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ANTIBIOGRAM OF SOME BACTERIA CONTAMINATING TILAPIA FISH AT EL-MANZALA LAKE IN PORT - SAID GOVERNORATE.

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SUMMARY

The antibacterial susceptibility of some bacterial isolates belonging to *E. coli*, coagulase positive Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas fluorescens and Vibrio anguillarum recovered from gills, skin, muscles and internal organs of Oreocochrormis niloticus fish was determined. Strains of these isolates were screened against ampicillin, chloramphenicol, oxytetrcycline, erythromicin, gentamicn, nalidixic acid, flumequine, neomycin, nitrofurantoin, penicillin and colistin using disc agar diffusion technique. High frequency of multiresistant strains in bacterial population of fish towards several commonly used antibacterial agents was observed. Most isolates were generally sensitive to gentamicin, nalidixic acid, flumequine and nitrofurantoin. On contrary, all tested isolates were resistant to the two used β–lactam antibiotics. The remaining drugs were effective but the level of activity was even lower than that of pervious four drugs.

The antimicrobial assay of the isolates using minimum inhibitory concentration (MIC) revealed a high sensitivity of most isolates to gentamicin Its MIC against the tested strains ranged from 0.25 to 8μg/ml. Moderate sensitivity was observed in flumequine and nitrofurantoin. The incidences of drug-resistance bacteria and public health importance of existing microorganisms were discussed.

INTRODUCTION

Fish are regarded as being most popular and more perishable than other high protein foods. The flesh of healthy fish is considered bacteriologically sterile. However, fish are sometimes contaminated with bacterial pathogens and thus can inflect heavy losses in fish and causing diseases in man.

Bacause chemotherapeutic have been widely used in fish farms, the incidence of drug-resistant fish pathogenic bacteria has increased and these organisms have caused many problems in fish culturing (Aoki, 1988).

Tilapia species are one of the most important species for Egyptian fisheries. Pollution of Tilapia with waste water is most common in El-Manzala lake. This pollution reduces the water quality and had been reported as a precursor to fish infections (Walters and Plumb 1980).

Therefore, the present investigation was undertaken to isolate some of common bacterial pathogens associated with disease in Tilapia fish and of public health importance and to determine their susceptibilities toward antibacterial agents.

MATERIAL AND METHODS

A total of fifty living Tilapia nilotica fishes were caught from El-manzala lake at El-Gamil region in Port-Said. Fish samples were transfered to laboratory and bacteriologically examined.

Bacteriological examination:

Specimens of fish were obtained from gills, skin, muscles and internal organs. Primary isolation was performed on trypticase soya broth and pepton water. The isolates were identified according to the scheme described by Finegold and Martin (1982).

In vitro sensitivity test:

Bacterial isolates were tested for their susceptibility towards eleven antibacterial agents by the agar disc diffusion test (Finegold, and Martin, 1982). The interpretation of the results was carried according to Bio-Merieux Manual (1986).

Determination of Minimum Inhibitory Concentration (MIC):

Tube dilution method for determination of MIC was conducted according to the method described by Bacticados et al., 1990). The following drugs were used as they showed high sensitivity in disc diffusion method to most bacterial isolates: gentamicin, nitrofurantoin and flumequine (CID, Giza, ARE).

RESULTS

Recovered bacterial isolates are tabulated in Tables (1) and (2). The results showed that E. coli and Staphylococcus aureus spp. were found in high frequency with rate of 79 % and 82.51 % respectively. With regard to fish pathogens, the obtained results showed that the predominant isolated bacteria was Pseudomonas fluorescence which was isolated from 41.5 % of the total examined samples followed by Pseudomonas aeruginosa (48 %). Aeromonas hydrophila (15.5 %) and Vibrio anguillarum (8.5).

The antibiogram of isolated micro-organisms against different antibiotics and antibacterial agents using disc agar diffusion methods are incorporated in Tables (3 a) and (3 b). Most recovered isolates showed a high susceptibility toward gentamicin, nalidixic acid, flumequine and nitrofurantoin. Meanwhile, the strains were moderalely sensitive to chloramphenicol, colistin, neomycin and doxycycline. All isolated

Table 1: Incidence and types of micro-organisms recovered from Tilapia nilotica.

