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Research Article
Isolation and Pathogenicity Determination of *Bacillus cereus* Associated with Ulcer Formation in African Catfish *Clarias gariepinus*

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Abstract

Background and Objective: *Bacillus cereus* is a toxin-producing facultatively anaerobic Gram-positive rods and could cause diseases in aquatic animals. The main objective of this study was to determine the isolation and pathogenicity of *Bacillus cereus* associated with ulcer formation in African catfish *Clarias gariepinus*. **Materials and Methods:** Live moribund African catfish *Clarias gariepinus* suffered from skin ulceration and hemorrhages were collected from a private fish farm at Kafr El Sheikh Governorate north Egypt, March, 2019. The bacterium isolated from moribund fishes was identified as a *Bacillus cereus* based on phenotypic, biochemical characteristics and confirmed with 16s rRNA sequence. **Results:** Experimentally infected fish showed external and internal clinical symptoms typical to those caused by natural infection. The clinical signs of the affected fish showed ulcers on the skin. The severity of ulcers was dose dependent. Antibiotic sensitivity tests showed that the bacterium was sensitive to all antibiotics but cefotaxime. *B. cereus* isolate was found to be positive for the production of protease, lipase, lecithinase and hemolysin. **Conclusion:** This study indicated that *B. cereus* was the causative agent responsible for skin ulceration and disease in African catfish *C. gariepinus*.

Key words: *Clarias gariepinus*, ulcer formation, *Bacillus cereus*, histopathology, molecular identification, antibiotic susceptibility, extracellular enzymes and hemolysis activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

North African catfish *Clarias gariepinus* (Burchell,1822) is known for its high growth rate, resistance to bad water conditions and give excellent meat quality. They are usually polycultured with Nile tilapia in earthen ponds to increase fish output from the same farm and to control undesired reproduction of tilapia[1]. Fish are susceptible to a wide variety of bacterial pathogens present in normal flora of water. Under stress conditions and water deterioration, these pathogens cause significant economic losses[2].

Bacillus cereus is a toxin-producing facultatively anaerobic Gram-positive rods forming a single heat resistant endospore. The endospores are generally oval or sometimes round or cylindrical, very resistant to extremely adverse conditions such as heating, freezing, drying and radiation. *B. cereus* is ubiquitous in nature because it does not have complex nutrient requirements. The bacteria are widespread in the environment and soils. Thus, processed and non-processed food ingredients are frequently contaminated with *B. cereus*, such as vegetables, sauces, rice, cereals, fish and dairy products[3-5].

B. cereus group also called *B. cereus sensu lato*, comprises the following closely related species: *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. cytotoxicus*, *B. mycoides*, *B. pseudomycoides*, *B. toyonensis*, *B. weihenstephanensis* and *B. Weidmannii* based on the similarity in 16S rRNA sequence[6]. The *B. cereus* is responsible for causing emetic and diarrhoeal food poisoning in humans. It has also been implicated in wound infections, ocular infection, meningitis, urinary and respiratory tract infections. The pathogenicity of *B. cereus* is associated with the production of tissue-destructive toxins and virulence factors. These secreted toxins are enterotoxins especially nonhemolytic enterotoxin, hemolysins, cytotoxins, phospholipases and proteases[5-7].

Bacillus species were used extensively in aquaculture practice as probiotics. The most famous examples are *B. subtilis*, *B. Licheniformis* and *B. cereus*. They initiate immunostimulation, besides having adhesion abilities they produce enzymes and vitamins as well as secrete antimicrobial peptides. Thus the application of Bacillus in aquaculture can improve feed utilization, reduce diseases and improve water quality for sustainable aquaculture[8,9].

However, some studies reported that *Bacillus* spp. could cause diseases in aquatic animals. In catfish, *Bacillus mycoides* was isolated from dorsal ulcer and muscle necrosis of channel catfish *Ictaluruspunctatus* in Alabama[10]. The *B. cereus* was claimed to be the causative agent of stinging catfish *Heteropneustes fossilis* mass mortality in West Bengal, India[11]. Moreover, *B. cereus* was mentioned as a fish pathogen other than catfish such as, common carp *Cyprinuscarpio*[12], striped bass *Morone saxatilis*[13], farmed European sea bass *Dicentrarchus labrax* in Greece[14] and Egypt[15] and White Sea bream *Diplodus sargus* in Egypt[16]. Furthermore, *B. cereus* was isolated from dying Chinese soft shell turtle *Pelodiscus sinensis*[17].

The main objectives of this study were to identify the causative agent responsible for catfish skin ulceration in the farm, to determine the extracellular enzyme production and to evaluate their antibiotic susceptibility profile along with the pathogenic capacity and histopathological effects in diseased fish.

