رقم البحث (19)

EFFECT OF POND SUPPLEMENTED WITH CHICKEN MANURE ON BACTERIAL BUILD UP AND ITS ANTIMICROBIAL RESISTANCE, BESIDES THE QUALITY AND SHELF-LIFE OF CULTURED NILE TILAPIA (OREOCHROMIS NILOTICUS)

BY

Salah Mesalhy Aly, Nashwa Mahmoud Abdel Atti * and Mohammed Fathi Mohamed

WorldFish Center, Research & Training Center for Africa & West Asia,Egypt.

**Food Hygiene Dept, Animal Health Research Institute, Ismailia Laboratory, Egypt.*

ABSTRACT

Twelve thousand fries of Nile Tilapia (*Oreochromas niloticus*) were stocked in 6 ponds, three ponds were supplemented with chicken-manure and others with artificial diet. The *Aeromonas*. and *Pseudomonas spps.* were isolated from all ponds while the *Salmonella* and *Enterococcus spps*. were isolated from the manure supplemented ponds. As a General observation, the antimicrobial resistance of the isolated bacteria was high with oxytetracyclin, low with ciprofloxacin. The *Pseudomonas spp*. seemed to be the highest resistant to the used antimicrobials, while the *Enterococcus spp*. was the lowest. The total psychrotrophic counts /g of fish-flesh, collected from the fish of manure supplemented pond, showed significantly higher mean value than that of fish supplemented with artificial diet. The psychrotrophic strains were isolated from all ponds with varied frequency and percentage at both 0 time and after 168 h of ice storage, however, *Coliform, Enterococcus spp., Flavobacterium spp., Staphylococcal spp.* and *Moraxella spp.* were only isolated from fish-flesh samples from manure supplemented ponds. It could be concluded that, using of the chicken manure supplement for aquaculture may transfer some food-borne or zoonotic bacteria to the aquaculture and consequently to the consumer. Moreover, may create multidrug- resistant bacterial strains and decrease the fish quality and shelf-life.

Key words: Antimicrobial resistance, fish, chicken-manure, quality and shelf-life.

INTRODUCTION

The bacterial infections are a major constrain to the development of aquaculture, thus the antimicrobials remain vitally important for treating and preventing some bacterial infections. The appropriate use of the antimicrobials will cure some sick animals and speed the recovery of others, and may improve the welfare of the treated animals and reduce the spread of infection to other animals and humans. The challenge is how to use the antimicrobials wisely to minimize the risk of resistance (**FAO/WHO/OIE 2006**). The antibiotics may gain access to the pond environment through using human and animal wastes or via the integrated fish farming system. Recently, there has been a surge in the number of food-borne infections, caused by antibiotic resistant bacteria (ARB) (**Teuber, 1999**). The antibiotics fed at low and generally subtherapeutic concentrations, improve the feed conversion efficiency and thus performance in food producing animals that may reflect a reduction in the subclinical disease (**Walton, 1983**). However, the use of antibiotics as feed additives may develop ARB in the environment (**Smith et al, 2003)**. The use of the antimicrobial drugs, for treating people and animals in many developing countries, is unregulated. The antibiotics can be purchased from pharmacies, general stores, and even market stalls (**Mamun, 1991).** The antibiotic resistance and possible treatment-failures in animal production, are fast involving diverse agricultural industries and species. A similar problem is taking place in the aquaculture industry where resistance-development has been reported in the freshwater and marine environments **(Schmidt et al, 2000; Miranda and Zemelman, 2001).**

The human and animal wastes have traditionally been used in Asia to supplement the fish culture ponds **(Tapiador, 1977).** The chicken manure has been used successfully as an organic supplement for Nile tilapia (*Oreochromas niloticus)* production in many parts of the world (**Shevgoor et al, 1994).** The livestock manure is either directly consumed by fish or supports the phytoplankton that produces high yields with low input **(Little and Edwards, 1999).** Such practice facilitate the dissemination of many pathogens **(Tacon, 1990).** There are some bacterial pathogens of fish that can induce diseases in human like *Aeromonas sp., Vibrio vulnificus, Streptococcus iniae* and Mycobacteriosis **(Joseph et al, 1991; Farooqui 1999; Ghittino et al, 2003).** Some bacteria especially enterobacteraceae, may reach the aquaculture through the manure and may induce infection in human upon handling or

consumption of carrier fish **(Jie-yi et al., 1988).** Microorganisms are the major cause of spoilage of most seafood products **(Gram & Dalgaard, 2002)** and total psychrotrophic counts are indicators used for seafood quality determination under chilling storage **(Antoine *et al.,* 2004 and Lopparelli *et al.,* 2004)**. The aquaculture may be a major source for ARB for human diseases. Therefore, there is a need for investigating the input of manure in the aquaculture environment (**FAO/WHO/OIE 2006**).