Micro-organisms	Samples		
	No. examined	No. positive	% positive
Aeromonas hydrophila	200	31	15.5
E. coli	200	158	79
Pseudomonas aeruginosa	200	96	48
Pseudomonas fluorescence	200	83	41.5
Staphylococcus aureus	200	165	82.5
Vibrio anguillarum	200	17	8.5

Table 2: Incidence of bacterial isolates recovered from different sites of Tilapia nilotica

Organisms	No. of isolates / Type of tissues				
	Gills	Skin	Muscles	Int, organs	Total
A. hydrophila	3	20	2	6	31
E. coli	25	31	12	90	158
P. fluorescence	0	29	33	21	83
P. aeruginosa	34	25	8	29	96
a,ureus	15	63	32	55	165
V. anguillarum	0	13	0	4	17

Table 3a: Antibiogram patterns of isolated micro-organisms aganist different antibiotics and chemotherapeutic.

Antimicrobial agents	Disc potency µg/ml	Aeromonas hydrophila (31)				E.coli (158)				Pseudomonas fluorescens (83)			
		R	I	S	S %	R	I	S	S %	R	I	S	S %
Ampicillin	10	31	0	0	0	158	0	0	0	83	0	0	0
Chloramphenicol	30	10	5	16	51.61	44	60	54	34.18	29	19	35	42.17
Erythromycin	15	12	11	8	25.81	10	25	63	39.87	55	10	28	33.73
Gentamicin	10	2	3	26	83.87	20	16	122	77.22	2	5	76	91.57
Oxytetracyclin	30	9	7	15	48.39	68	32	58	36.71	33	10	40	48.19
Nalidixic acid	30	3	6	22	70.97	15	25	118	74.68	2	3	78	93.98
Flumequine	30	2	4	25	80.65	15	21	122	77.22	4	6	53	63.86
Neomycin	30	5	4	12	38.71	85	22	51	32.28	38	7	38	45.78
Nitrofumntoin	300	3	5	23	74.19	0	23	135	85.44	4	7	72	86.75
Penicillin	10	31	0	0	0	158	0	0	0	83	0	0	0
Colistin	10	18	8	5	16.13	88	12	58	36.71	53	22	8	9.64

R: Resistance.

I: Intermediate.

S: Sensitive.

Table 3o: Antibiogram patterns of isolated micro-organisms aganist different antibiotics and chemotherapeutic.

Antimicrobial agents	Disc potency µg/ml	Pseudomonas aeruginosa (96)				S. aureus (165)				Vibrio anguillarum (17)			
		R	I	S	S %	R	I	S	S %	R	I	S	S %
Ampicillin	10	96	0	0	0	165	0	0	0	17	0	0	0
Chloramphenicol	30	50	32	14	14.58	99	31	35	21.21	4	2	11	64.71
Erythromycin	15	81	9	6	6.25	21	14	130	78.79	7	5	5	29.41
Gentamicin	10	2	3	91	94.79	38	14	113	68.48	2	3	12	70.59
Oxytetracyclin	30	50	6	40	41.67	24	46	95	57.58	3	6	8	47.06
Nalidixic acid	30	2	2	92	95.83	15	18	132	80	2	1	14	82.35
Flumequine	30	1	2	93	96.88	60	10	95	57.58	1	1	15	88.24
Neomycin	30	54	6	36	37.5	65	30	70	42.42	0	6	11	64.71
Nitrofumntoin	300	30	15	51	53.13	50	6	109	66.06	2	3	12	70.59
Penicillin	10	96	0	0	0	165	0	0	0	17	0	0	0
Colistin	10	93	3	0	0	153	4	8	4.85	12	4	1	5.88

R: Resistance.

I: Intermediate.

S: Sensitive.

Table 4: Minimum inhibitory concentrations (MIC) (µg/ml) of gentamicin, flumequine and nitrofurantoin against bacterial isolates.

Antibacterial agents	A. hydrophila (31)	E. coli (158)	P. fluorescens (83)	P. aeruginosa (96)	S. aureus (165)	V. anguillarum (17)
Gentamicin	0.25-2	0.25-2	2-4	2-4	4-8	2-4
Flumequine	2-4	4-8	4-8	4-8	8-32	8-16
Nitrofurantoin	2-4	2-4	4-8	8-16	16-32	8-16

strains were resistant to ampicilllin and penicillin. The in vitro antimicrobial assay of the isolates using minimum inhibitory concentration (MIC) exhibited a high sensitivity of most isolates to gentamicin. Moderate sensitivity were observed to flumequine and nitrofurantoin (Table 4).

DISCUSSION

The results of bacteriological examinations of fish samples demonstrate the presence of different micro-organisms as a contaminants in fish. Fish can retain in digestive tract or skin many human pathogens (Escherichia coli, Salmonella, Shigella, Staphylococcus and Clostridium) without becoming ill (Brown and Dorn, 1977, Osman et al., (1980) and Hefnawy et al., 1989). The present investigation revealed that E. coli and Staphylococcus spp were mostly recovered from tested fish samples.

