Journal of Kerbala University , Vol. 6 No.4 Scientific. 2008

Distribution of some aerobic bacteria in an infected Cyprinus carpio L. fish farm in Basrah and its resistance to antibiotics

Eman A. Al-Imarah
Department of Environmental Chemistry, Marine Science Center, University of Basrah, Basrah, Iraq

Abstract:

During April 2004, 30- carp male infected fishes and 25 water samples from fish farm ponds in Basrah city were collected. swabs were taken from: under scales , gills , air sac , intestine , spleen , liver , heart , gonads , and kidney. Both samples were tested for bacteria. The following Gram-negative bacterial species were isolated from all samples: Pseudomonas fluorescence (41isolates),Aeromonas hydrophila (28 isolates), Escherichia coli (27 isolates),Klebsiella pneumoniea (21 isolates), Pseudomonas sp.(18 isolates), and Proteus vulgaris(17 isolates). Pseudomonas fluorescence was the dominant among Gram- negative bacterial species isolated from fish samples while Escherichia coli was the dominant among Gram- negative bacterial species isolated from water samples. The Gram- positive bacterial species which isolated from all samples were: Staphylococcus aureus(31 isolates),Sterptococcus faecalis (25 isolates), S.faecium(19 isolates) , Staphylococcus epidermidis(11 isolates), and S.pyogenes(11 isolates). Numbers of Gram-negative bacterial species that isolated from all samples were greater than those of Gram-positive bacterial species. The Minimum Inhibitory concentrations (MICs) for 10 isolates of each bacterial species were determined; Gram-negative bacterial species showed more antibiotic resistance than Gram-positive bacterial species.

الخلاصة :

خلال شهر نيسان ٢٠٠٤، جمعت ٣٠ سمكة من يافعات ذكور الكارب الاعتيادي و ٢٥ عينة من مياه أحواض التربية من أحدى المزارع في محافظة البصرة للتحري عن وجود البكتيريا الهوائية في هذه المزرعة . شرحت الأسماك وأخذت مسحات من المناطق التالية: تحت القشور، الغلاصم ، كيس الهواء ، الأمعاء، الطحال ،الكبد ، القلب ، الأعضاء التكاثرية والكلية . فحصت المسحات بالإضافة إلى فحص عينات المياه وعزلت الأنواع البكتيرية السالبة لصبغة غرام التالية: Pseudomonas41)fluorescence عزلة)، Aeromonas hydrophila (28 عزلة)، Escherichia coli (27 عزلة). Klebsiella pneumoniea(21 عزلة)، Pseudomonas sp.(18 عزلة)و Proteus vulgaris (17 عزلة)، كان النوع Pseudomonas fluorescence هو النوع السائد ضمن البكتيريا المعزولة من عينات الأسماك في حين كان النوع السائد في عينات المياه هو Escherichia coli. عزلت الأنواع البكتيرية الموجبة لصبغة غرام التالية:Staphylococcus aureus (٣١ عزلة)، Sterptococcus faecails (٢٥ عزلة) , S.faecium (١٩ عزلة) ، ، Staphylococcus epidermidis (١١ عزلة) و S.pyogenes (١١ عزلة). حددت قيمة التركيز المثبط الأدنى MIC بعد اختيار ١٠ عزلة من كل نوع بكتيري تجاه خمسة مضادات حياتية وأظهرت البكتيريا السالبة لصبغة غرام مقاومة لهذه المضادات أعلى من تلك التي أظهرتها البكتيريا الموجبة لصبغة غرام.

Introduction

There is substantial evidence that fish and seafood may be vehicle for many bacterial pathogens (1).In assessing the risks from fish, it is important to have information on the incidence of these pathogens (2). As aquaculture has developed, a range of fish and shellfish diseases have been encountered that have led to major economic losses and the failure of the industry in some parts of the world. This has led to the increased use of veterinary drugs and vaccines in intensive production system to combat diseases in farmed fish. Antibiotics are commonly used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish such as Pseudomonas spp., Aeromonas spp., Streptococcus spp., etc. They are commonly used as in-feed medications or surface coated onto feed pellets and dispersed in water (3).There is a wide range of antimicrobial agents used in aquaculture; the use of antibiotics in fish farming is associated with new hazards in fishery products that are not encountered in wild captured species .The main hazards are antibiotic residues and the development of antimicrobial resistance in bacteria that may be transferred to consumers of farmed fish (4).Cyprinus carpio L.1758 (Cyprinidae) represents one of the most edible fish species which cultured in many countries, one of them is Iraq. Common carp farms are distributed in Basrah. The people like this species of fish, so the aim of this study is to isolate the aerobic bacterial pathogens from common carp farm and determination of their susceptibility to some antibiotics.

