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Enterobacteriaceae opportunism isolated from Caspian Turtle *Mauremys caspica* (Gmelin, 1774) suffering from a fracture of the external shell, East of Al-Hammar Marshes, Iraq

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Abstract - Caspian Turtles *Mauremys caspica* (Gmelin, 1774) are classified as an endangered species on the IUCN red list, by a variety of dangers including hunting, deliberate killings and infectious diseases. The present report included the Opportunistic bacterial isolation which caused clinical signs on the *M. caspica* Turtles caught from the East Al-Hammar freshwater marshes, during summer 2017. It was suffering from a fracture of the external shell, tissue damage, bacterial growth and acute inflammation of the body tissues. The common clinical signs were external hemorrhage, histopathological changes revealed degeneration and necrosis in all organs associated with chronic inflammatory cell infiltration and Melanomacrophage cells were detected in all turtle tissues. The most common macroscopic lesions found on the skin of the turtle and ulcerative skin, the result in abscesses and dermatitis, regenerative changes on skin lesions and ulcerative deep necrotic and gangrenous changes in the turtle examined. Four different genera of family Enterobacteriaceae which identified by the VITEK II system, with a probability level of 94-99%, *Citrobacter freundii* (29.06%), *Klebsiella oxytoca* (25.58%), *Serratia fonticola* (24.41%) and *Enterobacter cloacae* (20.41%). The result showed that of a total of sixty-five bacterial isolates from the necrotic lesions skin tissue, some of them which are considered a potential threat to the public health.

العائلة المعوية الانتهازية Enterobacteriaceae المعزولة من سلاحف بحر قزوين Caspian Turtle *Mauremys caspica* (Gmelin, 1774) التي عانت من كسر في الصدفة الخارجية، من هور الحمار العراق

عيسى طه محمد، نادية علي حسين الشمري و ماجد عبد العزيز بناي
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المستخلص - تعد سلاحف بحر قزوين *Mauremys caspica* (Gmelin, 1774) على انها مهددة بالانقراض على "القائمة الحمراء للطبيعة"، من خلال تناقص اعدادها بسبب انها محاطة بمجموعة متنوعة من المخاطر بما في ذلك عمليات الصيد، وعمليات القتل المتعمد والإصابة بالأمراض. جلبت عينة السلاحف من مستنقعات المياه العذبة في هور الحمار خلال موسم الصيف من عام 2017 حيث كانت تعاني من كسر في الصدفة، تلف الأنسجة والنمو البكتيري والتهاب حاد في أنسجة الجسم. العلامات السريرية المشتركة كانت من اهمها نزيف خارجي وقد كشفت التغيرات التشريحية المرضية على وجود تنكس وتنخر في جميع الانسجة على شكل التهاب مزمن وتم الكشف عن الخلايا Melanomacrophage في جميع انسجة السلحفاة، وأكثر الأفات العينية المشتركة وجدت على الجلد بشكل القرح. اظهرت نتائج الدراسة أن ما مجموعة خمسة وستين (65) عزلة بكتيرية من أنسجة الجلد تعد آفات نخريه، البعض منها التي تعتبر خطرا محتملا على الصحة العامة. أربعة أجناس مختلفة من عائلة Enterobacteriaceae تم تشخيصها بواسطة جهاز فاينك، على مستوى الانواع بمستوى احتمالات 94-99%، *Citrobacter freundii* (29.06%)، *Klebsiella oxytoca* (25.58%) و *Serratia fonticola* (24.41%) و *Enterobacter cloacae* (20.41%). أجريت الدراسة الحالية على هذا الكائن المصاب لتحديد العدوى البكتيرية المرتبطة معها لأنه يلعب دوراً مهماً في نقل العدوى إلى سائر الكائنات الحية كذلك لتحديد البكتيريا الانتهازية في هذه المنطقة التي قد تشكل خطراً على الصحة العامة في أهوار شرق الحمار، جنوب العراق.

الكلمات المفتاحية: *Serratia fonticola*; *Klebsiella oxytoca*; *Citrobacter freundii*; سلحفاة قزوين *Mauremys caspica*; شرق الحمار.

Introduction

The Caspian Turtle *Mauremys caspica* (Gmelin, 1774), family Geomydidae are of worldwide distribution, the recent population survey of species in Iraq is limited with less detailed information on the breeding and distribution (Fazaa *et al.*, 2015).

Most diseases in the ecosystem are affecting by different environmental factors, including infectious and stress, bacteria are important adapted to environments that have a wide range of conductivity (Hazen *et al.*, 1978).

Turtles are one of the most attractive animals of the reptile world, most diseases are the result of stress that occurs in their environmental pollution, water or incorrect diet or poor cultivation, causes bleeding into the skin or skin redness and lesions (Rawski *et al.*, 2017).

Gram-negative bacteria of the family Enterobacteriaceae are important causes of infection to humans and other animals. Most bacterial pathogens potentially transmitted by water and soil, within these opportunistic bacteria when stress these animals sing out many pathogen (Yagoub, 2009).

