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RESEARCH PAPER

First Occurrence of *Plesiomonas shigelloides* Bacteria on/ in *Carassius carassius* and *Silurus triostegus* Fishes from Greater Zab River in Kurdistan Region, Iraq.

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A B S T R A C T:

The present study shows occurrence of *Plesiomonas shigelloides* bacteria from *Carassius carassius and Silurus triostegus* that collected in Greater Zab River in Aski-Kalak in Erbil from October 2018 to November 2019. Swaps were taken from lesions on the skin, fins and internal organs of the 34 fishes (14 from *C. carassius* and 20 from *S. triostegus*) and inoculated in nutrient agar, blood agar and MacConkey agar. The agar plates were incubated at 37°C for 24-48 hours. Obtained 13 isolates, the bacterial isolates identified depending on some morphological, cultural, some biochemical test and further confirmed by Vitek 2 compound system. For characterization and identification the bacterial colonies were examined and then showed Gram negative reaction and motile. Biochemical tests were done by Vitek 2 compound system and result confirming. The study showed appearance of *P. shigelloides* on the skin lesions and intestine of the examined fish. The present existence of *P. shigelloides* bacteria regarded as first record for this bacterium in Iraq on and in fishes.

KEY WORDS: Fish pathogen, *Plesiomonas shigelloides*, *Carassius carassius*, *Silurus triostegus*.
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1.INTRODUCTION :

Bacterial diseases are the most frequent and major cause of mass death in fish worldwide. Several numbers of pathogenic bacteria have been reported that causes diseases in fish round the world (Pękala-Safińska, 2018), that responsible for economic losses in natural and cultured fishes (Khatun et al., 2011). Diseases have negative impacts on fish production due to mortality in the fish farms. Also, it can reduce reproductive performance and feed conversion efficiency leading to reducing growth and overall performance of cultured fish which it is serious economic losses due to increasing the cost thus have impact on the industry of country (Mustafa et al., 2001).

Plesiomonas shigelloides is a facultative, anaerobic, motile, Gram-negative rod aquatic bacterium recently recognized in the family Entrobacteriaceae and it is the only oxidase-positive member of this family also, as a potential human and animal pathogen of this family (Chen et al., 2013). Studies on concurrent infection with *P. shigelloides* and other microorganisms like *Vibrio* spp., *Aeromonas* spp., and *Edwardsiella tarda* (Butt et al., 1997).

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Common environmental reservoirs for plesiomonads include freshwater ecosystems and estuaries and inhabitants of these aquatic environs which particularly reported from tropical and subtropical countries, different aquatic organisms, water dwelling reptiles, poikilotherms, and birds can port *P. shigelloides* (Janda et al., 2016; Behera et al., 2018).

P. shigelloides has been involved in gastrointestinal infections in human (Bodhidatta et al., 2010), and in Iraq it was isolated from drinking, bath and sewage water (Mustafa, 2015) In addition it has been shown that also causes bacteremia, pneumonia, osteomyelitis, sepsis, keratitis and meningitis (Ozdemir et al., 2010; Klatte et al., 2012).

The aim of the presnt study was to identifying the bacterial flora which has been isolated from fish in the Greater Zab River in Aski-Kalak based on morphological, cultural and biochemical characterizations, also take notice of the prevalence and intensity of this bacteria on/in fishes.

2. MATERIALS AND METHODS

A total of 34 fish specimens (from 14 *Carassius carassius* and from 20 *Silurus triostegus*) were collected from Greater Zab River by fisherman through using gill netting were fishes transported with river water as a life. The fishes identified according to Coad (2010) and Froese and Pauly (2019).

For Bacterial isolation, specimens were taken from Lesion on the skin, fins and internal organs of fishes by using sterile swabs, and streaked in nutrient agar, blood agar and MacConkey agar. The agar plates were incubated at 37°C for 24-48 hours, for characterization and identification the bacterial colonies were examined. Morphological and cultural characteristics examined such as optical characteristics shape, color, size and edge, Gram staining reaction and motility test (Thongkao and Sudjaroen, 2017). The bacterial colonies were then subjected to. Vitek 2 compound system was used for result confirming (Pincus, 2010).

3. RESULTS AND DISCUSSION

The present study was revealed existence of 13 isolates of *P. shigelloides* bacteria which isolated from lesion on the skin, fins and internal organs of *C. carassius* and *S. triostegus* with a prevalence 14.2% and 15% respectively (Table 1).

