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Bacteriological and Immunological Study of *Aeromonas hydrophila* in Al-Samawa City

A thesis

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Summary

Aeromonas hydrophila is one of the most important bacteria that cause a diarrhea disease in humans, especially children whom their ages ranged between one month and six years. The objective of this study was to investigate the real role of *A. hydrophila* as one of the main causes of diarrhea and the ability to provide protection against *A. hydrophila*. This study was carried out in two stages, to study some of the bacteriological criteria of *A. hydrophila* that isolated from patients children whom suffering from a diarrhea disease, and to investigate its immune response in the lab animals. A total of 294 stool samples from Al-Muthana public health laboratory between December 2012 and February 2013 were collected. The results showed that, there were 12 (4.08%) positive isolates of *A. hydrophila*. These positive isolates were identifying by five methods such as (Culture method, biochemical tests, Api20E system, vitek system and polymerase chain reaction). Then investigation was done on some virulence factors and all isolates were showed its ability to produce haemolysin, protease, lipase, phospholipase, capsule, motility and biofilm formation. As well as, the antibiotic susceptibility of these isolates were taking in the account and showed that all the isolates were multiple antibiotic resistance to the Amoxicillin, Ampicilin, Cephalothin and Cefotaxime. While, all isolates were sensitive to the Nalidixic acid, Tetracycline and Gentamicin. In term of immunity response preparation of some antigens of *A. hydrophila* which involve heat killed bacteria and isolation outer membrane proteins antigens. The SDS-PAGE method was used to analysis OMPs profile. The result revealed that, the OMPs molecular weight was about 28 and 44 kDa. After that 18 rabbits were used in this experiment and divided into groups. The first group was used to study the immune response. This group was contained nine adults Newzealand male rabbits. Those rabbits were divided into three groups, each group composed from three animals. Group one was immunized by OMP antigen and group two were immunized by heat killed bacteria antigen, while group three was immunized by normal saline and considered as a control group. Then, after the immunization period the cellular and humeral immunity were investigated. In terms of cellular immune function test results revealed that, OMPs and heat killed bacteria antigen were induce the immune response that investigated by phagocytic activity. Where the results showed a significant increase ($P < 0.05$) in phagocytic activity in heat killed bacteria antigen (9×10^8 cell/ml) comparison with OMP antigen (200 μ g/ml).

While the control group showed a significant decrease at ($P < 0.05$) when compared with both antigens. In addition, IL-2 and IL-8 production were also investigated. The results revealed that there were no significant differences in IL-2 production when the rabbits groups were immunized by heat killed and OMP antigens comparison with control group at P-value ($P < 0.05$). However, the IL-8 production was increased in heat killed and OMP antigens comparison with control group but it's no significantly increase at P-value ($P < 0.05$). Moreover, the skin test was investigated as well by using the heat killed bacteria. The results showed induce hypersensitivity after 4 hr. and the maximum of induration diameter at 48 hr. However, after 72 hr. induration diameter was decreased with appears necrosis. While, maximum of induration with OMP antigen was after 24 hr but it was also decreased at 72 hr. As for the control group did not showed any signs of reactions. According to humeral immune function test that where investigated by tube agglutination and passive haemagglutination tests were used to detect antibody titer. Results showed that there were a significant increase in antibody titer (systemic and mucosal) at ($P < 0.05$) in both treatments heat killed bacteria and OMP when compared with control group. However, the second group was used to detect the challenge dose and this group also consisted of nine adult's local male rabbits. In this treatment after immunization period rabbits injected by live bacteria to investigate the ability of heat killed bacteria and OMP antigen to provide protection against *A. hydrophila*. Results showed the control group was died after 24hr. from injection; but, the rabbits that were immunized by heat killed bacteria and OMP showed a depressed with losing appetite. However, those groups were reactivation after three days from injection. As results of that, heat killed bacteria and OMP antigen have potential work as a vaccine.

Keywords: *A. hydrophila* isolation, virulence factors detection, OMPs profile, Immune response and the challenge does.