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CHARACTERIZATION OF SOME BACTERIA ISOLATED FROM AFRICAN CATFISH IN QENA, EGYPT, WITH STUDYING OF THEIR ANTIBIOTIC RESISTANCE

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ABSTRACT

Skin lesions, gills and liver, kidney, intestinal tissues of unhealthy wild catfish (Clarias gariepinus) captured from River Nile and canals at Qena, Egypt, were examined for presence of pathogenic bacteria and their resistance to antibiotics. Bacteria isolated from catfish (n=26) were identified based on physiological, biochemical characters and then identified by API system strips. The antimicrobial susceptibility test was carried out with 8 antibiotics: tetracycline, chloramphenicol, co-trimoxazole (trimethoprim-sulphamethoxazole), norfloxacin, nalidixic acid, erythromycin, neomycin and ampicillin; using the agar diffusion method. The predominant bacterial pathogens belonged to Gram-negative and oxidase positive bacteria (57.7%) as well as Gram-negative and oxidase negative bacteria (42.3%). The most effective antimicrobials were chloramphenicol, and neomycin. A higher frequencies of resistance were noted to tetracycline, co-trimoxazole, and rather low to ampicillin, nalidixic acid erythromycin and norfloxacin. Multiple antibiotic resistance were shown to two or more antibiotics tested. Simultaneous resistance to five antibiotic was observed in Pseudomonas aeruginosa. The results showed an occurrence of antibiotic resistance among some bacterial fish pathogens which is of a high concern in catfish cultured in the River Nile.

Keywords: bacteria: antibacterial resistance; unhealthy wild catfish, River Nile; microbial contamination, public health.

INTRODUCTION

Catfish species is considered one of the most popular fishes in upper Egypt, because of its cheaper price and palatable for consumers. Nowadays, bacterial diseases is a common problem faced by fish industry. Gram negative Bacteria were recognized as the causative agents of many bacterial diseases attacking fish. *Enterobacteriaceae*, *Pseudomonads* and *Vibrionaceae* are Gram negative families usually isolated from fish and their species are opportunistic and ubiquitous in the aquatic environment. Many factors could contribute to bacterial infection in fish including poor water quality, crowding, transportation and inadequate nutrition.

Many cases of bacterial infections in fish have been reported worldwide ***(Chung and Kou 1973; De Fegueiredo and plumb 1977; Taufik, 1990.; Figueiredo et al., 2005).*** In Egypt, ***Emeish, (2006)*** reported that bacteriological examination of gills of collected catfish revealed columnaris and bacterial gill diseases. ***Bader et al., (2003)*** stated that *F. columnare* infection has a worldwide distribution. ***Aly et al. (2008)*** identified *Aeromonas hydrophila*, *Citrobacter freundii* and *Pseudomonas* species. ***Sakr and Abd El-Rahman, (2008)*** isolated *Ps. anguilliseptica* from naturally infected *Oreochromis niloticus*.

Antibiotic resistance by bacterial fish pathogens has been reported in all areas of aquaculture from warm water to cold water, and freshwater to marine environments ***(Dixon, 2007). Dixon and Contreras (1999)*** showed the presence of multidrug resistance to *Edwardsiella tarda* in imported ornamental fish.***Sarter et al.,(2007)*** reported multiple antibiotic resistance among Gram negative bacteria isolated from farmed catfish. The antibiotic resistant in cultured fish have been reported ***(Aoki, 1992;***

Smith et al., 1994), but only few studies have dealt with resistant bacteria in wild fish *(Alves de Lima and Hofer, 1993)*. Therefore, this study was conducted to survey some bacterial species isolated from unhealthy wild catfish in Qena, Upper Egypt and analyze their resistance to antibiotics.

MATERIAL AND METHODS

Sampling:

Approximately thirty catfish showing growth clinical signs of skin ulceration, fin rot, pale gills were collected from River Nile and local canals at Qena, Egypt. They were brought to the lab using plastic bags filled with 2/3 water and processed within 2-6 h. from collection *(American Public Health Association ,1992)*.

Processing of samples:

For isolation of bacteria, fish were externally washed with sterilized water to reduce potential surface contamination *(Matyar et al., 2008)*. The abdominal cavity was opened aseptically and tissues from skin lesion, gills, liver, kidney and intestine were removed and prepared as previously described *(Miranda and Zemelman, 2001; Matyar et al., 2004; Mousa et al., 2008)*.

