The Antibiotic Resistant Patterns Of Bacterial Flora Of Cultured Catfish Fed With Poultry Hatchery Waste From Selected Farms In Ibadan, Nigeria

Adedeji O.B. and Onwenefah M

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria
Oluadedeji2001@yahoo.com

Abstract: Microbial quality of poultry hatchery wastes from three selected commercial poultry hatchery units and catfish (*Clarias gariepinus*) fed hatchery waste obtained from five purposively selected aquaculture farms in three local government areas in Ibadan, Oyo state, Nigeria were studied using standard microbiological methods. The antibiotic sensitivity test of the isolated organisms was also carried out to determine their sensitivity to seven different antibiotics commonly used by livestock farmers. The antibiotics were Nitrofuratoin (F), Augmentin (AMC), Ciprofloxacin (CIP), Nalidixic acid (NA), Erythromycine (E), Chloramphenicol (C) and Gentamicine (CN). The results of the samples examined were compared using both descriptive and inferential statistics. One-way analysis of variance was used to compare means among the three different hatcheries and organs. The level of significance was set as $p < 0.05$. The total bacterial count obtained ranged from 1.2×10^5 to 4.6×10^5 cfu/g and 1.2×10^5 to 5.6×10^5 cfu/g for the hatchery waste and catfish respectively. The Total enterobacterial count ranged from 6.0×10^4 to 3.0×10^5 cfu/g and 4.0×104 to 3.0×10^5 cfu/g for hatchery waste and catfish respectively. The Bacteria isolated from hatchery waste were *Staphylococcus epidermidis, Esherichia coli, Bacillus spp, Klebsiella pneumonia and Pseudomonas aeruginosa,* while those isolated from different organs (skin, stomach and intestines) of the catfish were *Salmonella subspp1, Leclercia adecarboxylata, Bacillus spp, Klebsiella pneumonia, Eschericia coli, and Staphylococcus aureus, Citrobacter spp, Pseudomonas aeruginosa. Salmonella arizonae subspp3A*. There were frequent occurrence of *Escherichia, Staphylococcus, Pseudomonas, Salmonella, & Klebsiella* in the samples (hatchery waste and catfish organs) analysed. *E coli* were sensitive to all the antibiotics except Erythromycine. *Salmonella arizonae subsp.3* was also sensitive to all the antibiotics except Nalixidic acid, Erythromycine, and Augmentin. Erythromycine was not active against any of the isolated organisms while *Bacillus spp* was not sensitive to any of the antibiotics. Gentamycin showed highest level activity against all the isolates while Erythromycin showed no activity against any of the isolates. This level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment.

Key Words : Antibiotic Sensitivity Tests , Resistance

INTRODUCTION

Population growth is accompanied by increasing demand for food. Direct human consumption of fish reached an estimated 103 million tons in 2003 (World Fish Center, 2009). Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas where they are widely accepted and form a much-cherished delicacy that cut across-economic, age, religious and educational barriers (Adeleye, 2003). Fish has always been a source of "rich food for poor people" and played an important role in improving a developing country's food security and nutrition for its people. Fish is a major source of high-quality dietary protein, essential vitamins, minerals, and other micronutrients for about 1 billion people, many isolated in rural communities of developing and low income countries (Toft, 2001 and FAO, 2003). About 25% of animal protein is obtained from fish and shell fish and this is required by the body for growth and maintenance of lean muscle and tissue (Ayoola, 2011).

Lack of nutrients is major constraint to aquaculture development and this remains true for much of the catfish culture practised in the developing world. Livestock production systems, and opportunities for reuse of wastes and byproducts, are changing. The intensive nature of modern poultry production and processing tends to concentrate high quality byproducts, and this has stimulated their reuse as livestock feeds.