MATERIALS AND METHODS

Fish sampling: Live moribund catfish suffered from skin ulceration and hemorrhages were collected from a private fish farm at Kafr El-Sheikh Governorate in North Egypt, in the period between March-April, 2019. Fish sample was packed into a sterile bag. Sample transported in icebox to the hydrobiology laboratory of the National Research Centre following standard protocol, for isolation of the causative agent(s). The fish was subjected to clinical and postmortem examinations.

Isolation and identification of the causative agent: Infected skin lesions were disinfected with 70% ethyl alcohol to avoid contamination from bond water. Skin ulcer scratched using sterile swabs and inoculated on Tryptone Soya Agar (TSA) (Oxoid), then incubated at 28°C for 24 hrs. The strain was characterized by Gram's staining. Biochemical tests were performed using API20E according to the manufacturer's instructions (bioMérieux, France). Then, 20% glycerol stock culture was prepared and stored at -80°C.

For the molecular identification, DNA template was amplified in T100 gradient thermal cycler (Bio-Rad). A total volume of 100 µL PCR reaction mixture containing 50 µL 2×DreamTaq PCR master mix (Thermo Scientific), 20 µg of eubacterial universal primer 63f (5'-CAGGCCTAACACATGCAAGTC-3) and 1387r (5'-GGGCGGWGTGTACAAGGC-3) was used as previously described[18]. The PCR products were purified with the Purelink™ Quick Gel Extraction (Invitrogen, USA) and sent for sequencing in both directions. Nucleotide similarity to the closest homolog of the microbes was conducted using BLAST Searching Tool (NCBI). The nucleotide sequence was deposited to GenBank.

Detection of extracellular enzymes and hemolysis activity: *Bacillus* spp. produce a variety of extracellular enzymes. Lecithinase, lipase and proteolytic activity of *B. cereus* was conducted on egg-yolk modified medium according to the method described by McClung and Toabe[19]. A positive lecithinase test is noted by the appearance of a white, opaque, diffuse precipitate into the medium surrounding the colony growth. Lipase broke down free fats present in the egg yolks, causing an iridescent sheen (oil on water) on the surface of the colonies when subjected to the light source. A positive proteolysis test was noted by the development of clear zones in the medium surrounding colonial growth. Furthermore, hemolytic activity was tested on blood agar including 5% defibrinated sheep blood.

Antibacterial susceptibility: Antibiotic susceptibility of the isolate was assessed by the disc diffusion method using Müller-Hinton agar (Oxoid). Different antibiotics used in the test were chloramphenicol (30), novobiocin (10), oxytetracycline (30), ampicillin (10), norfloxacin (10), nalidixic acid (30), streptomycin (10), kanamycin (30), erythromycin (15) and cefotaxime (30) (Oxoid). The diameters of the inhibition zone were measured following 24 hrs of incubation at 28°C according to standard guidelines for result evaluation[20].

Experimental infection: To evaluate the pathogenic potential of isolated strain, 25 apparently healthy *C. Gariepinus* free from any visible skin lesions with an average body weight of 120±20 g were randomly assigned into 5 groups with 5 per group. The fish were acclimatized to 25°C for 2 weeks. Catfish were intraperitoneally injected with 0.1 mL serial 10-fold dilution containing 2.7×10^5, 10^6, 10^7 and 10^8 CFU mL^{-1}. A control fish group was injected with 0.1 mL of 0.85% saline. Fish were not fed throughout the experiment and alterations in the fish were recorded. Re-isolation of the causative pathogen was done on TSA plates.

The animal experiment was carried in compliance with the National Research Centre Animal Care Committee and performed the following regulations and guidelines for the care and use of animals in research.

Histopathological examination: After complete necropsy of the fish, fresh tissue specimens, skin lesions, hepatopancreas, posterior kidney, spleen, gills and brain were collected from different experimental groups, fixed in Davidson's fixative, dehydrated in ascending grades of alcohol, cleared with xylene, embedded in paraffin, sectioned at 5 µm thickness, stained by hematoxylin-eosin (H and E) cover-slipped then visualized by Light Microscope (Olympus BX43)[21].

RESULTS

Clinical signs of naturally infected fishes: Naturally infected *C. gariepinus* revealed abnormal behavior, skin ulceration, bulging eyes (Fig. 1a), vent protrusion, abdominal distention. Internal lesions include enlargement and congestion of kidney and liver with an accumulation of fluid in the body cavity.

Bacterial characterization and identification: Morphological features coupled with biochemical profile revealed that the pathogenic isolate was motile, long bacilli arranged in pairs or long chains, endospore-forming, Gram-positive bacterium belonging to the *Bacillus* family. Colonies were large, white, flat with irregular perimeters and 2-5 mm in diameter. Biochemical tests revealed that *B. cereus* was positive to oxidase, catalase, Voges Proskauer, hydrolyzed carbohydrates, reduced nitrate to nitrite and negative to indole.

