# CONTAMINATION OF CLARIAS GARIEPINUS FISH WITH PSYCHROTROPHS PARTICULARLY AEROMONAS SPECIES

BRR, A. A. H<sup>a</sup>. and Sohair R. Basyoni<sup>b</sup>

a; Provincial Tanta Laboratory – b; Provincial Marsa Matrooh Laboratorby

Animal Health Research Institute, Egypt.

#### ABSTRACT

Fifty samples of fresh Clarias gariepinus fish were collected randomly from different markets at Tanta city, Gharbia governorate. These samples were examined for isolation and identification of psychrotrophic bact-eria, particulary Aeromonas species, the isolated strains of psychrotro-phic species were Acinetobacter 8%, Aeromonas 56%, Alcaligens 12%, Chromobacterium10%, Psuedomonas 28% and Flavobacterium 6%.

*Further identification of Aeromonas species were recovered as Aermonas A.hydrophla, 30% A.pumctata 16% and A.salmonicida 10%.* 

All Aeromonas speices were examined for their ability to produce lipolytic, proteolytic, hemolytic and Dnase activities in different percentages 78.6%, 71.4%, 82.1% and 67.9%, respectively.

Concerning the antibiotic sensitivity of the isolated Aeromonas strains, theywere sensitive to enrofloxacin, gentamycin, oxytetracycline, tetracycline and nalidixic acid on other hand, they have a high resistance to amoxi-cillin, ampicillin, pencillin, erythromycin, and lincospectin. The public health significance of isolated strains and recommended sanitary measures were discussed.

Key Words: Psychrotrophs. Aeromonas . Clarias gariepinus.

## **INTRODUCTION**

Psychrotrophs are very important group of organisms causing food spoilage, they are found in water and soil and their growth range from - 12 °C to 42 °C. (*Garbutt, 1997*).

Aeromonas, Acinetobacter, Alcaligens, Chromobacterium, Flavobacterium and Pseudomonas species are commonly psychrotrophs isolated from seafood especially fresh fish(Banwart, 1989 and Jay, 1996). From the economic point of view, these organisms can cause some defects as off-odor, fruity, ammoniacal and H<sub>2</sub>S odor (Banwart, 1989), moreover, these organisms have a public health hazards to human where Acinetobacter species may occasional serious opportunistic infections including septicemia, pneumonia and meningitis, also Flavobacterium species have been recovered from wound, urinary tract and spinal fluid infections (Robinson, 2000).

Aeromonads are ubiquitous organisms in fresh water fish and shell fish (*Isonhood and Drake, 2002*). A eromonas species have been recognized as potential food borne pathogens for more than 20 year These organisms such as Aeromonas hydrophila can cause abdominal cramps and gastroenteritis.

Aeromonas species are important to the food industry due to their psychrotrophic nature and their ability to express a range of virulence factors under refrigerated storage conditions. Aeromonas species such as A.hydrophila can be regearded as both spoilage and potentially pathogens (Robinson2000).A.salmonicida is the causative agent of the fish disease called furuncullosis but human disease has not been described (Isonhood Drake, 2002). and Food of animal origin such as seafood have been considered an important vehicle for Aeromonas species infections (Altwegg et al., 1991., Kirov, 1993; Mattick and Donovan, 1998).

A number of putative virulence factors such as autolysin, hemolysin, proteases, lipases and DNases that may play an important role in the development of disease cither in humans or in fish (*Santos et al., 1999 and soler et al.,2002*). All people are believed to be susceptible to *Aeromonas* gastroenteritis, although it is most frequently observed in very young children; individuals with impaired immune systems or underlying disease are susceptible to the more severe infections(*Brema et al.,2003*). In recent years *Aeromonas* group has received increasing attention as an agent of food borne diarrheal disease in human being (*Palumbo et al., 1985*).

The aim of this study was to determine the incidence of psychrotrophic bacteria in fresh water *Clarias gariepinus* with special reference to *Aeromonas* species and their ability to produce some virulence factors such as proteolytic, lipolytic, hemolytic and DNase activities, in addition to susceptibility of isolated *Aeromonas* to different types of antibiotics.

## MATERIALS AND METHODS

#### **1- Collection of samples:**

A total of 50 samples of fresh water fish, *Clarias gariepinus*, were collected randomly from the local markets at Tanta city, Gharbia govern-orate. The collected samples were transported aseptically to the laboratory without undue delay for bacteriological investigations.