Concept of utilizing poultry wastes is highly desirable since it will not only eliminate problem of waste disposal but also provide cheap fish feed (Ayoola, 2011). Poultry hatchery waste is an unconventional feed now increasingly used in freshwater aquaculture for economic reasons (Ayoola, 2010). Poultry industry produces large

amounts of hatchery waste which includes solid waste and wastewater. Use of livestock wastes in fish production may however, pose health risks to humans and aquatic organisms through possible introduction of pathogens (FAO, 2003; FAO/OIE/WHO, 2005). Aquaculture products like other foods have hazards that may adversely affect the consumers' health. The production system also presents risks to public health and major health risks of aquaculture products are biological especially for the organisms produced in waste water or water receiving animal and human wastes therefore; safety of consuming fish products from such environments becomes questionable (Erondu and Anyanwu, 2005). The overall sanitary quality of the waters from which these catfish are cultured is key to the overall microbial quality of their finished products. There is a serious risk of potential health hazards arising from pathogenic microbial contamination or parasites within the waste material and from these animal based feedstuffs there is a possibility of a hazard arising from the presence of viable and contaminating micro-organisms, including; bacteria (i.e. Salmonella contamination within animal by-products), fungi, and viruses.

In different studies, several types of microbial and bacterial pathogens have been associated with farmed fish. Many pathogenic microorganisms and parasites could conceivably be transmitted to humans through fish. (George, 2008). Bacterial flora isolated from eggs, skin, gills, and intestines have been described for a limited number of fish species. Generally, there is a relationship between the aquatic environment of the fish and the range of bacterial genera isolated and this varies with factors such as the salinity of the habitat and the bacterial load in the water. Bacteria recovered from the skin and gills may be transient rather than resident on the fish surfaces. The complexity of the fish digestive system appears to determine the microflora of fish intestines. The genera present in the gut seem to be those from the environment or diet which can survive and multiply in the intestinal tract, although there is evidence for a distinct intestinal microflora in some species. While obligate anaerobes have been recovered from carp and tilapia intestines, low ambient temperatures may prevent colonization by anaerobes in species such as rainbow trout (Cahill, 1990).

There is a serious risk of potential health hazards arising from pathogenic microbial contamination or parasites within the waste material and from these animal based feedstuffs there is a possibility of a hazard arising from the presence of viable and contaminating micro-organisms, including; bacteria (Salmonella contamination within animal by-products), fungi, and viruses (from faecal waste products). According to Apata (2009) it is the widespread use of antibiotics in the poultry industry that is the main risk factor for an increase in the occurrence of bacterial resistant strains. Bacteria display variable levels of resistance to antibiotics. The high incidence of antibiotic resistance among the bacteria populating poultry and rising frequency of the bacterial strains represent a public health hazard. Applications of antibiotics in poultry production bring about an increase in resistance to antibiotics not only in pathogenic bacterial strains, but also in commensal bacteria (Lukasova and Sustackova, 2003). In this respect, gastro-intestinal commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure for antibiotic use and for resistance problem to be expected in pathogens. Poultry products and meat are common reservoir of emerging antibiotic resistances available to bacteria inhabiting humans. It can be supposed that the transmission of antibiotic-resistant bacteria to people who got in contact with these sources through direct ingestion or handling results in an increase in the human reservoir of these strains which can rapidly spread to the community In theory, the birds' waste may serve as a vehicle for expanding the transmission of resistance bacteria to humans. In this direction, the waste ends up in water mainly wells and ponds which represent a significant source of natural water supply for rural population in the developing countries. Using hatchery waste from poultry where antibiotics have been use uncontrolled will further compound the problem of antibiotic resistance in the aquaculture industry.

This study sets out to identify the bacterial flora from the skin and digestive system of cultured catfish fed with poultry hatchery waste and also to evaluate and present the results of the antibiotic sensitivity test of the isolated bacterial flora from catfish fed with poultry hatchery wastes.

MATERIALS AND METHODS
Study Location
The study was conducted in five purposively selected fish farms and three commercial poultry hatcheries in Ibadan. The Poultry hatcheries were all located in Oluyole Industrial Estate in Ibadan Southwest Local Government of Oyo State while the fish farms were located in Ido, Oluyole and Southwest Local Government Areas of Ibadan, Oyo state, Nigeria. The choice of the study locations was based on their accessibility and owners' permit on the use of facilities.