This study was planned to determine the effect of chicken manure supplemented pond on the bacterial profile and antibacterial resistance, beside the quality and shelf-life of the cultured Tilapia nilotica (*Oreochromis niloticus*) and their possible effect on the human health.

MATERIALS AND METHODS

Experiment:

Twelve thousand fries, of 0.2 g average body weight were equally stocked in 6 ponds (each of 20 X 50 X 1 m), they classified into 6 groups (2 treatments each of 3 replicates). The ponds of groups (1-3) were supplemented with chicken-manure (100 kg / pond) weekly. The ponds of groups (4-6) supplemented twice a day by artificial food of 25% protein at a ratio of 3% body weight. Sampling was done weekly from the manure and artificial food and monthly from the pond-water and fish-flesh for bacteriological examination till the end of the culturing period (4 months).

Bacteriological examination:

a. Sampling:

Twenty four samples from each of water and Nile tilapia (*Oreochromas niloticus*) were collected from the ponds of the two treatment groups. The water samples (1 liter each) were collected in sterile bottle while the Nile tilapia were collected in sterile polyethylene bags, they delivered to the laboratory, without delay, for bacteriological examinations according to **Williams et al, (1975), WHO (1971) and APHA (1980).** Moreover, 16 samples, (each of 100 g) were collected from the diets (artificial food and manure) of groups (1-6). Thirty grams from each sample were placed in a sterile container with 270 ml of sterile distilled water and left for 20 minutes. The samples were shaken well and filtered in sterile containers through sterile gauze.

b. Isolation and identification of pathogenic bacteria:

Dilutions of 1 ml from each of the collected sample (water, intestinal content of cultured fish and chicken manure as well as the artificial food filtrate) were made in 9 ml of 0.85% (w/v) sodium chloride. 0.1 ml of the previous the dilutions and the liver swabs of cultured fish were spread on selective agar media. a) *Aeromonas spp*. was isolated on Aeromonas medium base (AMB) plates (Oxoid, Basingstoke, England), supplemented with ampicillin at a final concentration of 5 Ag/ml as recommended by the manufacturer. b) *Pseudomonas spp.* was isolated on Pseudomonas selective agar (PSA) plates (Biolife, Italy). c) *Salmonella spp*. was isolated by using methods described in the U.S. Food and Drug Administration Bacteriological Analytical Manual **(Andrews and Hammack 1998)**. d) *Enterococcus spp*. was isolated on Slanetz and Bartley (SB) medium (Oxoid). The AMB, PSA and SB plates were incubated at 30 ºC for 24–48 h. Individual colonies were randomly taken and subcultured on tryptone soya agar (TSA) plates (Oxoid). Then, the genus identity was confirmed by Gram reaction (KOH test), catalase and oxidase tests, glucose oxidation – fermentation test, and motility test in 0.25% (w/v) brain heart infusion agar (Oxoid) according to **Krieg and Holt (1984)**.

2.3. Determination of antimicrobial resistance:

The resistance of 56 isolated bacteria from each of *Aeromonas spp.*, *Pseudomonas spp.*, *Salmonella spp.* and 40 from *Enterococcus spp.* of manure supplemented groups (1-3) was determined by disk diffusion on The Mueller–Hinton agar (Difco.). Six antimicrobial agents were selected to represent different classes of antimicrobials relevant for therapy in human and animal medicine. Based on the distributions of the inhibitory zone diameters and, where available, recommendations from the Clinical and laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) **(CLSI/NCCLS, 2005a),** break point values were used to separate the sensitive isolates from the resistant. The concentration of antimicrobials in the disks and the inhibition zone break point values of the resistance are given; where isolated bacteria (*Aeromonas spp*., *Pseudomonas spp* , *Salmonella spp.*, *Enterococcus spp.*) were tested for the resistance to chloramphenicol C, (30 µg, < 13 mm); oxytetracycline OT (30 µg, < 15 mm); ciprofloxacin, CIP (5 µg, < 16 mm); Kanamycin K, (30 µg, < 13 mm); Sulphamethoxazole/Trimethoprim, SXT (25 µg, < 11 mm), and Nalidixic acid NA.(30 µg, < 14 mm). All the disks were purchased from Oxoid. The disk diffusion assays were prepared according to the recommendations of the **CLSI (2005 a & b).**