The 16S rRNA was partially sequenced and the isolate was presumptively identified as *B. cereus* and submitted to the GenBank database under the accession number MT084573. The blasting of 16S rRNA sequencing result demonstrated that it shared 99.76% homology with the nearest homologous species *B. cereus* and *B. anthracis*.

Extracellular enzymes and hemolysis activity: The results for the production of extracellular enzymes by the *Bacillus* isolate indicated that *B. cereus* isolate was found to be positive for the production of protease, lipase. In addition, *B. cereus* isolate was positive for lecithinase activity, which causes cell lysis by disrupting the membrane. Furthermore, *B. cereus* was β-hemolytic on blood agar.

Antimicrobial sensitivity testing: Antibiotic susceptibility tests indicated that the isolate was sensitive to all the used antibiotics except cefotaxime as shown in Table 1.

Experimental infection: In the experimental infection, LD_{50} value of *B. cereus* isolate was determined as

Table 1: Antimicrobial sensitivity testing of the isolated strain of *Bacillus cereus*

Antibiotic (µg)	Activity
Chloramphenicol (30)	+
Novobiocin (10)	+
Oxytetracycline (30)	+
Ampicillin (10)	+
Norfloxacin (10)	+
Nalidixic acid (30)	+
Streptomycin (10)	+
Kanamycin (30)	+
Erythromycin (15)	+
Cefotaxime (30)	-

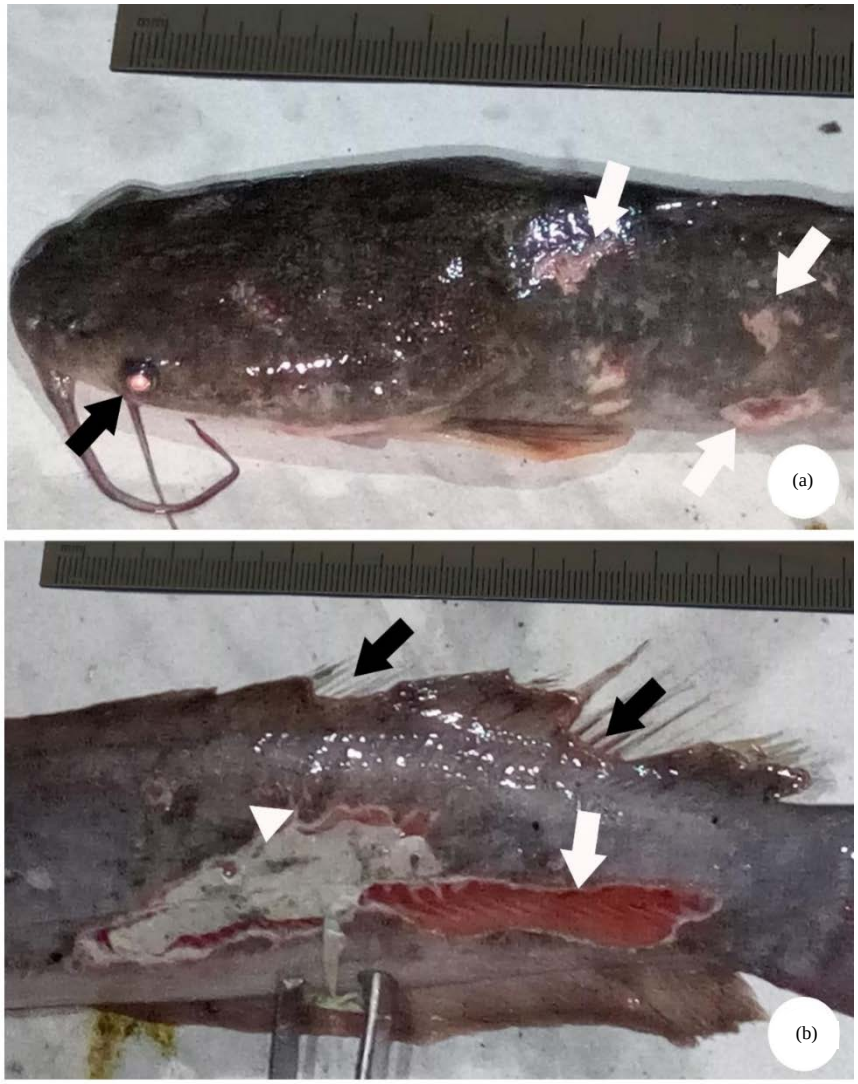

Fig. 1(a-b): Gross external lesions in *Clarias gariepinus*

(a) Naturally infected specimen and (b) Experimentally challenged specimen injected with *B. cereus* 2.7×10^8 CFU mL^{-1}, 1a-white arrows: Multiple small sized shallow skin ulcers, 1a-black arrow: Hemorrhagic exophthalmia, 1b-white arrows: Extensive deep ulceration along the fish trunk, 1b-white arrow head: Diphtheritic like membrane formation, 1b-black arrows: Distinctive multifocal fin erosions