Collection and Processing of Samples
15 samples of hatchery wastes were collected in a sterile polythene bags immediately after hatching

the chicken eggs. The samples were collected for a period of two weeks. 60 tissue samples (20 each from skin, stomach and intestines) were harvested aseptically from a total of 20 live catfish collected from the five selected catfish farms. All the samples were then transported in a cooler box containing ice packs to the Food and Meat Hygiene Laboratory of the Department of Veterinary Pubic Health and Preventive Medicine, University of Ibadan and Department of Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Oyo state.

1 cm³ of skin and 1g portion of stomach and Intestine were cut and weighed aseptically from each of the catfish tissue samples and 1g from each of the hatchery waste samples was aseptically weighed and taken for bacteriological examinations. Each of these samples was homogenized in 9ml peptone water that had been prepared aseptically according to manufacturer's instruction. 3-folds and 2-folds serial dilution of each homogenised sample from hatchery waste and fish tissues were further carried out respectively.

Bacteriological Examination of the Samples

Inoculation of diluted samples – 0.1ml of the desired solution of the sample was taken and inoculated (surface spread technic) aseptically unto already prepared cultured media namely; Nutrient agar, Eosin-methylene blue (EMB) agar, MacConkey agar and Salmonella-shigella agar (SSA). All the culture media were all prepared aseptically according to the manufacturer's instruction. The inoculated media plates were then incubated for between 18- 24 hours at 37°C. After incubation, distinct bacterial colonies were counted to determine the colony forming unit (CFU) per gram of the sample (Horsely, 1977, APHA, 1995).

Sub-culturing of isolates- distinct colonies were further subcultured on freshly prepared culture media; Nutrient agar, MacConkey, EMB agar and SS agar to obtain pure isolates of the organisms.

Identification of Pure isolates; after isolation of the pure colonies, the isolates were further identified morphologically and biochemically (Baron and Murray, 1999), using gram staining technique and Microbact Identification Kits (Microbact ™ GNB 12A/B/E,24E, Oxoid). Microbact™ Gram-Negative Identification Kits- it is designed to simulate convectional biochemical substrates used for identifcation of Enterobacteriaceae and common

miscellaneous Gram-negative bacilli. Organism identifiaction is based on pH change and substrates utilization (Cowan, 1977 and Farmer, et al, 1985;).

ANTIBIOTICS SENSITIVITY TEST OF THE ISOLATED BACTERIAL ORGANISMS

The test bacterial isolates were inoculated unto nutrient agar and followed by application of the discs (oxoid Ltd) impregnated with different antibiotics. Agar disc diffusion method (Baur et al., 1996; SFM, 2003) was employed. Antibiotic dics contained the following seven antibiotics Nitrofuratoin (F) -300μg, Augmentin(AMC)- 30μg , Ciprofloxacin (CIP)- 5μg, Nalidixic acid (NA)- 30μg, Erythromycine (E)- 10μg, Chloramphenicol (C)- 30μg, Gentamicine (CN)- 10μg .

3.6. **Statistical Analysis.**

Data entry and analysis was done using SPSS version 15. Both descriptive and inferential statistics were used. The mean total bacterial and enterobacterial counts in the hatchery waste samples and fish organs (skin, stomach, & intestine) were calculated. One-way analysis of variance was used to compare means among the three different hatcheries and organs. The level of significance was set as $p < 0.05$.

Results.

Table 1.0 shows the bacterial isolates obtained from the different catfish organs and the hatchery waste. The species of the bacterial isolated are those in the genera *Citrobacter, Esherichia, Staphylococcus, Pseudomonas, Salmonella, Bacillus, Leclercia* and *Klebsiella*.

All the samples of the hatchery waste were contaminated with the microbial load (log $_{10}$ CFU/g) in the range of 5.28-5.66. The enterobacterial load (log $_{10}$ CFU/g) of the hatchery waste samples was in the range of 4.30-5.48.

