SURVEY OF PATHOGENIC BACTERIA IN FRESHWATER CRAB (*POTAMON MESOPOTAMICUM*) COLLECTED FROM TIGRIS , BASRAH.

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(Received 20May 2014,Accepted 4July 2014)

Keywords; freshwater **,** Lincomycin**,** *E. coli***.**

ABSTRACT

The present study was carried out to determine the presence of pathogenic bacteria in freshwater crab (*Potamon mesopotamicum*) in Tigris at North of Qurna, as well as their antibiotic resistance. A total of 21 bacterial isolates consisting three bacterial species were isolated from freshwater crab, *P. aeroginosa, E. coli, A. hydrophila* kanamycin was found to be effective against all the bacterial isolated whilst the highest percentage of antibiotic resistance was shown beyond to Lincomycin (76.4%) followed by ampicilin(66.14%), Erythromycin(57.3%).

INTRODUCTION

The invertebrates, comprise over 95% of the animal species, some of them are live in environments rich with potentially harmful microorganisms. As a result, these animals have developed various competent strategies to defend their lives against invading pathogens [11]. Life in an aquatic environment rich in microorganisms.

Crustaceans have developed effective system for detecting and eliminating noxious microorganisms. The crabs are in intimate contact with aquatic environment rich in pathogenic microbes and are prone to infection by those microbes at various stages of growth, and losses due to disease can be enormous [10].

Microbial infections have been the major concern of aquaculturists worldwide. Various bacteria in marine and estuarine environment such as *Vibrio cholera*, and *Vibrio parahaemolyticus* and *Vibrio* species are potential human pathogens [3] . [16]. The use of antibiotics in aquaculture practices influenced the bacteria population in the environment [4]. Antibiotics such as oxytetracycline, oxolonic acid and quinolones are usually reported in fish farming[6 and 17]. As crabs are in close contact with the milien that rich in pathogenic bacteria, infection by bacteria can be vast [10]. In relation to health of crabs and concerning food safety for human consumption, it was crucial to understand the susceptibilities of bacteria flora in the crabs to antibiotics. Due to the

lacking scientific documentation regarding this aspect, antibiotics a study was conducted to isolated bacteria flora from crabs, and to evaluate the bacterial resistance to antibiotics .

MATERIALS AND METHODS

A total of 60 live crabs weighted from 250g to 400g were caught at Tigris river at north of Qurna. They were transported to microbiological laboratory of marine science center for analysis. The crabs were sacrificed according to [15]. The crabs were chilled in a refrigerator and then killed by spicking to destroy the nerve centers. Aseptically each body parts namely abdominal contents, gills, was swabbed by using sterile cotton bud then streaked onto universal agar which include Trypticase Soy Agar (TSA) and selective cytophaga agar (CA), Glutamate Starch Pseudomonas (GSP), and Xylose Lysine Deoxycholate (XLD) agar. The plates then incubated at 30C° for 24h. the colonies which showed different shapes per plate, were slectected from each sample and restreaked three times on to nutrient agar plates to receive pure culture. Phenotypic characteristics, Gram staining, and biochemical tests were determined for all isolates according to [19 and 9]. Another identification was carried out by using a commercial identification system Kit(BBL. Crystal, USA).

Antibiotics susceptibility test: was performed according to kirby –Baner disk diffusion method [2] by using Muller Hinton agar (Oxoid,England). The antibiotics include: Oxytetracycline(OT), 30µg, Furazolidone(FR), 15µg, Knamycin(K), 30µgm; Nalidixic acid (NA), 30 µg Chlroamphenicol(C) 30µg , ampicillin(AAP), 10 µg, Sulfhanethaxozole(RL)m 25 µg, Amoxicillin(AML), 25µg, Lincomycin(MY), 15µg, Novobiocin (NV), 30 µg Tetracycline(TE), 30 µg Erythromycin 15, Spiramycin 100, and Nitrofurntion 50.

RESULTS

Figure (1) shows freshwater crab (*Potamon mesopotamicum*). A total of 21 bacterial isolates were obtained from 60 crabs representing three species. *Aeromons hydrophla* 10(47.13%). Followed by *Pseudomnas aeroginosa* 6(28.12%) , *E. coli 5*(23.17%). As showed in table (1) Figure (2) colonies of A- *P. aeroginosa B- E. coli* C- *A. hydrophla.*

Table1: showed most bacteria species isolated from abdominal content than gills. All bacterial isolates in the present study were sensitive to kanamycin, chlormphenicol, erythromycin, nalidixic acid, Oxytetracycline, sulfamethoxizol, most of the present bacterial isolates were found to be resistant to Lincomycin, ampicillin and amoxillin. less than 10.% of the bacterial isolates were found to be resistant to the rest of the antibiotic.