2.7×10^6 CFU mL^{-1}. Experimentally infected fish showed external and internal clinical symptoms similar to those caused by natural infection in farm. Some signs and symptoms were observed such as skin depigmentation, hemorrhagic spots of skin, gills and fins, bulging eyes, swollen abdomens and abnormal behavior. The skin hemorrhagic spots welled gradually to form ulcerative lesions. The size of ulcer was dose-dependent. The small dose produced small ulcers, which were self-limited and completely healed within 2 weeks from infection. The ulcers of higher doses were observed to grow in size and underlying muscles become eroded (Fig. 1b). Finally, severely ulcerated fish died within first 3 days of infection. The internal lesions included ascites, oedema, hepatic congestion, enlarged spleen and posterior kidney and vent protrusion. To confirm the causative agent, *B. cereus* re-isolated from the skin lesions and other internal organs of experimentally infected fish.

Histopathological findings: Microscopic examination of tissue specimens revealed different lesions with various degrees of pathological harm in comparison to non-infected fish tissues (Fig. 2a-t). Skin lesions (ulcers) of all treated fish showed also various degrees of congestion, haemorrhages, oedema, mononuclear cell infiltrations and fibrous granulation

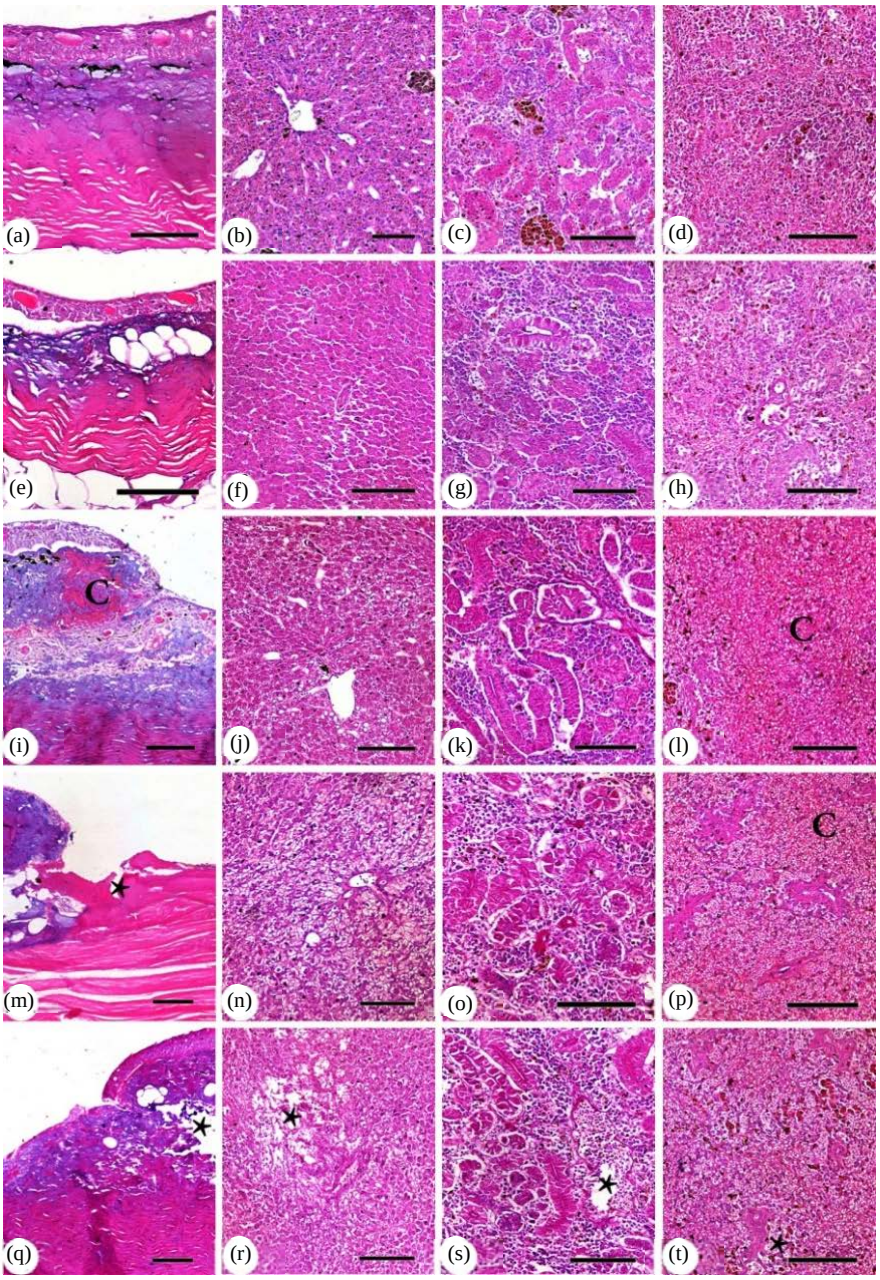

Fig. 2(a-t): Micrographs of various tissues of *Clarias gariepinus*

(a) Normal skin tissue, (b) Normal hepatic tissue with multifocal activation of MMCs, (c) Normal renal tissue with multifocal activation of MMCs, (d) Normal splenic tissue with some scattered pigmented macrophages, (e) Normal skin tissue with mild sub-epidermal oedema, (f) Normal hepatic tissue, (g) Renal tissue showing mild tubular vacuolar degeneration with mild mononuclear cell infiltration in interstitial tissue, (h) Splenic tissue with mild depletion of red pulp with appendance of scattered pigmented macrophages, (i) Skin tissue with severe congestion (C) and Hemorrhages in the sub-epidermal area with hyperplasia of fibrous connective tissue, (j) Hepatic tissue showing diffuse vacuolar degeneration, (k) Renal tissue showing oedema and mild tubular vacuolar degeneration with focal tubular necrosis, (l) Splenic tissue with severe degree of congestion (C), (m) Skin tissue with hyperplasia of fibrous connective tissue and necrosis of underlying muscular tissue (*), (n) Hepatic tissue showing diffuse vacuolar degeneration, (o) Renal tissue showing oedema and moderate diffuse tubular vacuolar degeneration with multifocal tubular and interstitial necrosis with mononuclear cell infiltration in interstitial tissue, (p) Splenic tissue showing congestion (C), Depletion of both red and white pulp and multifocal fibrosis, (q) Skin tissue with congestion and hyperplasia of fibrous connective tissue and necrosis of both