Keeping quality tests:

a. *Determination of shelf-life*:

One hundred and twenty five Nile tilapia (150 g average body weight), from each of manure and non-manure control groups, were collected at harvest time (by end of the experiment) and transferred immediately in sterile plastic bags on ice to the laboratory. The fish of each group were stored in an ice container after mixing with crushed ice that was replaced daily during the storage period (7 days). The total psychrotrophic count was determined in the fish flesh of the collected tilapia of the two treated groups after their storage in the ice for 0, 24, 72, 120 and 168 h (25 fish sample / each storage period). Ten grams of the fish-flesh were aseptically transferred into a sterile blender with 90 ml of sterile 10% peptone water. The blender was operated at a high speed (14000 rpm) for 2 minutes. The mixture was kept for 6 minutes at room temperature and decimal dilutions (10^{-1} – 10^{-6}) were prepared. One ml from each dilution of the previously prepared suspension was inoculated into duplicate plates, and then 10 ml of standard plate count agar was poured onto each plate. The inoculated plates were carefully shacked, left to solidify and then incubated at 20^{o}C for 48 h. The total psychrotrophic count was calculated according to **Thatcher and Clark (1975).**

b. Isolation and *identification of psychrotrophs:*

The psychrotrophic bacteria were isolated from the fish-flesh of the ice stored Nile tilapia of manure supplemented and non-manure control groups at 0 times and by the end of the storage period (7 days). The grown colonies, of the previously cultured plates from the fish-flesh that stored in ice at 0 time and 7 days, were picked up and purified by streaking of the isolate on nutrient agar which incubated at 20^{o}C for 2-3 days. The purified psychrotrophic isolates were identified using biochemical tests **(Krieg and Holt 1984)** and API 20 E strip system.

Statistical analysis:

Statistical analysis was performed using the one way and two ways analysis of variance (ANOVA) and ***Duncan (1955).*** Multiple Range Test was done to determine the differences among the six fish groups (mean at significance level of $P<0.05$). The standard errors were estimated. All the analyses were run on the computer using the SAS program ***(SAS, 2005).***

RESULTS

A total of 276 isolates were recovered from the investigated samples (176) and belong to *Aeromonas (*90), *Pseudomonas (*82), *Salmonella* (64) and *Enterococcus* (40) *spps.* Among those, 220 isolates (79.7%) were recovered from manure and water as well as fish samples from manure supplemented ponds while 56 isolates (20.3%) were obtained from artificial feed and water as well as fish samples from non–manure supplemented control ponds. *Aeromonas* and *Pseudomonas spps* were isolated from the feed, water and fish samples of all groups (1-6) while *Salmonella* and *Enterococcus spps* were only isolated from samples of manure supplemented groups (1-3). Sixty bacteria were isolated from the manure which showed significant high number than those from the artificial food (3 isolates). The pond water of manure supplemented ponds (1-3) revealed 71 bacterial isolates that was higher than those of control groups (4-6) which resulted 37 isolates. The water of manure supplemented ponds showed a significant high number of *Salmonella* and *Enterococcus spp*. when compared with those of control group. The isolated bacteria from the fish samples (liver & intestine) of manure supplemented groups (89) were higher than those of those of the control groups (16). The detailed data about the isolated bacteria from different samples and their significant value, based on the type of the bacterial isolate were reported in Table (1).