Materials and Methods

1-Sampling and culture conditions

Samples were collected in Apr. /2004. 30 male fish of common carp Cyprinus carpio.and 25 water samples one fish farm in Basrah.All samples were placed in ice box and transferred to the laboratory within 3hrs.

Fish Samples By using two ponds of fish farm, thirty infected fish samples were collected. weights of fishes were ranged l80-300 gms, whereas lengths ranged (20-25cm).In this farm there were high mortality, the symptoms which appeared on fishes were: hemorrhage, red spottiness along the body of fish. Fish were dissected by using sterile dissecting tools, swabs were taken from: under scales, gills, air sac, intestine, spleen, liver, heart, gonads, and kidney, the solid media which used in this study were listed in Table -1- . The sampling and culturing procedure had done according to (5).

Water samples:

Twenty five subsurface water samples were collected in sterile plastic flasks, 100 ml volume from the above mentioned tow ponds. Water samples were transferred to the lab in ice box .In the laboratory, 10 ml of each samples was filtered through 0.45 µm filter papers(WCN, Japan),cultured on the solid media mentioned in Table-1- .(6). All samples had incubated at 37° C for 24 hrs.The-grown colonies had identified according to (7). From each bacterial species, 10 isolates were randomly chosen to determine the minimum inhibitory concentration (MIC) of five antibiotics against these isolates.

2-Determination of MICsDetermination of MICs was done by serial dilution technique as described by (8), the five antibiotics used in the study were: Amoxicillin (Amx), Chloramphinicol(C), Tetracycline (Te), Sulphamethoxazole – Trimethoprime (S-T) combination, and gentamycin (G).This test was perfumed by preparing a stock solution with a concentration of 256 µg/ml, from it serial concentrations were prepared (128, 64, 32, 16, 8, 4, 2) µg/ml of each antibiotic,1.0 ml of each concentration was inoculated in a tube containing 10 ml of approximately 1.5x 108 cfu / ml(equal to McFarland tube no.0.5) of bacterial suspension. For each concentration, there was a control tube which consisted of the certain concentration of antibiotic without adding the bacterial suspension, all tubes then incubated at 35◦ c overnight, the tubes examined for visible signs of bacterial growth. The highest dilution without growth was the minimal inhibitory concentration (MIC).

Results

According to the data shown in Table 2, the origin of gram negative bacterial isolates tested during the period of this study were: Pseudomonas fluorescence was isolated from 3 sites of fish samples: under scales, liver, and heart, when the species Aeromonas hydrophila was identified in two different organs of fish samples (intestine and kidney). Ps.sp. had isolated only from under scales samples, whereas the species Klebsiella pneumoniae was isolated only from the liver samples. The species Escherichia coli was isolated from two organs of fish samples: intestine and kidney. The lowest percentage of isolation was for Proteus vulgaris which isolated only from kidney samples.Information derived from results reported in Table 2 indicated that Staphylococcus aureus was the most frequented species among Gram positive bacteria followed by S.faecalis, whereas S. pyogens was the least frequented species. S .faecium was isolated only from intestine samples; S.epidermidis was isolated from 2 sites (under scales and kidney).Table 3 showed the various percentage of distribution for Gram negative bacteria in water samples. The higher frequented species was Escherichia coli (92%), while the least frequented species was Ps.sp. (40%). Table 4 showed the various percentage of distribution for Gram positive bacteria in water samples. The higher frequented species was S.aureus (76 %), while the less frequented species was S.epidermidis (20%).Table 5 showed the numbers of Gram positive bacterial species collected during the period of the study. The species S.aureus was the most frequented Gram positive species (31 isolates) while both S.pyogens and S.epidermidis was the least frequented Gram-positive species (11 isolates for each species).The results of the determination of the minimum inhibitory concentration (MIC) for the bacteria against 5 antibiotics are summarized in Table 6 .The species Pseudomonas fluorescence showed a resistance to all antibiotics used in the study: Amoxicillin, (AMX) (90%). Chloramphenicol, (C) (80%). Tetracycline (TE) (90), sulphamethoxazole-trimethoprim, (S-T) (80%). gentamycin (GEN) (80%).The species A. hydrophila was fully susceptible to GEN (0%), highly susceptible to(S-T) combination (10%resistance). These findings were in agreement with (Ahmed et al., 1996).On the other hand, A. hydrophila was high resistant to AMX (90%), C (80%), and TE (90%). The species K. pneumoniae was resistant to all antibiotics: AMX (90%), C (80%) TE (80%), S-T (90%), and GEN (80%).The species Proteus vulgaris was resistant to three antibiotics: AMX (80%), C (60%), and S-T (50%) respectively, while the species was sensitive to both TE (30%) and GEN(10%).The species E.coli was resistant to all antibiotics used in the study: AMX (100%), C(80%), TE(90%), S-T(90%), GEN(80%).