fibrous connective tissue (*) and the underlying muscular tissue, (r) Hepatic tissue showing diffuse vacuolar degeneration and multifocal necrosis and fibrosis (*), (s) Renal tissue showing oedema and moderate diffuse tubular vacuolar degeneration with multifocal tubular and interstitial necrosis (*) with mononuclear cell infiltration in interstitial tissue, (t) Splenic tissue showing depletion of both red and white pulp (*) and multifocal fibrosis with some scattered pigmented macrophages, H and E. bar = 50 μm, (a-d) Control negative group, (e-h) group-2 (2.7×10^5 CFU mL^{-1}), (i-l) group-3 (2.7×10^6 CFU mL^{-1}), (m-p) group-4 (2.7×10^7 CFU mL^{-1}) and (q-t) group-5 (2.7×10^8 CFU mL^{-1})

Table 2: Histolopathological alterations and their respective degrees of damage to the tissue

		Detected lesions									
Groups	Organ	Circulatory disturbances	Degree	Degenerative changes	Degree	Proliferative changes	Degree	Necrosis	Degree	Infiltrations	Degree
(Control)	Skin	-ve		-ve		-ve		-ve		-ve	
	Heptopancreas	-ve		-ve		-ve		-ve		(M) MMCs	++
	Posterior kidney	-ve		-ve		-ve		-ve		(M) MMCs	++
	Spleen	-ve		-ve		-ve		-ve		(M) MMCs	++
	Gills	-ve		-ve		-ve		-ve		-ve	
	Brain	-ve		-ve		-ve		-ve		-ve	
2.7×10^{5} CFU mL^{-1}	Skin	C, O	+	(M) V	+	-ve		-ve		-ve	
	Heptopancreas	C	+	-ve		-ve		-ve		(M) MMCs	++
	Posterior kidney	C	+	(M) V	+	-ve		(F) Hyal.	+	(M) MMCs	++
										(F) M.Nuc.	+
	Spleen	C	+	(M) V	+	-ve		(M) Dep.H.T.	+	(M) MMCs	++
	Gills	-ve		-ve		(F) Hyperp.	+	-ve		-ve	
	Brain	-ve		-ve		-ve		-ve		-ve	
2.7×10^{6} CFU mL^{-1}	Skin	C, H, O	+++	(M) V	++	(F) C.T.	++	(M) Nec.	++	(F) M.Nuc.	++
	Heptopancreas	C	+	(M) V	++	-ve		(M) Nec.	++	(M) MMCs	+
	Posterior kidney	C, H, O	++	(M) V	++	-ve		(M) Tub.Nec.	++		
								(M) Dep.H.T.	++		
								(M) Tub.Cast.	+		
								(M) Dep.H.T.	++	(M) MMCs	++
	Spleen	C, H	+++	(M) V	++	-ve		(M) Dep.H.T.	++	(M) MMCs	++
	Gills	-ve		-ve		(D) Hyperp.	+	-ve		-ve	
	Brain	-ve		-ve		-ve		-ve		-ve	
2.7×10^{7} CFU mL^{-1}	Skin	C, H, O	++	(M) V	++	(F) C.T.	+++	(M) Nec.	+++	(F) M.Nuc.	++
	Heptopancreas	C, H	+	(D) V	+++	-ve		(D) Nec.	+++	(M) MMCs	+
	Posterior kidney	C, H, O	++	(M) V	++	-ve		(D) Tub.Nec.	+++	(M) MMCs	++
								(D) Dep.H.T.	++	(M) M.Nuc.	++
								(M) Tub.Cast.	++		
								(D) Dep.H.T.	+++	(M) MMCs	
	Spleen	C, H	++	(M) V	++	(D) C.T.	++	(M) Dep.H.T.	+++	(M) MMCs	++
	Gills	-ve		-ve		(D) Hyperp.	+	-ve		-ve	
	Brain	-ve		-ve		-ve		-ve		-ve	
2.7×10^{8} CFU mL^{-1}	Skin	C, H, O	++	(M) V	++	(F) C.T.	+++	(M) Nec.	+++	(F) M.Nuc.	++
	Heptopancreas	C, H	+	(D) V	+++	(D) C.T.	++	(M) Nec.	+++	(M) MMCs	+
	Posterior kidney	C, H, O	++	(M) V	++	(F) C.T.	++	(M) Tub.Nec.	+++	(M) MMCs	++
								(M) Dep.H.T.	++	(M) M.Nuc.	+++
								(M) Tub.Cast.	++		
								(M) Dep.H.T.	+++	(M) MMCs	++
	Spleen	C, H	+++	(M) V	++	(D) C.T.	++	(M) Dep.H.T.	+++	(M) MMCs	++
	Gills	C	+	-ve		(D) Hyperp.	++	(M)Spongiosis		-ve	
	Brain	C	+	-ve		-ve		-ve		-ve	

