Results
12.0 RESULTS

12.1 MORPHOLOGICAL AND BIOCHEMICAL CONFIRMATION OF
EDWARDSIELLA TARDA

*Edwardsiella tarda* was confirmed by morphological, biochemical confirmation tests and antimicrobial susceptibility test and the results were tabulated in Table 3 and 4 respectively. Morphological and biochemical characteristics of *Edwardsiella tarda* (ATCC 15947) were analyzed, confirmed and used for vaccine preparation.

12.1.1 GROWTH ANALYSIS

Temperature, pH and NaCl are important factors for the growth of bacterial strain. Growth was found at 25 °C and 35 °C in *Edwardsiella tarda* strain (Fig-23-A and B). Growth was not found in high temperatures (40 °C). *E. tarda* was able to grow at pH 4.0-10.0. *Edwardsiella tarda* can tolerate high pH like 10 but growth was not found at low pH. *Edwardsiella tarda* growth was found at 1.5% to 3% of NaCl. Growth was not occurring in high concentration of NaCl.

12.1.2 COLONY MORPHOLOGY

The *Edwardsiella tarda* strain was confirmed by small, circular, transparent and slightly raised colonies in Tryptic soy agar medium (Fig-24).

12.1.3 GRAM STAINING

The Gram staining (red colour) results have proved that the bacteria as gram negative bacterium. Thin rod shapes were found under the oil immersion microscope in 100x magnification (Fig-25).
12.1.4 MOTILITY TEST

Motility was determined at 48 hours in liquid medium cultures. The whole tube is turbid shows that the bacteria have moved far from the stab mark (are motile). Motility test confirmed that the bacterium was motile (Fig-26).

12.1.5 CATALASE TEST

Catalase test can be used for detecting the organism that can produce catalase enzyme. The bubbles resulting from production of oxygen gas clearly indicates that *Edwardsiella tarda* strain was catalase positive.

12.1.6 HYDROGEN SULPHIDE TEST

This test is commonly used to differentiate members of *Enterobacteriaceae*. A positive reaction is indicated by gas production and black colour forms in the medium (Fig-27). Positive results confirmed the presence of *Edwardsiella tarda*.

12.1.7 CYTOCHROME OXIDASE TEST

The enzyme cytochrome oxidase can be identified by using cytochrome oxidase test. It is normally used to recognize oxidase –ve *Enterobacteriaceae* and oxidase +ve *Pseudomonadaceae*. This test helps to identify aerobic Vs anaerobic metabolism. There is no color change observed in the medium which is indicated as negative result. Negative outcome shows that *Edwardsiella tarda* strain was distinguished as anaerobic bacterium (Fig-28).
12.1.8 CARBOHYDRATE FERMENTATION TEST

This is a test commonly used for gram negative enteric bacteria, all of which are glucose fermenters and non-fermenters of lactose. Red colour turns yellow indicates the presence of the organism with in few hours of incubation (Fig-29). In case of negative results the slant present in the tube will remain red (Fig-29). *Edwardsiella tarda* showed a positive result for glucose fermentation, where as it showed negative result for lactose fermentation.

12.1.9 SIMMON CITRATE TEST

The bacterial culture was streaked in to the Simmon’s citrate agar slant and it was incubated at 37°C for 24 hrs. After incubation period, the colour change was observed. *Edwardsiella tarda* strain was identified colourless in the medium and it showed negative result.

12.1.10 NITRATE TEST

This test is useful in identifying Gram-negative bacteria. The organism that has the capacity of reducing the nitrate to nitrite by means of the activity of the protein nitrate reductase and red color can be observed in the medium. The positive consequence of strain was recognized by developing red color in the medium (Fig-30).

12.1.11 VOGES PROSKAUER TEST

The Isolated culture was incubated into MR/VP medium for 24hrs. The medium will turn brownish yellow indicated that the strain is negative for Voges proskauer test (Fig-31).
12.1.12 INDOLE PRODUCTION TEST

This test can be used to identify the microbial strains that have the capacity to convert tryptohan into indole. A loop full of culture was taken from the slant and was inoculated into an indole broth and observed the color change. A positive reaction is indicated by a thin layer of red color ring in the medium (Fig-32). Positive result indicates that Edwardsiella tarda strain produces indole.