The organs were contaminated with bacteria load (log $_{10}$ CFU/g or cm^2) in the range of 5.08-5.75. The enterobacterial load (log $_{10}$ CFU/g or cm^2) was in the range of 4.06-5.66 with skin having the lowest. The result of total bacterial count of the fish are presented in table 2 while that of enterobateriaceae count in the skin, stomach and small intestine are presented in table 3. The zones of inhibition of the isolates from hatchery waste and the different organs in the catfish are as presented in tables 4 and 5 respectively.

Table 1. Bacterial isolates obtained from different catfish organs and hatchery waste

SAMPLES	BACTERIA ISOLATES
Skin	*Citrobacter spp, Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa*
Stomach	*Salmonella subsppI, Leclercia adecarboxylata, Bacillus spp, Klebsiella pneumonia, Eschericia coli, Staphylococcus aureus*
Intestine	*Eschericia coli, Salmonella arizonae subspp3A*
Hatchery waste	*Pseudomonas aeruginosa, Eschericia coli, Staphylococcus epidermidis, Bacillus spp, Klebsiella pneumonia,*

Table 2.0: Mean total bacterial load count in selected organs of cat fish fed hatchery waste.

	DESCRIPTIVE	ANOVA		
Organs.	Mean ± SD (n = 20)	F-value	P-value	Remarks
Skin	33.1 ± 9.0			
Stomach	37.1 ± 7.3	2.02	0.14	Not Significant.
Intestine	32.7 ± 6.3			

Table 3.0: Mean enterobacteria load count in selected organs of cat fish fed hatchery waste.

	DESCRIPTIVE	ANOVA		
Organs.	Mean ± SD (n = 20)	F-value	P-value	Remarks
Skin.	17.5 ± 8.6			
Stomach.	14.1 ± 9.0	1.38	0.26	Not Significant
Intestine.	13.3 ± 8.2			

Table 4.0 (a): Diameter (mm) of zones of inhibition to individual antibiotics.

Isolates from Poultry Hatchery samples	F(300μg)	NA(30μg)	CIP(5 μg)	C(30 μg)	E(10μg)	AMC(30μg)	CN(10 μg)
Escherichia coli	20	14	23	2	-	18	20
Klebsiella pnuemoniae	-	-	25	-	-	-	22
Bacillus spp	-	-	-	-	-	-	-
Staphylococcus epidermidis	8	17	-	-	-	14	21

F- Nitrofuratoin, AMC – Augmentin, CIP – Ciprofloxacin, NA - Nalidixic acid,
E- Erythromycine, C – Chloramphenicol and CN – Gentamicine.

Table 5.0 (b): Diameter (mm) of zones of inhibition to individual antibiotics.

Isolates from Catfish samples	F(300μg)	NA(30μg)	CIP(5 μg)	C(30 μg)	E(10μg)	AMC(30μg)	CN(10 μg)
Escherichia coli	20	15	25	20	-	20	20
Salmonella arizonae subsp.3	18	-	25	20	-	-	20
Escherichia fergusonii	18	12	20	-	-	18	12
Klebsiella pnuemoniae	16	-	25	-	-	16	12
Leclercia adecarboxylata	18	-	20	-	-	15	18
Citrobacter freundii	-	-	-	-	-	-	-
Salmonella subsp.1	-	20	26	-	-	25	20
Bacillus spp	-	-	-	-	-	-	--

F- Nitrofuratoin, AMC – Augmentin, CIP – Ciprofloxacin, NA - Nalidixic acid,
E – Erythromycine,   C – Chloramphenicol, CN – Gentamicine.

DISCUSSION

In the aquaculture industry, resistance to antibiotics has been commonly reported (for example, Rhodes et al. 2000; Schmidt et al. 2001; Kim et al. 2004; Hatha et al. 2005; Akinbowale 2006 and Adedeji et al 2011) all reported the resistance of different bacteria to different antibiotics in their studies. According to Apata (2009) it is the widespread use of antibiotics in the poultry industry that is the main risk factor for an increase in the occurrence of bacterial resistant strains. Bacteria display variable levels of resistance to antibiotics.