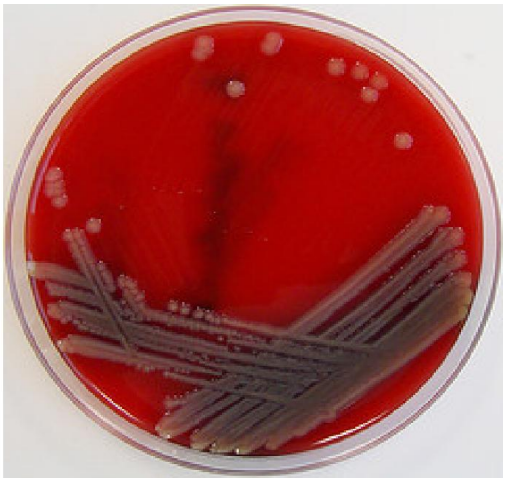
Table1: Bacteria showed in abdomen and gills of crab (*Potamon mesopotamicum*)

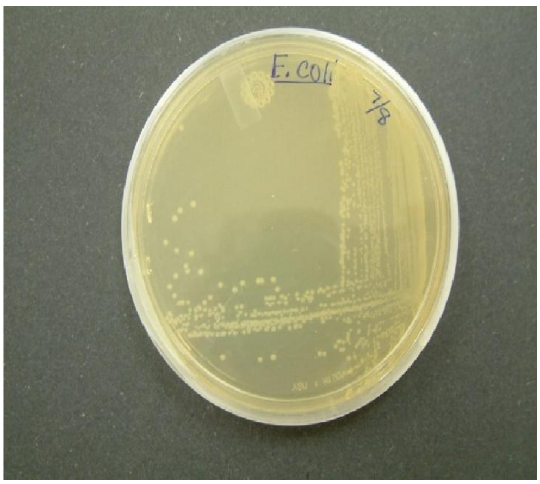
Bacteria	Abdomen	Gill	No.	%
P. aeroginosa	+	-	10	47.13
E. coli	+	-	6	28.12
A. hydrophila	+	+	5	23.17

+: presence of bacteria species -: Absence of bacteria species.

Fig (1): ***Potamon mesopotamicum***

A-





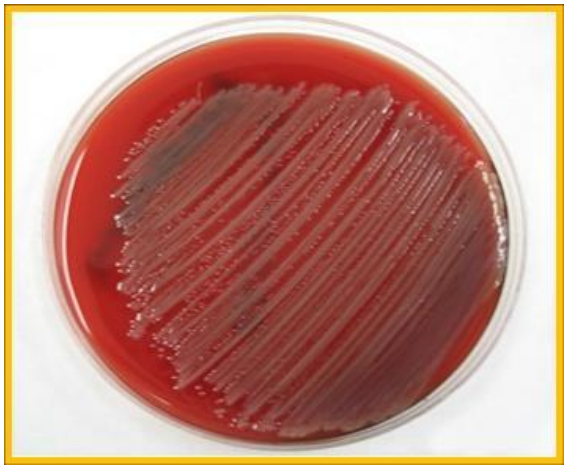


Fig (2): colonies of

A- *P. aeroginosa*
B- *E. coli*
C- *A. hydrophla*

Tables 2 : Morphological and Biochemical characteristics of bacterial strains from crab .

Reaction agent	*A. hydrophla*	*E. coli*	*P. aeroginosa*
Gram staning	-	-	-
Shape	Rod	Rod	Rod
Motility	M	M	M
Indole test	+	+	-
Methyl red test	+	+	-
Voges proskuer test	-	-	-
Citrate utilization test	-	-	+
Urease test	-	-	+
TSI test	K/A	A/A	K/K
H2S	-	-	-
Gas	-	+	+
Nitrate reduction test	+	+	-
Catalase test	+	+	+
Oxidase test	+	-	+
Carbohydrate test			
Glucose	+	+	+
Maltose	+	+	+
Sucrose	-	+	-

Table 3: susceptibility of 21 bacteria isolates of crab(*Potamon mesopotamicum*) against 12 antibiotics.