12.1.13 METHYL RED TEST

Methyl red indicator solution were added into the broth culture and observed for the colour change. Red colour indicates the presence of the Edwardsiella tarda strain (Fig-33).

12.1.14 ANTI MICROBIAL SUSCEPTIBILITY TEST

Antimicrobial Sensitivity means that the organism is inhibited by a clinically attained concentration of the antimicrobial; resistant means that the organisms were not inhibited if the antibiotic is to be used. Edwardsiella tarda (ATCC 15947) strains were found sensitive for tetracycline (Fig-34) and erythromycin (Fig-35) and resistant to erythromycin, novobiocin (Fig-36) and ampicilin (Fig-37). The result indicates the emergence of single and multiple antibiotic resistant strains in Edwardsiella tarda (Table-4).

12.2 MORPHOLOGICAL AND BIOCHEMICAL CONFIRMATION OF PSEUDOMONAS FLUORESCENS

Pseudomonas fluorescens was confirmed by morphological and biochemical tests and antimicrobial susceptibility test and the results were tabulated in Table -5 and 6 respectively. Morphological and biochemical characteristics of
Pseudomonas fluorescens (ATCC 13525) were analyzed, confirmed and used for vaccine preparation.

12.2.1 GROWTH ANALYSIS

There are different factors that can influence the growth such as temperature, NaCl and pH. In Pseudomonas fluorescens strain, growth was found at 25°C and 30°C (Fig-38 A and B) and no growth at high temperatures (40°C). Pseudomonas fluorescens was able to grow at pH 6.0-7.5. Bacterium can tolerate high pH like 7.5 but growth was not found at low pH. Pseudomonas fluorescens growth was found at 0% to 7% of NaCl. Growth was not occurring in high concentration of NaCl.

12.2.2 COLONY MORPHOLOGY

Pseudomonas fluorescens strain was confirmed by white cream coloured, smooth surface, transparent and glistening colonies with undulating edge. It produces a characteristic diffusible yellow-green pigment which fluoresces under UV light at 650 nm (Fig-39).

12.2.3 GRAM STAINING

The Gram staining (red colour) result showed that the bacteria (Pseudomonas fluorescens) as gram negative bacterium. Rod shape was observed under the microscope in 100x magnification (Fig -40).

12.2.4 MOTILITY TEST

Motility was determined at 48 hours in liquid medium cultures and identifies the ability of Pseudomonas fluorescens bacteria to move (ie, flagellated
cells) from the stab mark. Motility test results confirmed that the bacteria were motile (Fig-41).

### 12.2.5 CATALASE TEST

This test is utilized to detect the organism that has the ability to produce the enzyme, catalase. Catalase enzyme detoxifies hydrogen peroxide by breaking it up to water and oxygen. The bubbles coming about because of production of oxygen gas obviously show a catalase positive consequence of *Pseudomonas fluorescens* (Fig-42).

### 12.2.6 HYDROGEN SULPHIDE TEST

Triple Sugar Iron agar slant was inoculated by *Pseudomonas fluorescens* culture for 24 hours and observed the reaction. A positive reaction is indicated by a gas production in the medium (Fig-43).

### 12.2.7 CYTOCHROME OXIDASE TEST

The development of dark purple colour in the medium was considered as a positive result. This test will distinguish aerobic Vs anaerobic metabolism. Positive result indicates that *Pseudomonas fluorescens* strain was identified as aerobic bacterium (Fig-44).

### 12.2.8 SIMMON CITRATE TEST

The Simmon’s Citrate agar slant was inoculated with *Pseudomonas fluorescens* strain followed by incubating the agar slant at 37°C for 24hrs and color change was observed. The *Pseudomonas fluorescens* strain was identified by developing blue color in the medium and it showed positive result (Fig-45).
12.2.9 NITRATE TEST

This test is important in the identification of Gram-negative species. The negative result of *Pseudomonas fluorescens* strain was identified by unchanged colour in the medium indicating it as gram–negative strain.