Table 7 showed the results of in -vitro activities of the five antibiotics used in the study against 10 isolates of each Gram-positive bacterial species. The most resistant species was S.faecalis, it was resistant to all antibiotics used in the study, the percentage of the resistance were AMX (60%), C (50%), TE (50%), S-T (60%),and (50%) respectively, while S.pyogens was the least species in its resistance to the antibiotics, it was susceptible to all antibiotics used in the study(20%,20%,20%,10%,and l0%) respectively.

Discussion

As shown in Table 2, there is a significant variation in the distribution of the gram – negative bacterial isolates among the different tested fish samples. Pseudomonas florescence was recovered in this study; which is encountered in cases of spottiness of the skin and hemorrhagic bacterial septicemia (9).The results revealed isolation of several members of family Enterobacteriaceae from fish samples that had identified as Klebsiella pneumoniae, Proteus vulgaris, and Escherichia coli. These bacteria are potentially present in water and are not known as classical fish pathogens, yet the oxygen depletion and high water temperature rendered fish to be easily infected with these bacteria. The public health importance of Klebsiella species lies in the assumption of being a member of the food poisoning organisms and a cause of respiratory as well as urinary affection in human being (10).Aeromons hydrophila group is wide spread in the water environment; it has been isolated from water of rivers and cultured carp, Furthermore, A. hydrophila is considered to be the principle cause of hemorrhagic bacterial septicemia in fresh water fish. In recent years, A. hydrophila received increasing attention as an agent of food borne diarrhea disease in healthy people (11). These findings are in agreement with (12) who reported that some strains of A. hydrophila or species of Pseudomonas are etiological causes for systemic infections in common carp. An interspecific difference in vulnerability to these facultative bacteria infection reflects the degree of compatibility between fish and their environment rather in innate tolerance or susceptibility to a specific bacterial pathogen. The presence of detectable numbers of S.faecalis and S.faecium in the examined fish is an indication of pollution of water with sewage and animal wastes. Moreover, these organisms have been isolated from implicated in cases of food poisoning and the pathogenicity of such organisms on fish is not clearly recognized (13).

The presence of Staphylococcus aureus in fish indicated their contamination from polluted water or it is good indicator of the personal hygiene of food handlers suppurating lesions or from the nostrils of carrier "usually via the hand"(14).

The species S.epidermidis is one of the most important specific microorganisms responsible for congestion and ulceration on the tail of fish (15). The data presented in Table 3 revealed that Streptococcus pyogenes was isolated from examined fish in different percentage of distribution. Such pathogen was found to be an etiological significant in some epizootic among different fresh water fish (16).The affected fish are characterized by external hemorrhages around the anus and ventral body surface, secretion of abnormal slime on the gills, numerous hemorrhages in the intestine and accumulation of reddish fluid in the body cavity(17). The presence of some Gram negative bacterial species in water samples, which had previously isolated from fish samples, confirmed the probability that the source of infection in fish is surrounding water (16). From Table 4, it can be concluded that the frequency of Gram- positive bacteria was less than that of Gram negative bacteria, this is in agreement with (17) which reported that Gram- positive organisms can be found in various properties, but in general Gram negative bacteria dominate the micro flora of cultured carp. Data tabulated in Table 5, were in agreement with the following statement "Most pathogens of common carp are normally found in small numbers, both on the fish and in the environment. This constant low challenge helps to keep the immune system of the fish primed, as a kind of natural vaccination" (6) recorded this statement. The results of Table 6 were in agreement with (17) and (8) who reported that the resistance had been shown to occur more frequently in certain bacterial species, e.g. Pseudomonas spp.Since the ability to express resistance was almost evenly distributed among the different Gram-negative groups. (18) found similar results. They suggested that bacterial groups cohabitating a common environment share a pool of R-factor plasmids and therefore have a similar antibiotic resistance patterns. Similar results were obtained by (19) who attributed this high frequency of resistance to many years of exposure to drugs and their improper application. The above findings were in agreement with (18) and (3) who reported that conjugation is thought to be the principle way in which transfer of antibiotic resistance genes occur between bacteria. Large plasmids that encode resistance to several different antibiotics have been found in human pathogens such as the above bacterial species. The findings of Table 7 were in agreement with (19) who reported that the antibiotic resistance of the Gram- positive bacterial species isolated from common carp is somewhat lower than that of Gram- negative group. They had attributed these differences to genetic and environmental variations between these two groups of bacteria.