The high incidence of antibiotic resistance among the bacteria populating poultry and rising frequency of the bacterial strains represent a public health hazard. Applications of antibiotics in poultry production bring about an increase in resistance to antibiotics not only in pathogenic bacterial strains, but also in commensal bacteria (Lukasova and Sustackova, 2003). In this respect, gastro-intestinal commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure for antibiotic use and for resistance problem to be expected in pathogens. Poultry meat, products and waste are common reservoir of emerging antibiotic resistances available to bacteria inhabiting humans using hatchery waste from poultry where antibiotics have been use uncontrolled will further compound the problem of antibiotic resistance in the aquaculture industry.

In this study, the species of bacteria isolated are those in the genera *Citrobacter, Escherichia, Staphylococcus, Pseudomonas, Salmonella, Bacillus, Leclercia, Klebsiella.* .The bacterial load observed in the hatchery waste was in the range of 1.9×10^5 to $4.6 \times 10.^5$. The total number of bacterial count for fresh catfish (*Clarias gariepinus*) was in the of $1..2 \times 10^5$ cfu\g and 5.6×10^5 of fish organs, and this number fell within accepted limit according to Anon (1991) who said that the acceptability limit is 10^6 cfu\g for mesophilic aerobic bacteria. Also Sikorski (1990) reported that for high quality fresh fish, the number of bacteria present on the surface vary from $10^3 – 10^4$ cfu\g. These results agree with finding of Chou (1993) who reported that the total aerobic plate count of unwashed and washed catfish frame mince without cryoprotectants were 5×106 cfu\g and 106 cfu\g, respectively.

However, in spite of the normal bacterial load seen in this study, bacterial organisms isolated are pathogenic and capable of causing diseases in both fishes and humans under poor hygiene practice and in immuno-compromised individual and **animals**. This study has also shown that the enteric bacteria isolated from hatchery waste and fish were resistant to some antibiotics. *E coli* was sensitive to all the antibiotics except Erythromycine. *Salmonella arizonae subsp.3* was also sensitive to all the antibiotics except Nalixidic acid, Erythromycine, and Augmentin. Erythromycine was not active against any of the isolated organisms while *Bacillus spp* was not sensitive to any of the antibiotics. In this study therefore, a multidrug resistance pattern was observed in most of the bacterial isolates. However, the bacterial species were susceptible to the antibiotics Ciprofloxacin, followed by Gentamycine, Augmentin and Nitrofuratoin in that order.

Resistance to Erythromycine was wide spread in this study. Resistance to Erythromycine might be related to their overuse. Secondly, resistance of Gram-negative bacteria to erythromycin is to be expected because of intrinsic resistance of many such organisms to macrolide antibiotics. The emergence and dissemination of multiple antimicrobial resistance among *E.coli, Salmonella spp* is an increasing global health problem that is complicating the therapeutic management of severe salmonellosis and diarrhogenic diseases.

The variety of resistance pattern observed in this study can be correlated to antibiotic use in Nigeria and are similar from those seen in countries where antibiotics are known to be used in aquaculture. Chloramphenicol resistance has also been reported in Chile (Mirand and Zemelman 2002), France (Michel et al., 2003) and in a Western Mediterranean study (Chelossi et al. 2003). Quinolone resistance has been reported in environmental isolates at a relatively low frequency (McKoen et al. 1995; Guardabassi et al. 1998); however, Chelossi et al. (2003) reported resistance to nalidixic acid in 70% of their isolates. The result showed resistance to more than one class of antibiotic. Multiple drug resistance has been reported in a number of studies of fish pathogens and aquaculture environments (McPhearson et al. 1991; Schmidt et al. 2000; Hatha et al. 2005; Adedeji et al., 2011).