Similar findings have been reported previously (Chiltino, 1972, Shewan, 1972 and Lotfi and Lotfi et al., 1972). On the other hand, the otbained results confirmed that fish was carrier of various pathogenic bacteria including. Aeromonas, Pseudomonas and Vibrio spp., which have been reported as an etiological agents or co-agent in fish diseases. These results are inagreement with those of Collins (1970) who reported that Aeromonas and Pseudomonas spp are common in fresh water and can be isolated from healthy fish. Also Ahmed and Shoreit (1994) isolated Vibrio spp from internal organs of Tilapia spp.

The influence of different antibiotics against isolated species of bacteria varied in their degree of sensitivity. The results of the antimicrobial sensitivity tests using disc agar diffusion method indicated a dramatic resistance patterns of most bacterial strains to several commonly used antiobiotics. This may confirm that the extensive application of many antibiotics may lead to decrease in susceptibility of isolated strains to theier actions and also explain the possible therapeutic failure in man and fish.

Resistant strains of pathogenic and non pathogenic bacteria can transfer resistance factors between organisms even in the absence of antibiotics (Sojka and Hudson 1976). In this respect, Aoki (1988) reported the chloramphenicol and tetracycline resistant bacteria were isolated with higher frequencies from the internal tract of cultured ayu. Oxytetracycline reportedly enhanced the production of plasmid-mediated resistance in aquatic bacteria (Grondel et al., 1985). All isolated strains showed complete resistance to ampicillin and penicillin. These results are inagreement with those reported by Sanders and Sanders 1983, who stated that, the isolation of β-lactam resistant bacteria were likely become more frequent. They added that mutation resulting in reduced β- lactam susceptibility allowing organsms to persist in the presence of treatment with B lactam antibiotics.

It is evident from a formentioned results that, Aeromonas, Pseudomonas and Vibrio isolates were highly sensitive to gentamicin, nalidixic acid, nitrofurantoin and flumequine.

The achieved results are nearly similar to those of Hettiarachchi and Cheong (1994). They stated that, all isolates of A. hydrophila recovered from fresh water ornamental fish were resistant to tetracycline and penicillin while fleumequine, nitrofurantoin and gentamicin were proved to be the most efficacious on A. hydrophila isolates ; they added also that all Vibrio spp. isolates were sensitive to most tested antibiotics except colistin and streptomycin. MIC have already been shown to exert beneficial effect in clinical practice, the achieved data pointed to a much higher potency of gentamicin as compared with the other two tested antibacterial agents, suggesting its use in future studies. Similar results were reported by Wernicki and Rzedzicki (1988). They found that most E.coli isolates recovered from calves affected with diarrhoea were sensitive to gentamicin. These findings also are inagreement with Prescott et al., (1984) who found that, gentamicin was the most effective drug against P. aeruginosa.

The MIC value of gentamicin was found to be ranged from 0.25 to 8 µg/ml to the isolated species. In this respect, Heo and Lee (1995) found that the MIC of gentamicin against A. hydrophila, A. salmonicida, Edwardisiella tarda, Psweudomonas fluorescens, Streptococcous sp. And Vibrio anguillarum were 4-64 µg/ml.

The presence of E.coli in fish generally indicates direct and/or indirect faecal pollution as this bacteria is not known as a classical fish pathogens. Enteropathogenic E.coli are well recognized pathogens as a cause of infantile diarrhea and / or gastrointestinal illness in adult humans (Dupont et al., 1971. Edelman and Levine, 1983 and Ahmed 1992).

Contamination of fish with S. aureus usually occurs through surrounding water. If such fish is left without refrigeration for several hours or cools slowly in refrigeration, growth of S. aureus and entrotoxin formation may occur. However, growth of S. aureus in a cooked products is favoured by lack of competitive bacteria, which are destroyed by heat (Bryan, 1980).

Aeromonas and Pseudomonas species have low

virulence for men but treatment of infection in compromised host is often difficult because of a high natural resistance to many commonly used antibiotics (Nord et al., 1975).

In view of this study, it appears that the best method of controlling fish diseases would still be prevention, with particular concentrate on water management and sanitation, also the administration of the drug to fish should be carefully controlled and limited to the treatment and growth promotion.

The achieved results suggested that gentamicin can be used for therapy of bacterial diseases in Tilapia fish but from a therapeutic point of view a further information will be required about its effectiveness in vivo and comments on its safety and withdrawal periods.

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