The resistance of isolated *Aeromonas spp*. from manure against SXT and NA was significantly higher than those isolated from water and fish, while those against the C, OX and K were higher for the bacterial isolates from the water than manure followed by fish. On the other side, the isolated *Aeromonas spp*. showed no resistance to the CIP in all the investigated samples. The isolated *Pseudomonas spp*. was absolutely resistant to OX (100%) and highly resistant to K for those isolated from manure (100%) followed by water (90%) and fish (80%). The isolated *Enterococcus spp.* from water showed non-significant higher antimicrobial resistance than those isolated from the manure and fish (except for C & SXT). The *Salmonella spp.* that was isolated from both the manure and water, showed similar antimicrobiual resistance while was higher than the isolated salmonella from the fish (Table 2). The antimicrobial resistance of the isolated bacteria was higher with OX and lower with CIP than the other used antimicrobials. The isolated bacteria showed the minimal resistance to the CIP where *Aeromonas spp.* seemed to be the lowest resistant bacteria (0%). Overall, the *Pseudomonas spp*. showed the highest resistance while the *Enterococcus sp*. showed the lowest resistance to the antimicrobials used in this study (Table 3).

The mean value of the total psychrotrophic counts /g of ice-stored fish-flesh of manure supplemented groups (1-3) were higher than those of the artificial feed supplemented control groups (4-6). The increase was significant at 0, 24 and 168 h but non-significant at 72 and 120 h of ice-storage. The total psychrotrophic count/g of fish-flesh, among manure and non-manure supplemented control groups, showed an increase in the mean value by the prolongation in the ice-storage period. The increase was significant in the two groups after 24, 72 and 168 h and non-significant after 120 h of the ice-storage period (Tables 4). The bacteriological examinations in the fish-flesh of the manure supplemented and non-manure control Nile tilapia revealed 199 bacterial isolates after 0 and 7 days of ice storage, they all belong to 13 psychrotrophic bacteria. The frequency of these psychrotrophic isolates, from manures supplemented and non-manure control groups, were 50 and 20 at 0 time of ice-storage but 79 and 50 after 7 days of ice-storage; respectively. The type of the psychrotrophic bacteria and their frequency as well as percentage, of both manure supplemented and non-manure control groups, at 0 time and 7 days of ice-storage were reported in Table (5).

Table (2): Antimicrobial resistance of the isolated bacteria from manure and fish as well as water of manure-supplemented ponds.

Bacterial isolates		Antibiotics and antimicrobial resistance (%)					
Bacteria	**Source**	**C**	**SXT**	**CIP**	**NA**	**OX**	**K**
Aeromonas *(56 isolates)*	Fish (20 isolates)	20	20	0	30	40	20
	Water (20 isolates)	70	40	0	10	80	70
	Manure (16	60	80	0	70	70	40
Chi square value		11.2**	15**	0^{ns}	16.1**	7.5*	10.3**
Pseudomonas *(56 isolates)*	Fish (20 isolates)	20	70	0	70	100	80
	Water (20 isolates)	60	40	20	70	100	90
	Manure (16	70	80	30	80	100	100
Chi square value		11.2**	7.5*	6.7*	0.68^{ns}	0^{ns}	4.44^{ns}
Enterococcus *(40 isolates)*	Fish (6 isolates)	20	10	0	20	40	20
	Water (18 isolates)	40	60	30	60	80	90
	Manure (16	60	40	20	40	70	70
Chi square value		9.19**	7.5*	2.14^{ns}	2.14^{ns}	0.57^{ns}	2.17^{ns}
Salmonella *(56 isolates)*	Fish (20 isolates)	10	0	0	0	70	30
	Water (20 isolates)	50	30	10	10	60	50
	Manure (16	50	30	10	10	60	50
Chi square value		6.66*	10.9**	6.7*	6.67*	7.5*	21.6**

C = chloramphenicol, SXT = Sulphamethoxazole/Trimethoprim, CIP = ciprofloxacin, NA = Nalidixic acid, OX = oxytetracycline, K = kanamycine.

*** P value < 0.05 (significant). ** P value < 0.01(highly significant). ns. P value > 0.05 (non significant)**

Table (3): Resistance of the isolated bacteria from manure supplemented ponds to the tested antimicrobials.

Bacteria	Antibiotics and antimicrobial resistance						Chi square
	C	SXT	CIP	NA	OX	K	
Aeromonas	50.00	46.67	00.00	36.67	63.33	43.33	58.3**
Pseudomonas	50.00	63.33	16.67	73.33	100.00	90.00	119**
Salmonella	40.00	36.67	16.67	40.00	63.33	60.00	35.6**
Enterococcus	36.67	20.00	06.67	06.67	63.33	43.33	70.3**
Chi square	3.44 ns	24.40**	13.30**	56.20**	30.35**	36.10**	

*** P value < 0.05 (significant). ** P value < 0.01(highly significant). P value > 0.05 (non significant)**

Chi square value column: the value among the different antibiotics in the same bacteria.