Tjahjadi et al., 1994, Miranda and Zemelman (2002) reported that bacteria resistant to six to ten antibacterials were common. Studies on the antibiotic resistance in bacteria from shrimp ponds (Tendencia and de la Pena 2001) demonstrated a correlation between multiple bacterial antibiotic resistance levels and use of particular drugs. The types of bacterial organisms that are associated with the hatchery waste and catfish fed hatchery waste found in this study call for concern

In conclusion, the widespread use of antibiotics in fish farming for therapeutic, prophylactic purposes and as growth promoters can promote the emergence of resistance in bacteria. Many of the antibiotics investigated in the present study are known to be used in veterinary practice and the poultry industry. It is therefore necessary to monitor the usage of antibiotics in aquaculture practices to prevent the frequency of development of resistant fish pathogens and bacteria. Moreover the transferability of fish pathogens that are multi-drug resistant through horizontal gene transfer in the food chain has human health consequences (Nawaz *et al.*, 2001; Heuer *et al.*, 2009).

Corresponding Author:
Adedeji Olufemi B.
Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria.
Oluadedeji2001@yahoo.com

References
1. Adedeji O. B, Okerentugba P. O, Innocent-Adiele H.C, Okonko I.O (2012): Benefits, Public Health hazards and risks associated with fish consumption *New York Science Journal* 2012; 5(9) 33
2. Adedeji O.B., Tiamiyu A.M. and Emikpe B.O.: The Antibiotic Resistance Patterns of Bacterial Flora of Fish from Different Aquatic Environment from Ibadan, Southwest Nigeria. *Advances in Envronmental Biology*, 5(8) 2039-2047 2011.
3. Adeleye, O. A. conservation needs of fisheries resources and reorientation for sustainable captive and culture practices. Proceedings of the 10th annual Conference, Fisheries Society of Nigeria, 2003: 230-234.
4. Akinbowale O.L., Peng H. and Barton M.D. (2006) Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia Journal of Applied Microbiology 100:1103–1113.
5. ANONYMOUS. 1991. Su Ürünlerinde Mikrobiyolojik ve Ag˘ir Metal Kabul Edilebilir Deg˘erler. Resmi Gazete, 28 Mayis 20884, p. 5.
6. Apata D.F. (2009) International Journal of Poultry Science 8 (4): 404-408, 2009.
7. APHA (1995). Standard Methods for the examination of Water and Wastewater. 19th ed. American Public Health Association**.**
8. Ayoola, S.O 2010. Modern fish farming techniques (Aquaculture) (2nd edition). Glamour books Publishing, Ibadan;pp 180
9. Ayoola, S.O 2011. Heamatological characteristics of Clarias gariepinus (Buchell, 1822) Juveniles fed with Poultry hatchery watse. *Iranical Journal of Energy and Environment* 2(1); 18-23.
10. Baron, E.J., and P.R. Murray 1999. Bacteriology. In Manual of clinical microbiology, 7th Edition P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). ASM Press, Washington, D.C., pp. 246–831.
11. Bauer, A.W., Kirby W.M.M., Sherris, J.C.and Turck, M. (1966). Antibiotic susceptibility testing by a standard single disc diffussion method. *American Journal of Clinical Pathology* 45, 493-496
12. Cahill, M.M., (1990), Bacterial flora of fishes: A review. Microbial Ecology, 19: 21-41

13. Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Ricarrdi, G. and Banat, I.M. (2003) Antibiotic O.L. Akinbowale et al. Antimicrobial resistance aquaculture resistance of benthic bacteria in fish farm and control sediments of Western Mediterranean. Aquaculture 219, 83–97.
14. Chou C. 1993. Evaluation of Quality Changes in Frozen Catfish (Ictalurus punctatus) Mince[M.S. thesis]. Baton Rouge, La.: Louisiana State University.
15. Cowan, S. T. (1977). Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd edition. Cambridge University Press, London.
16. Erondu, E. S and Anyanwu, P. E 2005. Potential hazards and risks associated with the aquaculture industry. *African Journal of Biotechnology* 4 (1); 1622-1627
17. FAO (2003). Recycling of animal waste as a source of nutrients for freshwater fish cultured within an integrated livestock system.
18. FAO /OIE/ WHO 2005. Consultation on avarian influenza and human health. Risk reduction measures in producing,marketing and living with animals in Asia. FAO, Roma.
19. Farmer J J, III, Davis B R, Hickman-Brenner F W, McWhorter A, Huntley-Carter G P, Asbury M A, Riddle C, Wathen Grady H G, Elias C, Fanning G R, Steigerwalt A G,O'Hara C M, Morris G K, Smith P B, Brenner D J. Biochemical identification of new Species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J Clin Microbiol. 1985;21:46–76.
20. George J. Flick, Jr (2008); Microbiology Safety of Farmed Fish. Global Aquaculture Advocate .pp34
21. Guardabassi, L., Petersen, A., Olsen, J.E. and Dalsgaard, A. (1998): Antibiotic resistance in Acinetobacter spp. Isolated from sewer receiving waste effluent from hospital and a pharmaceutical plant. *Appl Environ Microbiol* 64, 3499– 3502.
22. Hatha, M., Vivekanandam, A.A., Joice, G.J. and Christol, G.J. (2005) Antibiotic resistance pattern of motile aeromonads from farm raised freshwater fish. *Int J Food Microbiol* 98,131–134.
23. Heuer O. E., Kruse H., Grave K., Collignon P., Karunasagar I., Angulo F. J. (2009) Human health consequences of use of antimicrobial agents in aquaculture. *Clin. Infect. Dis.* 49 1248–1253.
24. Horsley, R.W (1977). A review of bacterial Flora of teleosts and elasmobranches,including

methods for its analysis. *Journal of Fish Biology* 10,529-533

25. Kim S.-R., Nonaka L., Suzuki S. (2004). Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. FEMS Microbiol. Lett. 237, 147–156

26. Lukasova, J. and A. Sustackova, (2003). Enterococci and Antibiotic Resistance. Acta. Vet. Brno., 72: 315-323

27. McKoen, D.M., Calabrese, J.P. and Bissonnette, G.K. (1995) Antibiotic resistant Gram-negative bacteria in rural ground water supplies. Water Res 29, 1902–1908.

28. McPhearson, R.M., DePaola, A., Zywno, S.R., Motes, M.L. Jr and Guarino, A.M. (1991) Antibiotic resistance in Gramnegative bacteria from cultured catfish and aquaculture.

29. Michel, C., Kerouault, B. and Martin, C. (2003) Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *J Appl Microbiol* 95, 1008– 1015.

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

31. Nawaz, M.S., B.D. Erickson, A.A. Khan, S.A. Khan, J.V. Porthuluri, F. Rafii, J.B. Sutherland, R.D. Wagner and C.E. Cerniglia(2001): Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. Regul. Res. Perspect., 1: 1-8.

32. Rhodes G, Huys G, Swings J, McGann P, Hiney M, Smith P, Pickup RW (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. Appl. Environ. Microbiol. 66:3883-3890.

33. Schmidt, A.S., Bruun, M.S., Dalsgaard, I. and Larsen, J.L. (2001): Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile Aeromonads from a fish-farming environment. *Appl Environ Microbiol* 67, 5675–5682.

34. Sikorski ZE (1990). Seafood: Resources, nutritional composition and preservation. Boca Raton, Fla.: CRC press Inc. p. 248

35. Tendencia, E.A. and de la Pena, L.D. (2001) Antibiotic resistant bacteria from shrimp ponds. *Aquaculture* 195, 193–204.

36. Tjahjadi, M.R., Angka, S.L. and Suwanto, A. (1994) Isolation and evaluation of marine bacteria for biocontrol of luminous bacterial disease in tiger shrimp larvae (Penaeus monodon, Fab.). *Asia Pac J Mol Biol Biotechnol* 2, 347–352.

37. Toft M. (2003): The importance of Fish and other aquatic animals for food and nutrition security in the lower Mekong Basin (M.sci Thesis). Frederiksberg, Denmark. Department of Human Nutrition, The Royal Veterinary and Agriculture University.

38. World Fish Center. The threat to fisheries and aquaculture from climate change. World Fish Center. 2009: 8.

39. www.fao.org/docrep/field/003/AB468E. The nutrition and feeding of farmed fish and shrimp; a training manual 2- FAO Corporate Document Repository.

7/26/2013