Antibiotics should never be used as an easy alternative to good fish farming practices. National governments need to put in place control programmes for residues of antimicrobials in aquaculture productions. Such control programmes should control the approval or licensing of antimicrobials and should control their sale and use in fish farming. What is required at national level is up-to-date legislation and standards that are based on sound science, a monitoring program and adequate resources for enforcement of the legislation, consumers can protect themselves against antibiotic resistant bacteria, as these are just as susceptible to heat and hygiene as their non- resistant counterparts. Thorough cooking ,frequent hand washing, prevention of cross- contamination by separating raw sea foods from other foods and proper chilled storage will minimize the incidence of seafood poisoning.

References

1. Hosseini, H.; Cheraghali, A.M.; Yalfani, R.; and Razavilar, V. Food Control, (2004)15: 187.
2. Basti, A.A.; Misaghi,A.; Salehi,T.Z.; and Kamkar, A. Food Control, (2006). 17:183.
Reilly, A. In: Huss, H.H., Ababouch, L., and Gram, L. (Eds.) (2004). FAO, Rome, Italy, Chap. 5.
Spanggaard,B.; Jørgensen,F.; Gram, L., and Huss,H.H.. Aquaculture, (1993), 115: 195.
Andrews, W. Manual of food quality control 4. Rev.1.Microbiolgical analysis (1992). FAO Food and Nutrition Paper, Rome, Italy, pp. 347.
Schmidt, A.S.; Bruun, M.S.; Dalsgaard, I., Pederson, K., and Larsen,J.L.,. Appl. Environ. Microbiol. (2000), 66:4908.
Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley, J. T. and Williams, S. T. Bergey's manual of determinative bacteriology. (1994). 9th ed., PP. 787.
Baron, E. J., and Finegold, S. M. Diagnostic microbiology, (1990).7th Ed. The C. V. Mosby Company. Toronto, pp: 171.
Abo El-Alla, A.A.; and Bastawrows, A. F. Assiut.Vet. Med. J. (1999).., 42 (83): 174.
Bastawrows, A. F., and Mohammed, A.A., Assiut.Vet. Med. J., (1999). 40 (80): 197.
Ahmed, S.A.; Megid, R.M.A.; Marzouk, M.S.; Moustafa, M., and Gado,M. Vet. Med. J. Giza(1996).44 (11):11.
Tylor, P. W. (2003). Multiple antimicrobial resistance in a chronic bacterial infection of koi carp. North American J.Aquqculture. 65: 120.
Paperna, I.CIFA technical paper (1996). No.31, FAO, Rome, Italy.
Elwi, E.M. Ph.D. thesis, (1994). Fac. Vet. Med., Assiut Univ., Egypt.
Mousa, M. M.; and Mahmoud, Y.E. Assiut.Vet. Med. J., (1997). 37 (73): 219.
Al-Shatty,S.M. H. (2006). Ph.D. thesis, College of Agriculture, University of Basrah.
Arslan, A. Tr.J.Vet.Anim. Sci., (1992). 17:251.
Khater,A.A.; Abboud,L.O.A., and Fayad,A.A. Alex.J. Vet. Sci., (1997). 13(2):75.
Klein, B.U.; Siesnop, U.; and Boehm, K.H. Bull. Eur.Assoc. Fish Pathol(1996). 16:138.

Table 1: Media used in the study

No.	Media Type	Uses
1	MacConkey Agar	To isolate the Gram – negative bactria.
2	Pseudomonas F Agar	to isolate the bacteria which follow the genus Pseudomonas
3	Staphylococcus 110 Agar	to isolate the bacteria which follow the genus Staphylococcus
4	Brain Heart Infusion Agar	to isolate the Gram- positive bacteria

Table (2): Origin of Gram-negative and Gram-positive bacterial isolates and percentage of distribution for bacterial species in 30 fish samples tested