Chi square value row: the value among the different bacteria in the same antibiotics.

Table (4): Total psychrotrophic counts in the fish-flesh of Nile tilapia reared in manure-supplemented and non-manure control groups during ice-storage for 7 days.

Period/(h)	Manure-supplemented groups (1-3)			Non-manure control groups (4-6)		
	Min.	Max.	Mean X 10^3	Min.	Max.	Mean X 10^3
0 time	$2.7x10^2$	$6.11x10^2$	$00.43^{Ad} \pm 0.10$	$0.7 x10^2$	$1.3x10^2$	$00.11^{Bd} \pm 0.13$
24	$16.9x10^2$	$20.8x10^2$	$01.92^{Ac} \pm 1.19$	$5.2x10^2$	$8.5x10^2$	$00.68^{Bc} \pm 3.79$
72	$17.5x10^3$	$18.2x10^3$	$17.90^{Ab} \pm 0.20$	$10.2x10^3$	$11.7x10^3$	$11.10^{Bb} \pm 0.43$
120	$14.8x10^3$	$29.0x10^3$	$22.26^{Ab} \pm 4.24$	$13.2x10^3$	$18.0x10^3$	$15.56^{Bb} \pm 0.36$
168	$11.0x10^4$	$16.3x10^4$	$140.10^{Aa} \pm 0.66$	$8.7x10^4$	$11.8x10^4$	$97.93^{Ba} \pm 0.39$

Capital letter = comparison among treatment.

Small letter = comparison among time within same treatment.

Table (5): Frequency distribution of psychrotrophic strains isolated from fish-flesh of Nile tilapia reared in manure-supplemented and non-manure control groups at harvest time (0 time) and after 7 day of ice-storage.

Psychrotrophs	Frequency (%) at 0 time (at harvest)		Frequency (%) at 7 day of ice storage	
	Manure	Control	Manure	Control
Acinetobacter spp.	6 (12)	4 (20)	8 (10.08)	5 (10)
Aeromonas spp.	4 (8)	2 (10)	7 (8.82)	3 (6)
Bacillus spp.	5 (10)	3 (15)	6 (7.56)	4 (8)
Coliform	5 (10)	0 (0)	11 (13.86)	1 (2)
Corynebacterium spp.	5 (10)	4 (20)	7 (8.82)	6 (12)
Enterococcus spp.	4 (8)	0 (0)	5 (6.30)	0 (0)
Flavobacterium spp.	4 (8)	0 (0)	4 (5.04)	6 (12)
Micrococcus spp.	6 (12)	5 (25)	9 (11.34)	9 (18)
Moraxella spp.	6 (12)	0 (0)	7 (8.82)	6 (12)
Pseudomonas sp. spp.	0 (0)	1 (5)	2 (2.52)	0 (0)
Salmonella spp.	0 (0)	0 (0)	0 (0)	0 (0)
Shewnella spp.	5 (10)	1 (5)	9 (11.34)	10 (20)
Staphylococcus spp.	0 (0)	0 (0)	4 (5.04)	0 (0)
Total	50 (100)	20 (100)	79 (100)	50 (100)

DISCUSSION

The *Aeromonas* and *Pseudomonas spps.* were isolated from the feed, water and fish samples of both the manure and non manure supplemented ponds. The *Aeromonas spp.* is a group of aquatic bacteria with ubiquitous distribution in the freshwater environment **(Joseph and Carnahan, 1994).** Certain species of this group are pathogens of fish **(Austin and Adams, 1996)** and some are opportunistic or pathogenic to humans **(Janda and Abbott, 1996).** The *Salmonella* and *Streptococcus spps.*, in the present study, were only isolated from manure supplemented groups (1-3). The *Salmonella* is commonly associated with integrated fish farms **(Twiddy and Reilly, 1995).** Although no species of *salmonella* has been found pathogenic to fish **(Shewan**, **1962),** the fish infected with viable *salmonella* organisms, may be a vehicle of transmission to human. The *Enterococcus sp*. was isolated from many natural environments, including soil, water, plants, and birds besides insects, animals and humans **(Kanoe and Abe 1988; Bahirathan et al, 1998; Cai et al, 1999; Svec and Sedlacek, 1999).** The *Enterococci* are commensals in humans and animals, they are associated with gastro-intestinal tract disturbances **(Facklam et al, 1999).** The *Enterococcus spp*. is increasingly causing nasocornial infections and the isolates acquired resistance to a wide range of antimicrobials **(Murray, 1998)**, making the infections difficult to treat.