	Sensitive:		Resistant:	
Antibiotic	**Incidence**	**%**	**Incidence**	**%**
Ampicillin 10µg	**2**	**9.11**	**14**	**66.14**
Lincomycin 15	**1**	**4.19**	**16**	**76.4**
Erythromycin 15	**15**	**71.9**	**3**	**14.6**
Amoxillin 25	**1**	**4.16**	**12**	**57.5**
Sufamethoxazole 25	**1**	**28.12**	**4**	**19.1**
Chlroamphenical 30µg	**18**	**84.36**	**1**	**4.16**
Kanamycin 30 µg	**19**	**90.4**	**1**	**4.16**
Nalidixic acid 30 µg	**12**	**57.3**	**1**	**4.16**
Novobiocin 30	**2**	**9.11**	**1**	**4.16**
Oxytetracycline 30	**8**	**33.7**	**1**	**4.16**
Nitrofurntion 50	**1**	**4.16**	**2**	**21.6**
Spiramycin 100	**1**	**4.16**	**2**	**21.6**
Total	**86**	**267**	**58**	**297.54**

DISCUSSION

In the present study bacteria isolated could be pathogenic and involved in disease transmission to human [5]. who found pathogenic microorganisms in several tissues types of crabs such as tanner crab *Chionoectes opilio,* Dungeness crab *Cancer magaster*, king crab *Paralithodes camtschatia* and Rock crab *Cancer irroratus*, this could serve as accumulation sites for human pathogens particularly in crabs collected from contaminated area. [7 and 8] indicated that crab tissues contained higher number of bacteria than their surrounding environments. Most of the bacterial species found in the present study were comparable to bacteria found in cultivated oyster in Setia Wetland [13]. There have been less attention paid to the risk of antibiotic use in fish farming to human health. Other than marine system [18]. There has been a report on the occurrence of drug resistance microorganisms In freshwater eel farm system [1]. The presence *E. coli* in the present study further suggested that fecal contamination occurred in Tigris water . Being deposit-feeding animals which feed on plants and animal debris buried in water , fresh water crabs may accumulate microorganisms from the environment and therefore could serve as a vector for disease transmission , these risks further studies in the bacterial distribution in Tigris water . In addition to transfer of resistant microorganisms through consumption of contaminated crabs: there is substantial risk to environmental contamination due to the practice of using medicated feeds to treat bacterial disease, further more, do not only act against pathogenic bacteria but also against normal microbial flora in booth animals and humans , there fore , it is important to monitor the

antibiotic resistance incidence not only of pathogenic bacteria but also against normal bacteria flora. In this study, kanamycin was the most effective antibiotic in inhibiting the bacteria present in crab, relatively higher numbers of bacterial isolates were resist to lincomycin, ampicillin and erythromycin. In the present study, the bacterial species found in the gills and abdomen did not cause mortality to crabs, because the ability of Serum of crab to agglutinate bacteria which further indicate the involvement of humoral agglutinins in host-defense response [12].The loss of antibiotic susceptibility among the aquatic bacteria could also enhance by the physiochemical qualities of water and seasonal variation [14]. The present study concluded that fresh water crabs of Tigris water contain antibiotic resistances bacteria , these bacteria could transfer their antibiotic resistance gens to bacteria from other aquaculture sites and other organisms in the food chain , including human , therefore , comprehensive monitoring and regular analysis on crabs should be implemented to provide on early warning to the public for the presence of antibiotic resistances bacteria in freshwater crabs.

مسح للجراثيم المرضية في سرطان المياه العذبة *Potamon mesopotamicum* من نهر دجلة

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اجريت هذه الدراسة لتحديد تواجد الجراثيم المرضية في سرطانات المياه العذبة والتي تم جمعها من مياه نهر دجلة شمال القرنة . مع بيان المقاومة للمضادات الحياتية. تم عزل ٢١ جرثومة متضمة ثلاثة انواع من الجراثيم *P. aeroginosa, E. coli, A. hydrophila*. اظهرت الدراسة بان Kanamycin له فعالية على كل العزلات . بينما اعلى نسبة من المقاومة تعود الى Lincomycin (٧٦.٤ %) ويتبعها Ampicillin (٦٦.١٤ %) ثم Erythromicin (٥٧.٣ %) .

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