Bacterial isolation and characterization:

Samples were spread onto TSBA (trypticase soy 7% sheep blood agar), cytophaga agar, xylose lysine desoxycolate agar (XLD) (Merck, Germany), MacConkey agar (Difco, USA), and citremide agar (Oxoid, U.K.) plates. After incubation for 24-48h, at 22 and/or 35°C *(American Fisheries Society-Fish Health Section, 2005; Mousa et al., 2008)*, the inoculated plates were examined for the suspected single and pure

bacterial colony. Various colonies were selected and purified through successive streaking on trypticase soy agar (TSA) (Merck, Germany). The bacteria were then kept in TSA slant tube for further identification. All the isolates were characterized by Gram-staining, oxidase, catalase, KOH test (3% potassium hydroxide), oxidation/fermentation of glucose (O/F) ***(Hugh and Leifson, 1953)***, and motility test (motility test media, Difco), according to procedures recommended by ***(Cowan, 1974; Nicolas, 1984; AFS-FHS, 2005)*** and then identified by API system strips (Biomérieux at Marcy l'Etoile. France) as follows: API 20E for bacilli Gram negative oxidase negative (mainly *Enterobacteriaceae*), API 20NE for bacilli Gram negative oxidase positive (non-*Enterobacteriaceae*). All API strips were prepared according to the instructions of the manufacturer.

Antibiotic sensitivity assay:

Antibiotic susceptibility was determined by the agar diffusion method according to ***Bauer et al., (1966)***. The bacterial suspension were adjusted in sterile 0.9% saline to 0.5 McFarland standard (10^8 cfu/ml) ***(McFarland, 1907)*** and spread on Mueller-Hinton agar (Oxoid, U.K.). Eight antimicrobial agents were selected as representatives of the different classes of antibiotics: ampicillin (AM, 10 µg), erythromycin (E, 15 µg), nalidixic acid (NA, 30 µg), chloramphenicol (C, 30 µg), norfloxacin (NOR, 10 µg), neomycin (N, 30 IU), tetracycline (TE, 30 µg) and co-trimoxazole (trimethoprim-sulphamethoxazole) (SXT, 1.25 and 23.75 µg). All discs were from BIO-RAD (USA). After incubation for 24 h at 28 and/or 35°C, organisms were classified as sensitive, intermediate or resistant according to the inhibition zone diameter ***(National Committee for Clinical Laboratory Standards, 1997).***

RESULTS AND DISCUSSION

In the present study, 26 isolates were successively isolated from unhealthy fish. One isolates each (3.8%) of *Morganella morgani, Pseudomonas aeruginosa*; 2 isolate (7.7%) each of *Flavobacterium columnare, Edwardsiella tarda*; 3 isolates (15.4%) of *E.coli*; 5 isolates (19.2%) of *Enterobacter gergoviae*; and 6 isolates (23.1%) each of *Aeromonas hydrophila* and *Pseudomonas fluorescence* (Table 1). The bacterial isolates obtained from the fish samples in this study was consistent with the previously reported ***(Taufik, 1990; Miranda and Zemelman, 2001; Chelossi et al., 2003; Sarter et al., 2007; Musa et al., 2008)***. They isolated a high numbers of bacteria from skin lesions, gills and viscera of catfishes. The bacterial isolates obtained in this study belong to Gram-negative and oxidase positive strains as well as those of Gram-negative and oxidase negative bacteria. This was similar to the results obtained by ***Miranda and Zemelman, (2001)*** and in contrary to those reported by ***Chelossi et al., (2003)*** who isolated more Gram-positive bacteria. The majority of Gram-negative bacterial isolates are oxidase positive and this in agreement with ***Chelossi et al., (2003) and Musa et al., (2008).*** The most of the bacterial isolates obtained in this study were identified as *Aeromonas hydrophila, Pseudomonas fluorescence*, and *Enterobacter gergoviae*; others comprised only a small number of the isolates studied. This is mimic with those recorded by previous literature ***(Chung and Kou, 1973; Taufik, 1990)***. *A. hydrophila, Ps. fluorescence, E. tarda*, and *Flavobacterium* were bacterial species commonly isolated from diseased fish ***(Austin and Austin, 1985)***. The high recovery of which normally found in freshwater environment ***(McPherson et al., 1991; Schmidt et al., 2000)***. The infection by these ubiquitous organisms is usually associated with stress such as