The isolated bacteria from the manure treated ponds (groups 1-3), were significantly higher than those from control groups (4-6). The percentages of the isolated bacteria from the water and fish samples (liver & intestine) in manure supplemented groups (1-3) were higher than those of control groups (4-6), however the degree of the significance of the increase was varied according to the type of the isolated bacteria. **Coyne et al, (1994)** noticed that the overfeeding and water currents around the marine fish farms, particularly on the seafloor, significantly influenced the buildup of the antimicrobials in the sediment**.** Moreover, the microbial degradation and diffusion, beside the light and temperature conditions influenced the turnover of the antimicrobials in the sediment **(Samuelsen et al, 1992, Lunestad 1992 and Samuelsen 1989)**. Although, such analyses were beyond the scope of this study, it gave an idea by the multiple factors that contributed in the buildup of antimicrobials in the environment including aquaculture.

The antimicrobials, their residues, and antimicrobial resistant bacteria may enter the fish ponds through animal manure within the integrated fish farming systems. A high

prevalence of antimicrobial resistant bacteria has been found in fish farms and the surrounding aquatic environment **(Inglis et al, 1997, Schmidt et al, 2000).** The addition of broiler manure to the pond environment has established a selective pressure favoring growth of antimicrobial-resistant bacteria due to a slow degradation of the antimicrobials (Alderman and Hastings 1998). The motile *Aeromonas sp*. readily developed single or multiple antimicrobial resistance (**Hassani et al, 1992; Goni-Urriza et al, 2000b)**, making it suitable for monitoring the levels of the antimicrobial resistance in the aquatic environment. Thus, the motile *Aeromonas sp*. has been used as an indicator of the organisms which had developed antimicrobial resistance in the water farms (**Schmidt et al, 2000, Petersen and Dalsgaard 2003**) and in a river, receiving waste water (**Goni-Urriza et al, 2000a**). The resistance of currently isolated *Aeromonas* from manure against chloramphinicol and Sulphamethoxazole/Trimethoprim was higher than that obtained from water which was also higher than that isolated from fish. However, a resistance strain against oxytetracycline and kanamycine was higher in water than manure followed by fish. **Petersen et al, (2002)** noticed that the isolated bacteria from the water–sediment samples of the integrated farms showed a higher level of resistance, when compared with the isolated bacteria from the control fish farms. On the other hand, the *Aeromonas* showed no resistance to ciprofloxacin in all the investigated samples. The isolated *Pseudomonas sp*. was absolutely resistant to oxytetracycline, highly resistant to kanamycin (100% for the manure-isolate, 90% from water and 80% from fish). The *Enterococcus sp.* has been used as an indicator for the antimicrobial resistance in animals, humans and environment, including soil, manure and water samples (**Kuhn et al, 2000; Petersen and Dalsgaard 2003).** The currently isolated *Enterococcus* from water showed a higher antimicrobial resistance than those isolated from manure and fish. The emergence of antimicrobial resistant *salmonellae* is a problem for humans and animals worldwide. The isolated *Salmonella* from both manure and water, in the present study, showed a similar antimicrobial resistance which was higher than the isolated *salmonella* from fish. **Duijkeren et al, (2003**) mentioned that the resistance of *salmonella* strains, isolated from chickens, was increased to some antimicrobials (furazolidone and ampicillin) and decreased to others (tetracycline and chloramphenicol), but the Serovar Dublin isolates remained susceptible to flumequine and trimethoprim.