*Score value: -ve: None, +: Mild, ++: Moderate, +++: Severe, D: Diffuse, M: Multifocal, F: Focal, C: Congestion, H: Heamorrhages, O: Oedema, S: Separation in-between the epithelial cell lining of the secondary gill lamellae and the underlying capillary bed, V: Vacuolar degeneration, N: Necrosis, C.T: Connective tissue, MMCs: Activation of melanomacrophage centers, Tub.Nec.: Tubular necrosis, Dep.H.T.: Depletion of heamopoeitic tissue, Hyperp.: Hyperplasia of the epithelial lining at the base of the secondary gill lamellae, :Spongiosis: Necrotic changes in-between the proliferated malpighian cells, :Hyal.: Hyaline droplet deposition, :Tub.Cast.: Tubular casts, :M.Nuc.: Mononuclear cells infiltration

tissue at the periphery of the ulcerative tissue, with prominent necrosis inside the ulcer either in dermal tissue layers or in the underlying musculature, the degree of harm is progressive with increasing the injected bacterial doses (Fig. 2 a, e, l, m, q). Hepatopancreas of all treated fish showed various degrees of congestion in main blood vessels, hemorrhages, oedema with some vacuolar degeneration and necrosis in hepatocytes which are replaced with fibrous connective tissue in higher injected doses (Fig. 2b, f, j, n, r). The posterior kidney also revealed increasing tissue pathology with the gradual increase in the injected bacterial doses in all treated fish groups, the lesions varied between congestion, oedema and minor vacuolar degenerative changes to reach higher degrees of tubule-glomerular and interstitial necrosis infiltrated with mononuclear cells and haemorrhages (Fig. 2c, g, k, o, s). Spleen in lower doses showed signs of hypraemia and congestion while in higher doses it turned to show severe degrees of depletion of both white and red pulps replaced by fibrous tissue (Fig. 2d, h, l, p, t). Gills were not affected by lower injected doses, while in higher doses hyperplasia of malpighian cells lining the base of secondary gill lamellae supervenes. Finally, brain tissue was not affected in all treated groups, except that the highest injected dose causes moderate degree of congestion of meningeal blood vessels. The lesions scoring in different groups as well as the degrees of damage are summarized in Table 2.

DISCUSSION

The African catfish (*C. gariepinus*) (*Siluriformes*: *Clariidae*) has increasing economic importance because of its extensive use in polycultured with Nile tilapia in earthen ponds in Egypt. Its main role in this farming system is to control the unwanted fish reproduction in the grow-out ponds by devouring tilapia hatchlings. Earthen pond type culture system depends mainly on increasing the natural food availability by the addition of organic fertilizers such as livestock manure, with the increase in water pollution and high organic loads in fish ponds, fish skin and gills are usually attacked by waterborne bacteria[22,23]. The *B. cereus* is common in soil and different environment and has the capacity to spread easily[3]. In this study, the causative agent was identified to be Gram-positive *B. cereus* bacterium based on its characteristic and 16s rRNA sequence analysis.