## 2- Preparation of samples: -

Each sample was based on its side over sterile plate holded by sterile forceps, the skin was sterilized by burning with ethyl alcohol 70%, then removed. Ten grams of each fish muscle sample were aseptically transferred to 90 ml of sterile trypticase soy broth containing 10  $\mu$ g ampicillin /ml then incubated at 28°C for 24 hours.0.1 milliliter from enriched broth was streaked into trypticase soy agar supplemented with ampicillin and incubated at 28°C- 30°C for 48 hours (*FAO*,1979).

## **3-Isolation and Identification of psychrotrophic species:**

The suspected pale yellow colonies were picked upon nutrient agar slants and incubated at 28-30° for 48 hours for further identification. The

isolated strains were identified morphologically according to *Cruickshank et al.*(1975)and biochemically according to *Macfaddin*(1976), *A.P.H.A* (1992) and koneman, et al. (1994).

# 4- Detection of some virulence factors associated with isolated *Aero-monas* species:

#### a- Detection of lipolytic activity (Anguita et al., 1993).

It was determined by streaking of suspected *Aeromonas* species on tributyrin plate agar and incubated at 37° for 24 hours The presence of a transparent zone around the colonies indicated lipase activity.

#### b- Detection of proteolytic activity (Castro et al, 2003):

Protolytic activity was tested on skim milk agar by streaking suspected *Aeromonas* species on to the plates and incubating at 37°C for 24 hours, the plates are flooded with 1% hydrochloric acid. The presence of clear transparent zone around the colonies indicated caseinase activity.

### c- Detection of DNase activity (Cruickshank et al., 1975):

DNase agar plate was inoculated with a loopful of suspected colony by spotting and incubated at 37° C for 18 hours, then plate was flooded by normal hydrochloric acid(N HCL)which precipitates deoxyribonucleic acid (DNA) and turns plates cloudy. The appearance of clearing zone and absence of turbidity around colonies indicates DNase production.

#### d- Hemolytic activity:

Aeromonas species were plated onto sheep blood agar and incubated at 37°C overnight. The hemolytic activity was detected by presence of a clearing zone around colonies. (Quinn et al., 1994).

#### 5- Antibiotic susceptibility test:

The isolated strains of *Aeromonas* species were suspended in trypticase soy broth for 18 hours, and then the suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard by adding sterile saline then it was suitable for sensitivity testing by disc diffusion method (*Quinn*)

*et al.,1994*). The discs contain the following antibiotics: amoxycillin, 10µg; pefloxacin,5µg; Rifampicin, 5µg; ampicillin, 10µg; pencillin, 10µg; tetracycline, 30µg; gentamycin, 10µg; oxytetracyclene, 30µg; enrofloxacin, 10µg; danofloxacin, 5µg; norfloxacin, 10µg; streptomycin, 10µg and nalidixic acid,30µg and neomycin, 10µg(Oxoid Limited, England). The entire surface of trypticase soy agar was streaked by a sterile cotton swab soaked in test strain suspension. After complete drying, antibiotic discs were placed on the surface of inoculated plates gently and after incubation overnight at 37°C, the diameter of inhibition zone of *Aeromonas* species were measured and interpretated by referring to tables recommended by National committee for clinical laboratory standards (NCCLS ) (**Finegold and Martin, 1982**). Cultures were characterized as sensitive or resistant.

#### RESULTS

Table (1): Incidence of psychrotrophic species isolated from Clarias
<i>gariepinus</i> fish samples $(n = 50)$ .

Psychrotrophic species	NO	%
Acinetobacter species		
A.caloaceticus	4	8
Aeromonas species	28	56
Ahydrophila	15	30
A.punctata	8	16
A.salmonicida	5	10
Alcaligens species		
A. faecalis	6	12
Chromobacterium species	8	16
C. lividum	5	10
C.violceum	3	6
Pseudomonas species	14	28
Ps. vesicularies	2	4
Psputrifaciens	5	10
Ps .diminuta	3	6
Psauriginosa	4	8
Flovobacterium species	3	6

Aeromonas species	NO. of species	Lipolytic		Proteolytic		Hemolytic.		DNase	
		NO	%	NO	%	NO	%	NO	%
A.hydrophila	15	13	86.6	14	93.3	14	93.3	12	80
A.punctata	8	5	62.5	4	50	6	75	3	37.5
A. salmonicida	5	4	80	2	40	3	60	4	80
Total	28	22	78.6	20	71.4	23	82.1	19	67.9

**Table (2):** Incidence of some virulence factors of *Aeromonas* species isolated from *Clarias gariepinus* fish samples.