The present study was conducted on the Turtle *Mauremys caspica* to identify the bacterial infections associated with it because it is infections to human being in East Al-Hammar marshes.

Materials and Methods

Samples Collection:

An infected Caspian Turtle *M. caspica* was caught from East Al-Hammar marshes during the summer 2017, it had suffered clinical signs, the specimen was acclimatized in a glass tank fresh water at the laboratory conditions at approximately 25°C.

Microbiological Examination:

Swab samples were collected from the skin lesions and then were inoculated onto MacConkey agar media (HiMedia-India), Tryptic soy agar (TSA) (HiMedia-India), and Nutrient agar (Himedia-India), using the spread plate method and the plates were incubated (Binder) for 24h at 37°C.

The Enterobacterial colonies bacterial appropriate pink on MacConkey gar. Once grown and pure cultures on Nutrient agar were obtained, after 24 h, these isolates were taken to the Laboratory and then identified by VITEK II system (Biomerieux-USA).

It is an automated microbiological system used for biochemical identification of isolates according to the manufacturer's procedure imparts an automated computer in a microbiology system utilizing growth based technology designed for the identification and susceptibility testing of wide range of microorganisms including Gram-negative and Gram positive bacteria. Species identifications technique relies on advanced colorimetric technology, the measurement of light attenuation associated with each biochemical reactions in VITEK II system cards (Gram-positive and Gram-negative bacteria).

Suspension Preparation of VITEK II:

A sterile swab is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube, the VITEK II compact system

identified all the isolates with a probability level of 94-99%, final identification results are available in approximately 10 hours or less (Pincus, 2005).

Results and Discussion

The Enterobacteriaceae is a large family of Gram-negative bacteria belonging to the order of Enterobacteriales, class Gammaproteo bacteria, phylum Proteobacteria. A total of 65 isolates were made from the necrotic lesions skin, of the Caspian Turtle *Mauremys caspicawas* (Fig. 1) with the result of predominance of Gram-negative microorganisms and unidentified Gram-positive.

All bacterial were isolated from Testudines' incubated at 37°C. The isolated species were *Citrobacter freundii* with probability of 29.06%, *Klebsiella oxytoca* (25.58%), *Serratia fonticola* (24.41%) and *Enterobcter cloacae* (20.41%) (Table 1). The data of bacteria are useful tool in understanding the role of bacteria as pathogenic agents in wild turtles.



Figure 1. Caspian Turtle *Mauremys caspica* (Gmelin, 1774) East of Al-Hammar Marsh-Iraq showing Skin lesions.

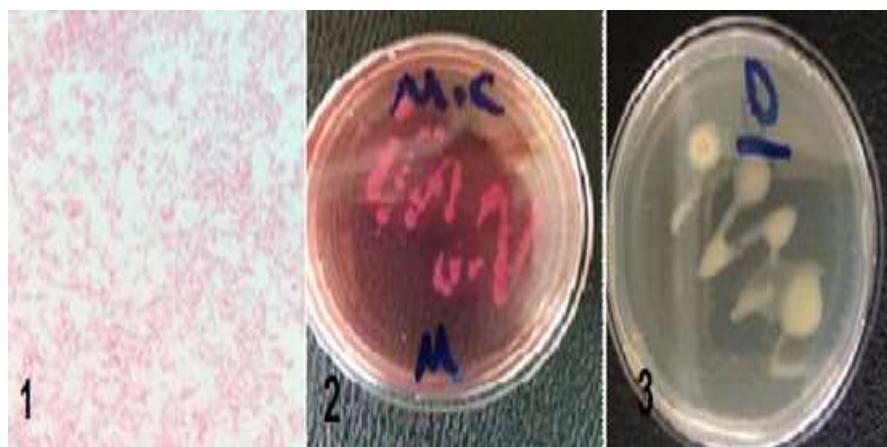


Figure 2. Cultural of Enterobacteriaceae infection on Caspian Turtle *Mauremys caspica*. 1-Oil immersion at 1000x microscopy, 2-In MacConkey agar, 3-Tryptic soy agar.

Table 1. Identification of bacterial isolates with Probability (%).

Bacterial isolates	No.of isolates	Probability %	Analysis time /hour	Unidentified of total number of species
<i>Citrobacter freundii</i>	20	94	5	2
<i>Klebsiella oxytoca</i>	12	99	2.75	1
<i>Serratia fonticola</i>	15	98	4	3
<i>Enterobacter cloacae</i>	18	92	7	4

1- *Citrobacter freundii* (Table 2):

A facultative anaerobic gram-negative bacterium of the family Enterobacteriaceae cause a number of opportunistic infections (Abbott, 2011). *C. freundii* is generally considered as an opportunistic pathogen in wild and aquaculture organisms, may cause fatal septicemia in humans and animals and associated with a wide spectrum of infectious diseases with population declines in reptiles and free-ranging turtles living under stressful conditions (Fernandez *et al.*, 2011). Certain diseases studied in trout and cyprinids are also caused by *C. freundii*. *C. freundii* causes abnormal inflammatory changes in the intestine of trout and inflammatory and necrotic changes in the internal organs of cyprinids. The illness was discovered by means of artificial infection with a pure culture of *C. freundii*. This discovery established *C. freundii* as a cause of fish disease (Drelichman and Band, 1985).