Bacterial species	Under scales %	Gills %	Air sac %	Intestine %	Liver %	Heart %	Gonads %	Kidney %	Spleen %	Total
Pseudomonas fluorescence	13 (43.33)	Nil	Nil	Nil	6 (20)	4 (13.33)	Nil	Nil	Nil	23 (76.66%)
Aeromonas hydrophila	Nil	Nil	Nil	4 (13.33)	Nil	Nil	Nil	7(23.33)	Nil	11 (36.66%)
Pseudomonas sp.	8(26.66)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	8 (26.66%)
Klebsiella pneumonia	Nil	Nil	Nil	Nil	6 (20)	Nil	Nil	Nil	Nil	6 (20%)
Escherichia coli	Nil	Nil	Nil	1(3.33)	Nil	Nil	Nil	3(10)	Nil	4 (13.33%)
Proteus vulgaris	Nil	Nil	Nil	Nil	Nil	Nil	Nil	3(10)	Nil	3 (10%)
Staphylococcus aureus	3(10)	Nil	Nil	7(23.33)	Nil	Nil	Nil	2(6.66)	Nil	12 (39.99%)
Streptococcus faecalis	Nil	Nil	Nil	10(33.33)	Nil	Nil	Nil	Nil	Nil	10 (33.33%)
Streptococcus faecium	Nil	Nil	Nil	7(23.33)	Nil	Nil	Nil	Nil	Nil	7(23.33%)
Staphylococcus epidermidis	2(6.66)	Nil	Nil	Nil	Nil	Nil	Nil	٤(13.33)	Nil	6(19.99%)
Streptococcus pyogens	Nil	2(6.66)	Nil	Nil	2(6.66)	Nil	Nil	Nil	Nil	4(13.32%)

* Nil : No bacterial growth

Table (3): Percentage of distribution of various Gram-negative bacterial species in 25 water samples tested

Bacterial species	Percentage of distribution
Escherichia coli	23(92%)
Pseudomonas fluorescence	18(72%)
Aeromonas hydrophila	17(68%)
Klebsiella pneumonia	15(60%)
Proteus vulgaris	14(56%)
Ps.sp.	10(40%)

Table (4): Percentage of distribution of various Gram-positive bacterial species in 25 water samples tested

Bacterial species	Percentage of distribution
Staphylococcus aureus	19(76%)
Streptococcus faecalis	15(60%)
Streptococcus faecium	12(48%)
Streptococcus pyogens	79(28%)
Staphylococcus epidermidis	5(20%)

Table (5): Number of isolates of Gram-positive bacterial species collected during the period of the study (for both fish and water samples)

Bacterial species	No. of isolates	Percentage of total
Staphylococcus aureus	31	31.95
Streptococcus faecalis	25	25.77
Streptococcus faecium	19	19.58
Staphylococcus epidermidis	11	11.34
Streptococcus pyogens	11	11.34

* Total of Gram-positive bacterial isolates was 97 isolates.

Table (6): In vitro activities of five antibiotics against 10 isolates of each Gram-negative bacterial species

Bacterial species	AMX	%	C	%	TE	%	S-T	%	GM	%
	MIC range		MIC range		MIC range		MIC range		MIC range	
Pseudomonas fluorescence	32-128	90	32-256	80	16-256	90	128-256	80	64-256	80
Aeromonas hydrophila	256	90	2-128	80	128-256	90	16-64	10	-	0
Ps.sp.	16-256	70	4-128	60	16-64	30	8-128	70	2-64	20
Klebsiella pneumonia	16-128	90	32-64	80	16-256	80	32-128	90	8-256	80
Proteus vulgaris	4-64	80	8-128	60	2-32	30	2-64	50	2-128	10
Escherichia coli	8-128	100	4-128	80	16-64	90	32-128	90	2-128	80

Table (7) In vitro activation against 10 isolates of each Gram-positive bacteria species

Bacterial species	AMX	%	C	%	TE	%	S –T	%	Gen.	%
	MIC range		MIC range		MIC range		MIC range		MIC range	
Streptococcus faecalis	8-64	60	16- 128	50	4-32	50	16-128	60	4-128	50
Streptococcus faecium	4 -32	30	8 -128	40	16-128	40	2-64	30	2-32	20
Streptococcus aureus	4 -64	20	2 -32	30	8-128	30	8-32	40	2-64	20
Streptococcuse pideridis	8 -32	30	4 -64	50	4-32	30	8-64	20	4-32	30
Streptococcus pyogens	4 - 32	20	16 -128	20	8-128	20	4-64	10	2-32	10

AMX: Amoxicillin
C : Chloramphenicol
TE : Tetracycline
S-T : Sulphamethoxazole- Trimethoprim
GM : Gentamycin
MIC : Minimum Inhibitory Concentration