The antimicrobial resistance of the isolated bacteria in this study, was higher for the oxytetracycline than the other used antimicrobials. The *pseudomonas* was showed the highest resistance (100%) to this antibiotic than the others. Although the increased levels of the

bacterial antimicrobial resistance are transient in and around the fish farms, there is a potential risk that the antimicrobial resistance genes could be disseminated into a wide range of the aquatic environmental bacteria. Nevertheless, the resistance to one antimicrobial, within a class of antimicrobials, often confers resistance to the other members of the same group (cross-resistance). The use of the antimicrobials as growth promoters, in animal husbandry, has been linked with certain antimicrobial resistance patterns among human bacterial pathogens **(Bager et al, 1997). Wegener et al, (1999)** suggested that, there is a possible flow of antimicrobial resistance genes between animal and human pathogens through the direct consumption of the antimicrobial-resistant bacteria, present in fish and associated products. **Twiddy and Reilly (1995)** isolated 118 antibiotic-resistant strains, of salmonella, Aeromonas and pseudomonas from fish samples of integrated fish farms, to nalidixic acid (11%), oxolinic acid (12%), chloramphinicol (17%), neomycin (6%), oxytetracycline (74%), tetracycline (75%), furazolidone (47%) and sulphamethazol combined with trimethoprim (25%).

The isolated bacteria, in the present study, showed the minimal resistance to the ciprofloxacin where the *aeromonas* was the lowest resistant (0%). This finding could be due to the recent use of ciprofloxacin in aquaculture. Overall, the pseudomonas seemed to be the highest resistant while the *enterococcus* displayed the lowest resistance to the antimicrobials used in this study. **Petersen et al, (2002**) mentioned that, the levels of the resistance to most antimicrobials were higher for the *Enterococcus sp.* isolated from the water-sediment samples of the integrated farms. The differences were significant for the resistance to the erythromycin, oxytetracycline, streptomycin and ciprofloxacin. **Tapiador (1977)** mentioned that, the extensive use of antimicrobials in human and veterinary medicine has led to an increase in the multidrug- resistant strains where *Salmonella* strains may acquire resistance in food animals before being transmitted to humans through the food chain.

It has been claimed that the risk to public health from the use of antimicrobials in aquaculture probably is very low (Alderman and Hastings, 1998). This is likely true in North American and European aquaculture where the use of antimicrobials is generally decreasing. In contrast, legislation and enforcement of antimicrobial use in animal husbandry and aquaculture in developing countries may be less strict. The combination of animal husbandry and aquaculture as in integrated fish farming or using animal manure makes new routes of contamination of fish and fish products with antimicrobial-resistant bacteria possible.

Furthermore, environmental bacteria from warmer climates may be acclimatized to temperatures near human body temperature and may upon ingestion survive and transfer antimicrobial resistance genes to human gut bacteria (Alderman and Hastings, 1998). However, the practice of using chicken manure as pond fertilizer may represent a route of transmission of antimicrobial-resistant bacteria and antimicrobial resistance genes from animal husbandry to humans. However, this study did not assess whether the presence of antimicrobial-resistant bacteria in the pond environment (feed, water and fish samples) represents any risk to humans. Such risk assessment should take into consideration the impact of other routes of transmission of antimicrobial-resistant bacteria from animals to humans, e.g. from poultry and cattle meat and their products.

The bacteriological examination revealed a significant increase of the psychrotrophic count/g of fish-flesh in the fish of the manure supplemented groups (1-3) when compared with the control. These results could indicate the effect of manure in impairment of the keeping quality and shelf-life of fish as these microorganisms could deeply penetrate the underlying muscles, fastening the spoilage of the product **(El Mossalami and Wassef, 1971).** The examination of the fish for the shelf-life indicated a low quality meat in the fish of the manure treated-ponds than those of the control throughout the period of the study. However, the psychrotrophic count of all groups throughout the period of examination was still within the accepted level of the **Egyptian Standard (2000)** for the fresh chilled fish. The bacteriological examination of fish-flesh samples from both manure-supplemented and control groups revealed the isolation of psychrotrophic bacteria with varied frequency and percentage at both 0 time and after 7 days of ice-storage. However, the psychrotrophic bacteria in the fish-flesh of Nile tilapia reared in manure-supplemented ponds were higher than those reared on the artificial feed-supplemented ponds. These findings could indicate the role of manure in the contamination of fish and pond environment that consequently associated with the increase in the bacterial load of the investigated fish-flesh samples. The bacterial examination revealed no isolation of *Salmonella spp.* from the fish-flesh of both groups. However, *Coliform, Enterococcus spp., Flavobacterium spp.* and *Moraxella spp.* were only isolated from the fish-flesh samples of manure-supplemented ponds at 0 time of ice-storage and *Staphylococcal spp.* was isolated from the same groups after 7 days of ice-storage. These microorganisms were found to be related to the use of manure where fish grown in contaminated ponds with bacteria could facilitate their penetration to the fish-flesh and in turn could represent a threat to the consumers (Thomas et al., 1983). The results of the