temperature and water quality. The bacterial species identified as *A. hydrophila* in this study was in constituent with those reported a high number of this organisms among fish samples ***(Taufik, 1991; Musa et al., 2008)***. *Aeromonas hydrophila* is the causative agent of the disease known as "haemorrhagic septicaemia", "ulcer disease" or "red-sore disease" ***(Guz and Kozinska, 2004). Emeish, (2006)*** isolated *F. columnarae* from catfish captured from River Nile that showed skin lesion and gill affection. These results were confinement to those of our study. Columnaris disease is one of the bacterial diseases affecting gills and fins. ***Evelyn and McDermott (1961)*** isolated Pseudomonas as the predominant bacterium from internal organs of various freshwater salmonid fishes from Ontario, Canada. These results mimic those of our study and differ from that reported by ***Taufik, (1991)***, where Pseudomonas constitute only small percentage of bacterium isolates from catfishes. The isolation of enteric bacteria as *E.coli* may due to pollution of water. Faecal coliforms in fish reflect the level of pollution of their environment, as the normal flora of fish do not include coliforms ***(Cohen and Shuval, 1973).***

Data of the inhibitory zone obtained by the agar diffusion method indicated that, the strains showed high susceptibility for several antimicrobial agent tested, including erythromycin, norfloxacin, ampilicin, neomycin, and chloramphenicol. All of the bacteria isolated were varied in resistance to the various antibiotic tested and the resistance frequencies were moderate between isolates (Table 2). Multiple antibiotic resistance to two or more different antibiotics was observed among isolates and a total of 5 antibiotic resistance patterns were recorded (Table 3). Most of strains analyzed showed higher resistance to co-trimoxazole, ampicillin, and tetracycline. Simultaneous resistance to 5 antibiotics was observed in *Pseudomonas aeruginosa*. The findings that the majority of isolates were

susceptible and have varied resistance to the antimicrobial tested, agree with the report of previous study ***(Mousa et al., 2008)***. The resistance to antibiotic tested rather low and this differ from those obtained by ***Sarter et al., (2007)*** that detected more resistant isolates among Gram-negative bacteria isolated from freshwater farmed catfish. The lower resistance and higher susceptibility among isolates in our results might owed to differences in environment and fish be as farm reared or wild. Most of the isolates were resistant to co-trimoxazole (STX). Furthermore, norfloxacin, chloramphenicol, and neomycin were found to be effective in the isolates. Antibiotic resistance by bacterial fish pathogens has been reported in all areas of aquaculture from warm water to cold water, and freshwater to marine environments ***(Dixon, 2007). Dixon and Contreras (1999)*** showed the presence of multidrug resistance *Edwardsiella tarda* in imported ornamental fish. ***Sarter et al., (2007)*** reported multiple antibiotic resistance among Gram- negative bacteria isolated from farmed catfish. Multiple antibiotic resistance of *A. hydrophila* strains from organs of infected catfish, *Clarius batrachus* has been reported ***(Pathak et al., 1993).*** Furthermore, ***Orozova et al., (2008)*** indicated that all examined *A. hydrophila* strains displayed multiple drug resistance. The presence of high numbers of antibiotic resistant bacteria in gills and intestines of fish may have an ecological and public health implications ***(Matyar et al., 2004).*** Most of the present isolates were resistant to co-trimoxazole. This was in constituent with those reported by ***Mousa et al. (2008). Emeish, (2006)*** found that *F. columnarae*, isolated from catfishes with gill affection, were moderate sensitive to STX. The simultaneous resistance of isolates to antibiotics, may be due to the dissemination of antibiotic resistance plasmids in the aquatic environment, as reported by ***Stewart and Koditschek, (1980).***

The presence of potentially pathogenic and antibiotic-resistant bacteria in gills and viscera of fish may have ecological and public health implications.

Table (1): Identification of bacterial isolates (n = 26) from unhealthy freshwater catfish.