Table (3): Suceptibility of Aeromonans species isolated from Clariasgariepinus for different types of antibiotics.

Antibiotic disc	A. hydrophila	A. punctata	A.salmonicida
Amoxycillin	R	R	R
Ampicillin	R	R	R
Danofloxacin	V	S	Ι
Enrofloxacin	S	S	S
Erythromycin.	R	R	R
Gentamycin	S	S	S
Neomycin	S	Ι	R
Pefloxacin	Ι	V	R
Pencillin	R	R	R
Rifampicin	V	V	S
Streptomycin	S	Ι	V
Tetracyclene	S	S	S
Oxytetracyclene	S	S	S
Lincospectin	R	R	R
Nalidixic acid	S	S	S

 ${\bf S}$  : sensitive

I: intermediate.

**R:** resistant.

V: variable (some strains were sensitive and other were resistant)

## DISCUSSION

Fresh water fish acts as a vehicle for many types of microorganisms. The chief source of fish contamination was water, soil and fish handlers (*Youssef et al., 1985*). Fish can acquire pathogenic microorganisms from the natural aquatic environments, from sewage contaminating harvesting areas as well as from contamination by workers, utensils, equipments during harvesting and transportation. (*Frazier, 1967 and National Academy of Science, 1985*).

A total of 63 psychrotrophic strains isolated from 50 samples of *Clarias gariepinus* fish collected from local markets at Tanta city,the most isolated species were *Aeromonas* 56% followed by *pseudomonas* 28% then *Alacaligens* 12%, *Chromobacterium* 16%, *Acinetobacter* 8%., and *Flav-obacterium* 6% as shown in table(1). Nearly similar results were obtained by *Youssef et al.* (1985); *Banwart* (1989); *Gram and Huss* (1996); *Jay* (1996); *and El-Sheikh* (2001), while a high incidence of psychrotrophic species, especially *Flavobacterium* 25% and *Pseudomonas* 36% isolated from *Clarias lazera* by *Mousa and Mahmoud* (1997).

Members of the genus *Aeromonas* are tyrpically aquatic bacteria which may be isolated from fresh water and estuarine fish(*Janda, 1991*). The results of our study recorded in table (1) indicated that *Aeromonas* species was the most strain (56%) and the most prevalent isolates were *A.hydrophila* 30 %,*A.punctata* 16% and *A.Salmonicida* 10%, these results agree with those reported by *Jay* (1996) and Brema (2003). A high incidence of *Aeromonas hydrophila* (36.1%) in cat fish obtained by Wang and Silva (1999), while, Bastawrows and Mahmoud (1999) and El-Sheikh(2001). isolated *A. hydrophila* from fresh *Clarias lazera* fish in low incidence 18% and 6%, respectively. Khalil (2004) isolated and identified 24 Aeromonas hydrophila strains from the muscles of *Clarias lazera* in 10%, while *El-Sheikh* (2001) isolated *A.punctata* in high incidence 24%. The high incidence of *Aeromonas* species in examined fish samples may be attributed to water contamination with human and animal Kaft El-Sheikh Vet. Med. J. Vol. 3 No. 1 (2005) effluents, during handling and distribution in local markets, where motile *Aeromonas* including *A.hydrophila* are common aquatic inhabitants and recognized as pathogens in fish (*Shotts et, al.1972 and Rippey and Cabelli, 1979*), also, *A. hydrophila* is one of the bacterial flora of surface water and considered as indicator of water pollution (*Schubert, 1987*). Another source of *Aeromonas* species may be asymptomatic human carriers particularly those working as food handlers (*A.P.H.A, 1992*). So, the harvesting of fish from polluted areas must be avoided, clean pans and utensils are used in handling of fish, strict hygienic measures during handling, transport and storage of fish.

Aeromonas species are considered as emerging food borne pathogens that associated with septicemia, gastroenteritis, endocarditis and wound infections in human being (Janda, 1991).

Pseudomonas species produce wound infection, burns, blue green pus, meningitis, urinary tract infections and may lead to rapid eye destruction (*Aly*, 1997). These bacteria may invade blood stream resulting in fatal sepsis. This occurs commonly in infants and debilitated persons (*Brooks, et al., 1995*).

A number of potential virulence factors which have a highly significant public health problem as well as economic importance, these factors include enterotoxins, cytotoxins, haemolysins, lipases and proteases production (*Trust and Chipman*, 1979).