12.2.10 VOGES PROSKAUER TEST

The isolated *Pseudomonas fluorescens* culture was incubated into MR/VP medium for 24hrs. The medium turned brownish yellow indicating that the *Pseudomonas fluorescens* strain is negative for Voges proskauer test (Fig-46).

12.2.11 ANTI MICROBIAL SUSCEPTIBILITY TEST

Antimicrobial sensitivity means that the organism is inhibited by a clinically attained concentration of the antimicrobial; resistant means that the organisms were not inhibited if the antibiotic is to be used. *P. fluorescens* (ATCC 13525) strain was found to be sensitive to neomycin (Fig-47) and kanamycin (Fig-48) and resistant to chloramphenicol (Fig-49), penicillin (Fig-50) and erythromycin (Table-6). These results indicate the emergence of single and multiple antibiotic resistant strains in *Pseudomonas fluorescens*.

13.0 QUALITATIVE PROTEIN ANALYSIS OF PREPARED VACCINES – SDS - PAGE METHOD

13.1 PROTEIN PROFILE OF WHOLE CELL VACCINES – (FORMAIN INACTIVATED) EDWARDSIELLA TARDA AND PSEUDOMONAS FLUORESCENS

The SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) profile of *Edwardsiella tarda* (ATCC 15947) WC vaccine had 8
polypeptide bands with the molecular weight of 220.14, 172.71, 104.00, 50.79, 38.98, 28.62, 22.96 and 18.01 KDa (Fig-51, Table -7) and Pseudomonas fluorescens (ATCC 13525) WC vaccine had 11 poly peptide bands with the molecular weight of 248.00, 188.92, 142.65, 99.53, 72.56, 53.84, 40.65, 22.98, 17.05, 11.79 and 9.89 KDa (Fig-56, Table -8).

13.2 PROTEIN PROFILE OF OUTER MEMBRANE PROTEIN VACCINES – (OMP VACCINES) EDWARDSIELLA TARDA AND PSEUDOMONAS FLUORESCENS

The SDS-PAGE profile of OMP vaccine had 7 poly peptide bands with the molecular weight of 210.64, 138.53, 89.12, 65.45, 42.57, 31.96 and 21.49 KDa (Fig-51, Table-7) and Pseudomonas fluorescens (ATCC 13525) OMP vaccine had 8 poly peptide bands with the molecular weight of 121.80, 76.49, 59.30, 31.52, 25.31, 18.45, 15.08, 3.70 KDa (Fig-56, Table-8).

13.3 BAND ANALYSIS OF SDS-PAGE OF EDWARDSIELLA TARDA AND PSEUDOMONAS FLUORESCENS

10% SDS - PAGE WC and OMP Protein profile of E. tarda and P. fluorescens was analysed by G image software analyser to determine the molecular weights (Fig - 51 and 56, Table -7 and 8).

14.0 QUANTITATIVE PROTEIN ANALYSIS OF PREPARED VACCINES LOWRY’S METHOD (EDWARDSIELLA TARDA + PSEUDOMONAS FLUORESCENS)

14.1 PROTEIN ESTIMATION OF WHOLE CELL VACCINES

The protein concentration of prepared WC and OMP vaccines were estimated by Lowry’s method. The total protein concentration of Edwardsiella tarda
WC vaccine was 58µg/ml (Fig-62, Table-9) and *Pseudomonas fluorescens* WC vaccine was 66µg/ml (Fig-63, Table-10).

### 14.2 PROTEIN ESTIMATION OF OUTER MEMBRANE PROTEIN VACCINES

The total protein concentration of *Edwardsiella tarda* OMP vaccine was estimated as 32µg/ml (Fig-62, Table-9) and *Pseudomonas fluorescens* OMP vaccine was estimated as 100µg/ml (Fig-63, Table-10).

### 15.0 PATHOLOGICAL SYMPTOMS

Edwardsiellosis and *Pseudomonas* septicemia were observed in the control groups after the bacterial infections. The rate of developing infection and mortality varied among the fingerlings. The experimental pathogenicity caused by *Edwardsiella tarda* and *Pseudomonas fluorescens* were different in nature. This might be due to the differences in the biochemical characteristics, virulence and host immunological system.