The morphological characteristic of this bacteria is non-lactic fermenting, round, yellow to pale-brown colony in MacConkey Agar. On the blood agar non hemolysis, white to gray in color and smooth round colonies. In addition it is whitish jelly like, round, flat colony on nutrient agar, colonies are oxidase positive (Fig.1). The microscopic appearance of bacterium is Gram-negative, polymorphism, round ended, non-endospore forming (Fig.2). Excellent identification was done by using vitek 2 compact system (Table 2).

In Iraq for the first time Saleh (1997) isolated six types of bacteria namely: *Aeromonas* sp., *Streptococcus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp. and *Alcaligenes* sp. in the skin, gills, blood, intestine, liver, kidney, spleen and muscle on *Cyprinus carpio*. Worth to mention there were other bacteria have been isolated from the fishes such as *Myxobacterium* spp. was isolated on *C. carpio* by Mohammad-Ali et al. (2000) and *Staphylococcus* sp. from skins, muscles, intestine and liver of *C. carpio* by Ali (2014). It is the first record of *P. shigelloides* from the fish in Iraq.

In Kurdistan region *Aeromonas hydrophila*, *A. sobria*, *P. shigelloides* and *A. salmonicida* were isolated from fishes (Ibrahim, 2008; Mustafa and Mustafa, 2015; Mustafa, 2016; Mustafa et al., 2019; Ibrahim, in press). The first record of *P. shigelloides* in Iraq was done by Mustafa (2015) that isolated from drinking, bath and sewage water.

P. shigelloides have been recognized as potential fish pathogens (González et al., 1999). It was isolated from diseased *Hypophthalmichthys molitrix* which associated with mortality in India (Behera et al., 2018). *P. shigelloides* pathogen has been identified as one of the main pathogen in the sturgeon's culture in Beijing area (Wang et al., 2013). Hu et al. (2014) was isolated *P. shigelloides* from clinical cases of fishes during mass mortality of *Ctenopharyngodon idellus*, Nisha et al. (2014) was reported 100% mortality of cichlid ornamental fish by *P. shigelloides*. Also, by Liu et al. (2015) isolated *P. shigelloides* as severe pathogen of *Orechromis nilotica* cultured fish.

4. CONCLUSIONS

The present study showed first record of *P. shigelloides* as a fish pathogen in Iraq which was isolated from skin, fins and intestine lesions of *C. carassius* and *S. triostegus* with prevalence 14.2% and 15% respectively in the Greater Zab River in Aski-Kalak _ Erbil/Iraq. The recorded bacteria showed lesions in skin, fins and intestine in infected fishes.

Table 1: Showing the prevalence of *P. shigelloides* from fish hosts.

Fishes	No. of examined fishes	No. of infected fishes	Prevalence (%)	Locations
Carassius carassius	14	2	14.2	Skin, fins and intestine
Silurus triostegus	20	3	15	Skin, fins and intestine

Table 2: Biochemical details of *P. shigelloides*.

Biochemical Details																	
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	BAIap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
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	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