Regarding the results recorded in table (2), 82.1%, 78.6% and 71.6% of *Aeromonas* species have hemolytic, lipolytic and proteolytic activities whilemean, 67.9% of these species showing DNase activity.

*Aeromonas hydrophila* isolates were the most prevalent strains producing hemolytic activity at 93.3%, proteolytic at 93.3%, lipolytic at 86.7% and DNase at 80% activities than those obtained by *A.punctata* and *A.salmonicida*. These results are nearly agreed to great extent with those reported by *Santos et al. (1988); Aly, (1997); Wang and Silva* (1999) and Castro et al. (2003). Hemolytic and proteolytic activities act Kafr El-Sheikh Vet. Med. J. Vol. 3 No. 1 (2005)

as a marker of pathology in fish (*Rogulska et al., 1994 and Austin and Adams, 1996*), also, *Aeromonas* organisms which had hemolytic and proteolytic in food of animal origin indicate that this food may play a significant role in the epidemiology of *Aeromonas* associated with gastroenteritis.(*Aly,1997*).

The extensive use of antibiotics in veterinary practice and the addition of antibiotics as feed additives are considered to be the main cause of the high incidence of antibiotic- resistance organisms, especially among Gram-negative bacteria in domestic animals (*Anderson, 1968*).

The subtherapeutic use of antimicrobial drugs has played an important role in animal husbandry for control of disease efficiency of feed conversion. (*Franco et al., 1990*). *Aeromonas* species are zoonotic important. So, antimicrobial sensitivity test for isolated *Aeromonas* species are performed and found that *A. hydrophila* was sensitive to gentamycin, enrofloxacin, neomycin,nalidixic acid, streptomycin and oxytetracyclene, while *A.punctata* was sensitive to danofloxacin,enrofloxacin,gentamycin, oxy and tetracycline, moreover, *A. salmonicida* was sensitive to enrofloxacin, gentamycin, rifampicin and oxytetracyclene. On the other hand, amoxycillin, ampicillin, erythromycin, pencillin and lincospectin were resistant to isolated species of *Aeromonads*. Nearly our results agree to a great extent with those reported by *Gado (1988);Radwan (1995);Bastawrows and Mahmoud(1999);Wang and Silva (1999);Megahed (2000); Badawy (2002) and Khalil (2004)*.

In conclusion our results indicated that psychrotrophic species were prevalent in *Clarias gariepinus* samples and all *Aeromonas* isolates have a high hemolytic, lipolytic, proteolytic and DNase activities, furthermore they are resistant to amoxycillin, ampicillin, pencillin, erythromycin and lincospectin antibiotics. The risk of illness associated with consumption of fish is reduced by good handling practices in home including thoroughly cooking of fish. Finally proper cleaning and sanitizing all utensils and contact surfaces.

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أجريت هذه الدر اسه على خمسين عينه من سمك القرموط تم جمعها من الأسواق بمدينة طنطا ، لاستبيان مدى تلوثها بالميكروبات المحبة للبرودة وخاصة ميكروبات الأيروموناس ودراسة بعض الخواص الممرضة المتعلقة بها، وكذلك مدى حساسية ومقاومة هذه الميكروبات للمضادات الحيوية

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المختلفة ، وأوضحت الدر اسه على انه قد تم عزل وتصنيف الميكروبات المحبه للبروده بنسبة 8% للأ سينيتوباكتر 56% للأيروموناس و12% للألكاليجينز و 10% للكروموباكتر 28% ،المسودوموناس ،6% للفلافوباكتيريم كما تم عزل وتصنيف أنواع االآيروموناس بنسبة 30% للايروموناس هيدروفيلا و16% للايروموناس بنكتاتا و10% للايروموناس سالمونيسيدا كما تضمنت النتائج على الأنشطه المحلله للدهون و البروتين وتكسير كرات الدم الحمراء و حمض DNA لمعزولات الايروموناس و هي 78.6% و 71.4% و 82.1% و67.9% على الترتيب. باجراء اختبار الحساسية للمضادات الحيويه على معزولات الايروموناس اتضح إنها حساسة للجنتاميسين والانروفلوكساسين والاوكسيتتراسيكلين وحمض النالدكسيك وغير حساسة للبنسلين و الأمبسلين و الاموكسيسلين والارثرومايسين واللينكوسبكتين ونوقشت الاهميه الصحية والاقتصادية للميكروبات المعزولة و كذلك الاحتياطيات الواجب اتخاذها لحماية المستهلك.