#### 15.1 EDWARDSIELLOSIS

The challenged fingerlings exhibited symptoms of Edwardsiellosis like spiraling movement, sign of lethargy, loss of escape and defence reflexes, died with the mouth agape and opercula flared, which may be due to the development of anemia leading to oxygen insufficiency. The fingerlings has pale appearance in body colour, lesions on the head (Fig-64) pale gills, body swelling, lesions and protruding eyes (Fig-65), haemorrhage throughout the body and gills (Fig-66), lesions on the head and gills (Fig-67), excessive mucus secretion, scale erosion and ulcers in a few cases. Swelling and bleeding of the body leading to reddening was
often noticed. The fingerlings continued to feed even when they were severely affected with diseases.

15.2 PSEUDOMONAS SEPTICEMIA

The fingerlings did not feed properly when they were severely affected with Pseudomonas septicemic diseases. The infected fingerlings exhibited lesions on the gills and eyes (Fig-68), sluggish movement; abnormal swimming behavior (Fig-69), gasping at the surface, Fin rot and lesions on the base of fins (Fig-70), dull coloration and congestion of dorsal fin and fin rot and tail rot (Fig-71) darkening of the skin (Fig-72), darkening of the skin and detached scales (Fig-73) and hemorrhagic oedema at the base of fin was observed. Opercula movement was feeble, lesions on the head, lesions on the body, abdomen swellings and lesions on the gills (Fig-74) were noticed.

16.0 RELATIVE PERCENT SURVIVAL

The efficacy of the vaccine that was used in this study was determined by using the relative percent survival (RPS) through the application of challenge test (Bath challenge route) with the virulent strain of Edwardsiella tarda, Pseudomonas fluorescens and mixed pathogens. The relative percent survival rate in each group was calculated by the following formula:-

\[
RPS = 1 - \frac{\% \text{ of mortality in vaccinated group}}{\% \text{ of mortality in unvaccinated group}} \times 100
\]

The Bacterial challenge of experimental groups resulted in the highest RPS rates in vaccinated groups when compared to control groups. The RPS value of
Control group was recorded as 0% in all the experimental groups. The Blank control groups had unvaccinated fishes without bacterial challenge.

The results of Edwardsiella tarda primary challenge shows that the RPS in Cirrhinus mrigala fingerlings vaccinated by OMP vaccine was higher than other types of vaccines. The RPS value of monovalent Edwardsiella tarda OMP vaccine was higher as 72% than the monovalent Edwardsiella tarda WC vaccinated group which was recorded as 60%. Moreover, fingerlings vaccinated with vaccine plus adjuvant increased the RPS rates than fingerlings vaccinated with vaccine alone. The RPS rate recorded for OMP vaccines with adjuvant was 80% and WC with adjuvant was 64%. The RPS rate was shown in Table-11 and Fig-75. There has been a significant increase in the RPS rate of fingerlings vaccinated with OMP +adjuvant than the other experimental groups.

The results of Pseudomonas fluorescens challenge study shows that the RPS value after 30dpv with monovalent OMP vaccines in Cirrhinus mrigala fingerlings was observed to be higher (68%) than monovalent Pseudomonas fluorescens WC vaccinated groups which was observed as 60%. The RPS rate after 30dpv, observed in OMP vaccines with adjuvant was 76% and in WC vaccine with adjuvant was 64%. The RPS rate of this study was presented in Table-12 and Fig-76. From the above result, it has been observed that OMP vaccines confer higher protection rate than WC vaccines in both adjuvanated and non adjuvanated groups.

The result of mixed bacterial challenge study shows that the RPS rate after 30dpv in OMP vaccines was higher (72%) than in WC vaccines (64%). The RPS rate was increased in vaccines with immunoadjuvanated groups when compared with vaccines administered without adjuvant. The RPS rate after 30dpv
was recorded as 80% in adjuvant groups vaccinated with OMP vaccines and in WC with adjuvant as 68%. The RPS values were shown in Table-13 and Fig-77. In all the above results, both monovalent *E. tarda* OMP vaccinated fishes and bivalent OMP vaccinated (*E. tarda*+*P.fluorescens*) groups showed similar RPS rate (72%) at 30 dpv. Above all, it has been recorded that OMP vaccines confer more protection when combined with adjuvants showing a significant increase of 80% RPS rate at 30 dpv in mixed bacterial challenge group. The above result signifies that OMP of *E. tarda* plays a major role in conferring protection against both pathogens with reference to the similar RPS results shown by monovalent OMP and bivalent OMP which was 72% in both cases.