Bacterial species	Total numbers of isolates	% of 55 fish samples
Aeromonas hydrophila	6	23.1
Pseudomonas fluorescence	6	23.1
Enterobacter gergoviae	5	19.2
Escherichia coli	3	15.4
Flavobacterium columnare	2	7.7
Edwardisella tarda	2	7.7
Pseudomonas aeruginosa	1	3.8
Morganella morgani	1	3.8
Total	26	47.3

Table (2): Prevalence of antibiotic resistance among bacterial fish isolates.

Antibiotic	Percentage of resistant strains							
	A.hydrohila (n=6)	Ps. Fluorescence (n=6)	E.gergoviae (n=5)	E.coli (n=3)	F.columnarae (n=2)	E.tarda (n=2)	M.morganiae (n=1)	Ps.aeruginosa (n=1)
AM	0.0	0.0	20	100	0.0	50	0.0	100
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	0.0	0.0	20	0.0	50	0.0	0 0	100
N	0 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NA	0.0	0.0	0 0	0 0	0 0	50	0.0	100
NF	0 0	0 0	40	0.0	0.0	0.0	0.0	0.0
STX	50	16 7	60	33.3	50	50	0.0	100
TE	100	50	20	33.3	50	0 0	0.0	100

N.B. 0.0% = all isolates sensitive ; 100% = all isolate resistant.

AM (Ampicillin); C (Chloramphenicol); E (Erythromycin); N (Neomycin); NA (Nalidixic acid); NF (Norofloxacin), STX (co-trimoxazol); TE (Tetracycline).

Table (3): Multiple antibiotic resistance patterns encountered in resistant bacterial isolates.

Antibiotic resistance patterns	Bacterial species	Number of isolates
STX-TE	*A. hydrophila*	2
	E. gergoviae	1
	Ps. fluorescence	1
E-STX	*F columnarae*	1
AM-STX	*E. tarda*	1
	E.coli	1
AM-E-STX	*E. gergoviae*	1
AM-E-NA-STX-TE	*Ps. aeruginosa*	1
Total		9

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توصيف أو خصائص بعض البكتيريا المعزولة من القرموط الأفريقي في قنا ـ مصر مع دراسة مقاومتها للمضادات الحيوية

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أخضعت عينات من جلد، خياشيم، الأعضاء الداخلية تم الحصول عليها من أسماك القرموط المصابة والمجمعة من مناطق مختلفة في محافظة قنا- مصر للفحص البكتيريولوجي للبكتيريا الممرضة ودراسة مقاومتها لفعل المضادات الحيوية.تم تعريف البكتيريا المعزولة وعددها 26 بالاختبارات البيوكميائية التمهيدية واستخدام أطقم التحليل API . تم فحص الحساسية الدوائية للميكروبات المعزولة لعدد ثمانية من المضادات الميكروبية باستخدام طريقة الانتشار في الآجار وتشتمل علي الأمبيسيلين، التتراسيكلين، الكلورامفينكول، كو-ترامیثاكسزول ، النوروفلوكساسين، النيومايسين، حمض النالدكسيك و الأريثرومايسين. كانت غالبية البكتيريا المعزولة تنتمي إلي البكتيريا سالبة الجرام – موجبة اختبار الأكسدة (57.7%) وأخري سالبة لاختبار الأكسدة (42.3%). لم يتم عزل ميكروب السالمونيلا. أظهرت نتائج اختبار الحساسية الدوائية أن العزلات حساسة للمضادات الميكروبية المختبرة ، وكذلك تنوعا في مقاومة بعض العترات البكتيرية لبعض المضادات الميكروبية. كان النيومايسين، الكلورامفينكول، النوروفلوكساسين من أكثر المضادات الميكروبية تأثيرا. كان النمط الشائع في مقاومة الميكروبات للمضادات الميكروبية خليطا من الأمبسيلين،التتراسيكلين كو-ترامیثاكسزول وقليلا للأرثرومیسين. وجد أن ميكروب الزائفة الزنجارية مقاوما لفعل خمسة من المضادات الحيوية في ذات الوقت. من النتائج السابقة تم الاستدلال علي وجود البكتيريا الممرضة أو الانتهازية والمعزولة من الأسماك المصابة، وكذلك إمكانية حدوث مقاومة بعض العترات البكتيرية لفعل المضادات الميكروبية من إسماك القرموط في مياه نهر النيل كبير الأهمية للصحة العامة.