bacteriological examination prove the role of the environmental conditions in the growth and multiplication of the various microorganisms that adversely affect the keeping quality and shelf-life of the fish via the rapid deterioration and may consequently constitute a public health hazard. Therefore, strict hygienic measures should be imposed to ensure safety and improve quality of the fish and fish products.

Conclusion:

It could be concluded that, the chicken- manure-supplemented fish-ponds build-up the bacterial contaminants in the aquaculture, such contaminant are hazardous for the fish and human health. They may be zoonotic and frequently build-up the antimicrobial resistant strains and decreases the quality and shelf-life of the products. It is recommended to avoid contaminating fish ponds by enforcing strict hygienic measures. Also the use of antibiotics should be limited to avoid the build-up of the resistant bacterial strains.

Acknowledgements

The authors thank Dr. Patrick Dugan, DDG at the WorldFish center and Dr. Malcolm Beveridge, ADD at the WorldFish center and all other colleagues for their generous support.

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الملخص العربي

تأثير تخصيب أحواض الأسماك بسبله الدواجن على النمو البكتيري ومقاومتها للمضادات الميكروبية بجانب تأثيرها على جودة وفترة صلاحية اسماك البلطى النيلي المستزرع

صلاح الدين مصيلحى على ، نشوه محمود عبدالعاطى* ، محمد فتحي محمد

المركز الدولي للأسماك– مركز تدريبي وبحثي لأفريقيا وغرب آسيا- العباسة شرقية- جمهورية مصر العربية

***قسم المراقبة الصحية على الأغذية -معهد بحوث صحة الحيوان – معمل فرعى الإسماعيلية**

تم استزراع ١٢ ألف من اصبعيات البلطي النيلي في ستة أحواض ترابية تم تزويد ثلاثة منها بسبله الدواجن لنمو الهائمات لتغذية الأسماك أما الثلاثة الأخرى فقد تم تزويدها بغذاء صناعي خلال فترة التجربة (٤ أشهر). كما تم اخذ عينات (مياه، اسماك، سبله، علف) أسبوعيا حتى نهاية التجربة.

وقد تم عزل ميكروب الأيروموناس والسيدوموناس من جميع الأحواض المستخدمة فى التجربة أما ميكروب السالمونيللا والأنتيروكوكس فقد تم عزلها من الأحواض المزودة بسبله الدواجن فقط. وكان ملاحظا أن مقاومة جميع الميكروبات المعزولة كانت عالية ضد الأكسي تتراسيكليين ومنخفضة ضد السبروفلوكساسين. كما لوحظ أن ميكروب السيدومونس المعزول كان أكثر مقاومة لجميع مضادات الميكروبات المختبرة أما الأنتيروكوكس فكان اقل الميكروبات مقاومة. وقد كان العدد الكلى للميكروبات المحبة للبرودة في لحوم الأسماك المستزرعة في الأحواض المزودة بسبله الدواجن أعلى بقيم معنوية عن تلك الأسماك المستزرعة في الأحواض المزودة بغذاء صناعي (علف). وقد اختلف معدل تكرار ونسبة تلك الميكروبات المحبة للبرودة في لحوم الأسماك فور التخزين (ساعة الصفر) وحتى ١٦٨ ساعة من التخزين في الثلج المجروش. كما تم عزل الكلى فورم ، الانتيروكوكس ، الفلافوبكتيريا ، الاستافيلوكوكس والميروكزيللا من لحوم الأسماك المستزرعة في الأحواض المزودة بسبله الدواجن.

وقد انتهى البحث إلى أن استخدام سبله الدواجن في تسميد أحواض الاستزراع السمكي قد يؤدى إلى نقل بعض بكتيريا التسمم الغذائي و الأمراض المشتركة لكل من مزارع الأسماك والمستهلك كما أن تلك الممارسة قد تؤدى إلى مقاومة البكتيريا للعديد من الأدوية وتقلل جودة الأسماك وفترة صلاحيتها.