Booster vaccination or 2nd dose of vaccination was applied after 30th day of the experimental period. After 60dpv, 2nd bacterial challenge study was applied in all the experimental groups. *Edwardsiella tarda* monovalent OMP vaccinated groups challenged with *Edwardsiella tarda* results showed that the RPS rate was 84% and in WC vaccines as 64%. The RPS rate of OMP vaccines with adjuvant was higher as 88% and WC with adjuvant was 76% respectively. The RPS values were shown in Table-14 and Fig-78.

The challenge studies after the booster vaccination of monovalent OMP vaccine of *Pseudomonas fluorescens* results revealed that the RPS rate was higher as 80% than the RPS values of WC vaccines which were lesser at the rate of 64%. The RPS rate of OMP vaccines with adjuvant showed slightly higher values (84%) with not much significant difference and WC with adjuvant was 72% which was significantly higher than non adjuvanated groups. The RPS rates were presented in Table-15 and Fig-79.
After 60 dpv, the RPS rate of (*E. tarda*) vaccinated group was higher (84%) than the OMP (*P. flourescens*) vaccinated group (80%). The RPS results of *E. tarda* OMP vaccinated group and *P. flourescens* adjuvanated groups were similar. Also the WC vaccinated groups of both *E. tarda* and *P. flourescens* showed similar RPS rate (64%) which was significantly lesser than the OMP vaccinated groups in both bacterial challenges. Similarly, after 60 dpv, the OMP vaccinated with adjuvant groups showed significantly higher value, 88% and 84% respectively for *E. tarda* and *P. flourescens*. But in the case of WC vaccines with adjuvants it showed a slighter change when compared with the monovalent WC vaccinated groups [showing similar results (64%)], here the RPS rate was 76% and 72% for both adjuvanated groups where not much difference was seen.

The result of mixed bacterial challenges shows that the RPS rate in bivalent vaccinated groups after 60dpv was recorded as 84 % in OMP vaccines which was higher than WC vaccines as 72%. The RPS rate was increased in vaccines with herbal adjuvanated groups when compared with vaccines without adjuvant. The RPS rate after 60dpv was recorded in groups administered with OMP vaccines and adjuvant as 92% and in WC with adjuvant as 76%. The RPS values were shown in Table-16 and Fig-80.

The results of monovalent *E. tarda* OMP vaccinated and bivalent OMP vaccinated groups after 60 dpv showed similar values (84%). There was a significant increase in the RPS rate of OMP + adjuvant against mixed bacterial challenge which was recorded as the highest (92%) showing higher efficacy of OMP vaccine when compared to WC vaccines.

The RPS values in vaccinated *Cirrhinus mrigala* fingerlings by OMP vaccine were higher than other types of vaccines in all experimental groups. The
Cirrhinus mrigala fingerlings vaccinated with vaccine plus adjuvant showed higher RPS value than the fishes vaccinated without adjuvant in all the experimental groups. Booster vaccination should enhance the RPS value than the fish vaccinated with 1st dose or prime dose. The Results indicated that vaccination with adjuvant could enhance the resistance of WC and OMP vaccines in Cirrhinus mrigala fingerlings. It might be due to the enhancement of the non-specific immune system of fish by vaccination. The result reveals that the WC and OMP vaccines enhanced the immune protection against Edwardsiella tarda (Edwardsiellosis) and Pseudomonas fluorescens infection (Pseudomonas Septicemia) in Cirrhinus mrigala fingerlings. Moreover, the results also showed that the Bivalent/mixed vaccine through the immersion route have had higher efficacy in comparision to other types of vaccines and the RPS values reached 72,76,84 and 92 for Bivalent WC, Bivalent adjuvanated WC, Bivalent OMP and Bivalent adjuvanated OMP respectively.