Table 2. Biochemical details identification of isolates bacterial species (*Citrobacter freundii*) by VITEK II system.

2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	+
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	DMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CiT	+	37	MNT	-	39	SKG	+
40	ILATK	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	0129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

2- *Klebsiella oxytoca* (Table 3).

Klebsiella, Gram-negative bacteria may cause different of healthcare-associated infections (Onori *et al.*, 2015). Isolation and diagnosis of some data of bacteria (*Klebsiella pneumoniae*, *Enterobacter cloacae*, *Shigella*) are down by Al-Shemmari (2017) who suggested that the disease infections are associated in some fishes in Basrah Governorate with *Aeromonas* sp., *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia marcescens*, *Staphylococcus* sp. and *Vibrio* sp., which matched our results.

Table 3. Biochemical details identification of isolates bacterial species (*Klebsiella oxytoca*) by VITEK II system.

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNA G	-	12	AGLT p	-	13	dGL U	+	14	GGT	-	15	OFF	-
17	BGL U	-	18	DMA L	+	19	dMA N	+	20	dMN E	+	21	BXY L	-	22	BAla p	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CiT	-	37	MNT	-	39	SKG	+
40	ILAT K	-	41	AGL U	-	42	SUCT	-	43	NAG A	-	44	AGA L	-	45	PHO S	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGU R	-
	0129 R	-	59	GGA A	-	61	IMLT a	-	62	ELL M	-	64	ILAT a	-			

3- *Serratia fonticola* (Table 4).

Serratia is a Gram-negative, facultative anaerobic, rod-shaped bacterium, wide variety inhibition cause disease in plants, vertebrates and invertebrates (Kampfer, 2016).

Table 4. Biochemical details identification of isolates bacterial species (*Serratia fonticola*) by VITEK II system.

2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	+	7	dCEL	+	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	DMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CiT	-	37	MNT	-	39	SKG	-
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	0129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

4-*Enterobacter cloacae* (Table 5).

A facultative anaerobic Gram-negative bacterium, widely found in nature, saprophytic in the environment, found in soil and sewage (Mezzatesta *et al.*, 2012). *E. cloacae* was described for the first time in 1890 by Jordan as *Bacillus cloacaev* surname, and then underwent numerous taxonomical changes. According to Mezzatesta *et al.* (2012). *E. cloacae* is considered as ubiquitous in terrestrial and aquatic environments, wide variety inhibition cause disease because of these strains occur as commensal microflora in the intestinal tracts of humans and animals and play an important role as pathogens in plants and insects (Al-Bahry *et al.*, 2011).

Table 5. Biochemical details identification of isolates bacterial species (*Enterobacter cloacae*) by VITEK II system.

2	APPA	+	3	ADO	+	4	PyrA	+	5	IARL	+	7	d CEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGL Tp	-	13	d GLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	DMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	d TAG	-	35	d TRE	+	36	CiT	-	37	MNT	-	39	SKG	-
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	0129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			-

The aquatic animals are prone to bacterial infection in the same way as terrestrial animals, especially when stressed or result lesion (Buller, 2014). The genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* opportunistic pathogens responsible for a wide range of infections.

Our results indicate that the *Citrobacter freundii*, *Serratia fonticola* and *Klebsiella oxytoca* are pathogenic agents, secondary invaders and they seem likely that they are capable of producing disease in turtles. In addition to that, they may be pathogens for humans and risk to public health. So, people who live near this area, fishermen and the aquarist who sell ornaments turtle should be more careful when dealing with these turtles.

Coliform is the name of a test adopted in 1914 by the Public Health Service for the Enterobacteriaceae. It is the commonly used bacterial indicator of sanitary quality of foods and water. Coliform bacteria are not a single type of bacteria, but a group of bacteria that includes many strains, such as *E. coli*. They are ubiquitous in nature, and many types are harmless. Coliform bacteria belong to the genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pantoea* and *Serratia* (WHO, 2003).

Some of the Enterobacteriaceae can cause disease, if they contaminate regions such as the body cavities. The strains within each bacterial species are overt pathogens and are regularly associated with disease (Linton and Hinton, 1988). The diversity and contamination of many bacterial species, which cause many infectious diseases that directly threaten human health and threaten the fish industry. Sekar *et al.* (2008) had shown that the *E. cloacae* were the causative agent for the mortality of *M. cephalus*, found in Muttukadu lagoon.

The particular isolation of most pathogenic organisms such as *Salmonella* spp., *E. coli* and potential pathogenic organisms as *Klebsiella* spp., *Citrobacter* spp. and *Proteus* spp., from fish and fish products gives an indication about environmental fecal pollution of fish (Wogu and Maduakol, 2010).

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