Summary

Motile aeromonas septicemia (MAS) one of the bacterial infection caused by *A. hydrophila* and its biotrophes. The disease reported to cause severe economic loss in aquaculture industry and affected many fish species not only freshwater but also marine ones. Antibiotics are the only way to overcome MAS outbreaks and as a result the antibiotic resistances of the causative pathogen (s) become problematic. Thus, researchers have been trying to develop vaccines to protect fish from the disease outbreaks; however, the problems of the bacterial heterogenicity arise to obstacle the vaccine development.

In the presented study, 240 diseased fish, which suspected to be infected with MAS were collected from khor Abo-Sleem, Abo-Saleh fish hatchery and private fish farms at the Beni-Seuf Governorate. Results revealed that the prevalence of the *A. hydrophila* infection were17.5, 10 & 13.75 % and 25, 12.5 & 20 % among cultured, wild *O. niloticus* and cultured *C. gariepinus,* in spring and summer seasons, respectively.

The most important external clinical signs of *A. hydrophila* infection were scale-loss, fin rot, skin erosions in head and trunk regions with diffusible and/or scattered hemorrhages on body surface. In addition, the postmortem examination of the diseased fish revealed generalized enlargement together with hemorrhages in internal organs including kidneys, liver, gonads and spleen. Some cases showed distended gall bladder and yellowish mucoid exudates tinged with blood within the body cavity.

For isolation of the causative agent (s), the study was designated to isolate Aeromonads. For the purpose, samples from kidneys, liver, spleen and skin lesions of the diseased fish were streaked on BHIA, TSA and Aeromonase selective

media. The isolation results revealed isolation of small, rounded, flat colonies that were suspected to be belonging to aeromonads. Depending upon the morphological, biochemical characteristics, 79 strains were primarily identified as *A. hydrophila*. The identification was confirmed on both genus and species levels by polymerase chain reaction (PCR) assays. Individual multipelex PCR (M-PCR) assays using primer sets for detection virulence genes of isolated strains were performed to select the most virulent ones. The results revealed 20 out of 79 were molecularly confirmed as highly virulent ones.

Depending upon pathogenicity values, *A. hydrophila* BNS 01614 was selected as highly virulent one. The pathogenicity of selected strain was carried by IP injection of catfish with bacterial suspension in PBS at concentrations of 3×10^8, 1.5×10^8, 1.5×10^7, 1.5×10^6 and 1.5×0^5 cfu/mL. The cumulative mortalities were 100, 60, 50, 40, and 30% within 5, 7, 9, 10 and 12 days post infection, respectively. In addition, the toxicity of the concentrated crude ECPs of the selected strain were investigated also in catfish and the LC_{50} was determined as 20µg.

The overall aim of the represented study is to prepare and select vaccine candidate for preventing *A. hydrophila* infection. For the purpose, 2 ECPs vaccine candidates were prepared and evaluated for their protective efficacies, humeral and cellular immune response parameters in *C. gariepinus.* The results revealed that both intra-peritoneal (IP) and oral immunization with the 2 ECPs vaccine candidates could produce RPS of over 80% after challenge with *A. hydrophila* and induce stronger immune responses. Vis-a-Vis, DNA vaccines (pcDNA-MAS) was constructed and evaluated at the same manner of ECPs vaccines. As a result, DNA vaccine candidates could offer RPS of over 75 % in the IP and intra gastric (IG) vaccinated fish.