Similar to these results, some studies reported that *Bacillus* sp. could cause diseases in catfish, *B. mycoides* was isolated from dorsal ulcer and necrotic musculature in an epizootic of disease in cultured channel catfish (*Ictaluruspunctatus*)[10]. The *B. cereus* was isolated during mass mortality of stinging catfish (*Heteropneustes fossilis*) in West Bengal, India. The affected fish showed numerous ulcers on the skin which gradually increase in size to form ulcerative dermatitis[11]. Moreover, *Bacillus* spp. is considered as pathogens of fish other than channel catfish. Pychynski *et al.*[12] reported that *B. cereus* and *B. subtilis* can cause gill arch necrosis, branchionecrosis, in common carp (*Cyprinus carpio*). *B. Cereus* retrieved from apparently healthy striped bass (*Morone saxatilis*) was lethal to other healthy individuals when injected at 10^7 CFU/fish[13]. Furthermore, *Bacillus* spp. are common bacterial fish pathogens affecting European sea bass *Dicentrarchus labrax* aquaculture in Greece[14]. Moreover, a highly lethal *B. cereus* pathogen was isolated from dying Chinese softshell turtle (*Pelodiscus sinensis*)[17].

In a previous study,[4] *Staphylococcus epidermidis*, [2]*B. cereus* and one *Pseudomonas stutzeri* isolates were identified as the causative agents responsible for mortalities in European sea bass (*Dicentrarchus labrax*) in Egypt[15]. Also, in another study[15] *S. epidermidis* and[11] *B. cereus* bacterial isolates were obtained from septicemic cases in a hatchery broodstock of white sea bream (*Diplodus sargus*) in Egypt[16].

In this study, the experimentally injected fish showed symptoms of lethargy, skin discoloration, superficial hemorrhagic ulcers, deep ulcers that reached the underlying muscle and tail erosions. Some fishes showed hemorrhages on the fins and vent, exophthalmia and congested internal organs. These clinical signs and postmortem findings were approximately similar to the previously recorded findings in *B. cereus* infections in white seabream[16] and stinging catfish[11]. The present study indicated that *B. cereus* isolate was sensitive to antibiotic used but cefotaxime. This finding was in line with previous reports that *B. cereus* is sensitive to the most antibiotics especially chloramphenicol, ciprofloxacin and tetracycline[11,16]. The results obtained in this study and others showed that *B. Cereus* infection in aquaculture could be effectively controlled by antibiotic addition.

Bacillus spp. produce a variety of extracellular enzymes such as protease, lipase, lecithinase, gelatinase, hemolysins, enterotoxins, cytotoxins, phospholipases responsible for food spoilage and their pathogenesis in human[5,6,24]. In this study, *B. cereus* isolate was found to be positive for the production of protease, lipase, lecithinase and hemolysin. Similarly, Ozdemir and Arslan[24] found that all *Bacillus* spp. isolated from retail fish and ground beef secreted protease, lipase, gelatinase and DNase. While 69.2% were positive for lecithinase activity.

CONCLUSION

This study confirmed that *B. cereus* was responsible for skin ulceration and disease in African catfish *C. gariepinus*. Fish experimentally re-infected confirmed that the isolate was considered the causative agent of the condition. The pathogen could be controlled using antibiotics. The production of extracellular enzymes participated in *B. cereus* pathogenicity and tissue damage.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the causative agent(s) of African catfish *C. gariepinus* ulcerative dermatitis in Egypt, with revealing of more detailed pathological changes observed later after experimental challenge using such pathogen(s).

