of *Aeromonas hydrophila* Isolates To Antimicrobial Drugs. *Lucrări Stiinlifice Medicină Veterinară* Vol. Xliii (1), 132-136.

Swann, L. and White, M. R. (1989). Diagnosis and treatment of *Aeromonas hydrophila* infection of fish. *Aquaculture extension*- Illinois-Indiana Sea Grant Program, pp.91-92.

Trust, T. J. (1986). Pathogenesis of infectious diseases of fish. *Annual Review of Microbiology*, 40: 479-502

Trust, T. J., Bull, L. M., Currie, B. R. and Buckley, J. T. (1974). Obligate anaerobic bacteria in the gastrointestinal microflora of the grass carp (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*. 36: 1174- 1179.

Vivekanandhan, G., Savithamani, K., Hatha, A. A. M. and Lakshmanaperumalsamy, P. (2002). Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int. *J. Food Microbiol.* 76, 165- 168.

Xia, C., Ma, Z.-H., Habibur Rahman, M. and Wu, Z.G. (2004). PCR cloning and identification of the h-haemolysin gene of *Aeromonas hydrophila* from freshwater fishes in China. *Aquaculture*, 229, 45-53.