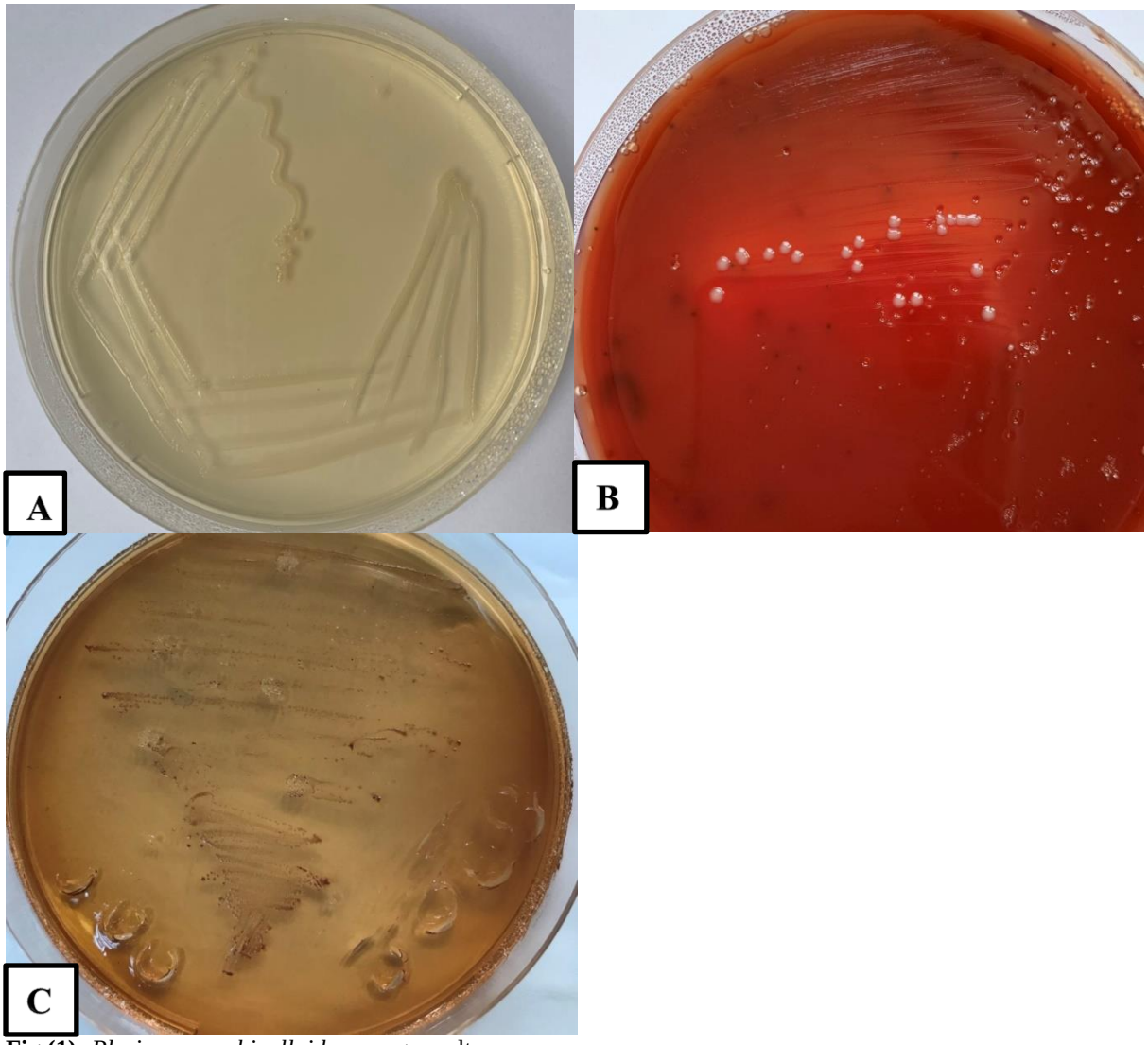

Fig (1): *Plesiomonas shigelloides* on agar cultures.
A- Colony of *Plesiomonas shigelloides* cultured on nutrient agar
B- Colony of *Plesiomonas shigelloides* cultured on blood agar
C- Colony of *Plesiomonas shigelloides* cultured on MacConkey agar

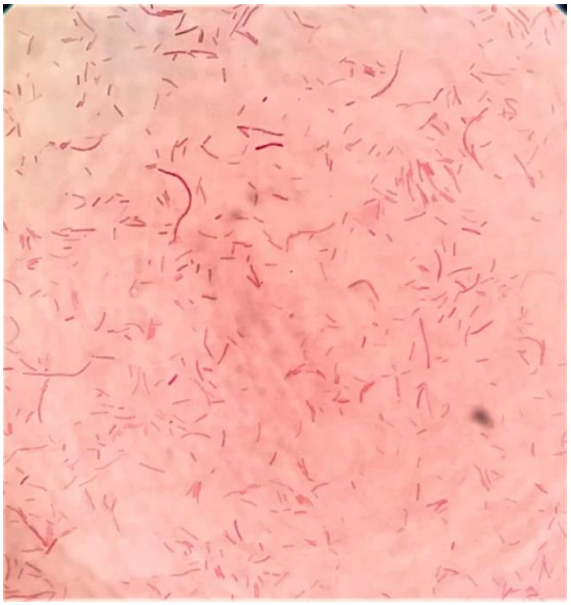

Fig (2): Photomicrograph of *Plesiomonas shigelloides* showing polymorphism of bacterial cells (1000x).

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