REFERENCES

1. El Naggar, G., 2007. Efficiency of African catfish *Clarias gariepinus* in controlling unwanted reproduction of Nile tilapia *Oreochromis niloticus* in low input production system. Egypt. J. Aquatic Biol. Fish., 11: 105-113.
2. Younes, A.M., A.Y. Gaafar, A. El-Din Z. Abu-Bryka, L.A. Mohamed and El-Sayed M. Bayoumy, 2019. Genotyping and pathogenicity of Streptococcus iniae strains recovered from cultured Oreochromis niloticus at Kafr El-Shiekh Governorate, Egypt. Egyptian J. Aqu. Biol. Fishe., 23: 467-474.
3. Kotiranta, A., K. Lounatmaa and M. Haapasalo, 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes Infect. 2: 189-198.
4. Ceuppens, S., N. Boon and M. Uyttendaele, 2013. Diversity of *Bacillus cereus* group strains is reflected in their broad range of pathogenicity and diverse ecological lifestyles. FEMS Microbiol. Ecol., 84: 433-450.
5. Nguyen, A.T. and S.M. Tallent, 2019. Screening food for *Bacillus cereus* toxins using whole genome sequencing. Food Microbiol., 78: 164-170.
6. Webb, M.D., G.C. Barker, K.E. Goodburn and M.W. Peck, 2019. Risk presented to minimally processed chilled foods by psychrotrophic *Bacillus cereus*. Trends Food Sci. Technol., 93: 94-105.
7. Kovac, J., R.A. Miller, L.M. Carroll, D.J. Kent, J. Jian, S.M. Beno and M. Wiedmann, 2016. Production of hemolysin BL by *Bacillus cereus* group isolates of dairy origin is associated with whole-genome phylogenetic clade. BMC Genomics, Vol. 17. 10.1186/s12864-016-2883-z.
8. Kuebutornye, F.K.A., E.D. Abarike and Y. Lu, 2019. A review on the application of *Bacillus* as probiotics in aquaculture. Fish Shellfish Immunol., 87: 820-828.
9. Soto, J.O., 2017. *Bacillus* Probiotic Enzymes: External Auxiliary Apparatus to Avoid Digestive Deficiencies, Water Pollution, Diseases and Economic Problems in Marine Cultivated Animals. In: Advances in Food and Nutrition Research, da Cruz, A.G., E.S. Prudencio, E.A. Esmerino and M.C. da Silva (Eds.)., Elsevier, New York, pp: 15-35.
10. Goodwin, A.E., J.S. Roy, J.M. Grizzle and M.T. Goldsby, 1994. *Bacillus mycoides*: A bacterial pathogen of channel catfish. Dis. Aquat. Org., 18: 173-179.
11. Chandra, G., I. Bhattacharjee and S. Chatterjee, 2015. *Bacillus cereus* infection in stinging catfish, *Heteropneustes fossilis* (*Siluriformes*: *Heteropneustidae*) and their recovery by *Argemone mexicana* seed extract. Iran. J. Fish. Sci., 14: 741-753.
12. Pychynski, T., T. Malanowska and M. Kozlowski, 1981. Bacterial flora in branchionecrosis of carp (particularly *Bacillus cereus* and *Bacillus subtilis*). Med. Weterynaryjna, 37: 742-743.
13. Baya, A., T. Li, B. Lupiani and F.M. Hetrick, 1992. *Bacillus cereus*, a pathogen for striped bass. Eastern Fish Health and American Fisheries Society Fish Health Section Workshop, June 16-19, 1992, Auburn University, Auburn, Alabama. pp: 67-67.
14. Yiagnisis, M. and F. Athanassopoulou, 2011. Bacteria isolated from diseased wild and farmed marine fish in Greece. Recent Adv. Fish Farms, 27: 61-69.
15. Aboyadak, I.M., N.M. Sabry, N.G. Ali and H.S. El-Sayed, 2016. Isolation of *Staphylococcus epidermidis*, *Bacillus cereus* and *Pseudomonas stutzeri* from diseased European sea bass (*Dicentrarchus labrax*) for the first time in Egypt. Egypt. J. Aquatic Biol. Fish., 20: 103-114.
16. Ali, N.G.M., I.M. Aboyadak and H.S. El-Sayed, 2019. Chemotherapeutic control of gram-positive infection in white sea bream (*Diplodus sargus*, Linnaeus 1758) broodstock. Vet. World, 12: 316-324.
17. Yuan, X.M., S.J. Lyu, H.Q. Zhang, X.Y. Hang, W.D. Shi, L. Liu and Y.L. Wu, 2020. Complete genome sequence of novel isolate SYJ15 of *Bacillus cereus* group, a highly lethal pathogen isolated from Chinese soft shell turtle (*Pelodiscus sinensis*). Arch. Microbiol., 202: 85-92.
18. Younes, A.M., L.A. Mohamed, M.F. Eida and A.Y. Gaafar, 2015. Characterization and pathogen challenge of *Pseudomonas* species from *Oreochromis niloticus* in Egypt. Res. J. Pharm. Biol. Chem. Sci., 6: 312-317.
19. McClung, L.S. and R. Toabe, 1947. The egg yolk plate reaction for the presumptive diagnosis of clostridium sporogenes and certain species of the gangrene and botulinum groups. J. Bacteriol., 53: 139-147.

20. Gray, J.T. and T.R. Shryock, 2005. Antibiotic susceptibility testing of bacteria isolated from animals. Clin. Microbiol. Newslett., 27: 131-135.
21. Suvarna, K.S., C. Layton and J.D. Bancroft, 2018. Bancroft's Theory and Practice of Histological Techniques. 8th Edn., Elsevier Health Sciences United States Page: 672.
22. El-Gamal, A.M., M.S. El-Gohary and A.Y. Gaafar, 2018. Detection and molecular characterization of some bacteria causing skin ulceration in cultured nile tilapia (*Oreochromis niloticus*) in Kafr El-Sheikh governorate. Int. J. Zool. Res., 14: 14-20.
23. Elgendy, M.Y., W.S. Soliman, W.T. Abbas, T.B. Ibrahim, A.M. Younes and S.T. Omara, 2017. Investigation of some virulence determents in *Aeromonas hydrophila* strains obtained from different polluted aquatic environments. Jordan J. Biol. Sci., 10: 265-272.
24. Ozdemir, F. and S. Arslan, 2019. Molecular characterization and toxin profiles of *Bacillus* spp. isolated from retail fish and ground beef. J. Food Sci., 84: 548-556.