17.0 MORTALITY RATE

After 30 and 60 days of post vaccination (dpv), 25 fingerlings from each group were challenged with virulent Edwardsiella tarda; Pseudomonas fluorescens and mixed pathogens separately by bath challenge method and the mortality rate was recorded. The mortality was recorded up to 10 days post-challenge. All experimental groups showed that the cumulative mortality rates were decreased in vaccinated fingerlings when compared with control.

In blank group, 0% mortality was recorded and unvaccinated control group attained 100% mortality rate for single and mixed bacterial challenge in C. mrigala fingerlings in all experimental periods. The mortality rate of control group challenged with Edwardsiella tarda was recorded as 100% on 7th day and 100% on 10th day when challenged in the Pseudomonas fluorescens. Mortality rate
was observed to be 100% on 7th day in mixed bacterial challenge (*Edwardsiella tarda* + *Pseudomonas fluorescens*). The Mixed bacterial challenge caused death of fish started within 24 hours.

A challenge study after 30days of post monovalent OMP vaccinated *Edwardsiella tarda* group’s mortality rate was recorded to be lower as 28% than WC vaccines which was 40%. The adjuvant has helped to reduce the mortality rate in OMP vaccines+A as 20% and in WC vaccines+A as 36%. The mortality rate was presented in Table-11 and Fig-75.

After 30dpv, the mortality rate in *Pseudomonas fluorescens* monovalent OMP vaccinated group was recorded to be lower as 32% and the mortality rate was recorded as 40% in the *Pseudomonas fluorescens* monovalent WC vaccinated group. The vaccine plus immunoadjuvant group showed decreased mortality rate in the case of OMP vaccines with adjuvant as 24% and WC with adjuvant as 36%. The mortality rate was illustrated in Table-12 and Fig-76.

The Bivalent vaccinated group’s Mortality rate after 30dpv challenge with mixed pathogens was recorded. The Bivalent OMP vaccinated group after mixed bacterial challenge was 28% than Bivalent WC vaccinated group challenged with mixed pathogens which showed lesser mortality as 36%. The Mortality rate was recorded in OMP vaccines with adjuvant group challenged with mixed pathogen as 20% and WC with adjuvant group challenged with mixed pathogens was recorded as 32%. Moreover vaccine with adjuvant should decrease the mortality rate when compared with vaccines without adjuvant. The mortality rate was shown in Table-13 and Fig-77.
The Booster vaccination was efficient to reduce the cumulative mortality in *Edwardsiella tarda* OMP vaccines as 16% when compared with WC vaccines which was 36%. The Booster OMP vaccine plus adjuvant groups (60dpv) Challenged with *E. tarda*, the mortality rate was recorded as 12% and 24% in WC vaccines along with the adjuvant. The mortality rate was presented in Table-14 and Fig-78.

After 60dpv (Booster vaccination), the mortality rate of *P. fluorescens* monovalent OMP vaccines was recorded as 20% and WC vaccines was recorded as 36%. The mortality rates in OMP vaccines with adjuvant were observed as 16% and in the WC vaccine with adjuvant it was recorded as 28%. The results are illustrated in Table -15 and Fig-79.

In mixed bacterial challenge, experimental groups after 60dpv (Booster vaccination), the results showed that the mortality rate of OMP vaccines was observed as 16% and WC vaccines was observed as 28%. It was interesting to note that the results attained from OMP vaccine (*Pseudomonas fluorescens*) with adjuvant and WC vaccine (*P. fluorescens*) with adjuvant were similar with respect to the results of mixed bacterial challenge experimental groups. The mortality rates in both cases were 16% for OMP vaccines and 28% for WC vaccines. It was shown in Table-16 and Fig-80. The above results confirm that *Edwardsiella tarda* was more virulent than *Pseudomonas fluorescens*.

The mortality rate was low in all the vaccinated groups when compared with control groups. Moreover, the Mortality rate was lower in OMP vaccines when compared with WC vaccine in all experimental groups. The Booster vaccine helped to reduce the mortality rate when compared with prime dose. The vaccine plus adjuvant significantly reduced the mortality rate when compared with vaccine alone.
in 30 and 60dpv in all experimental groups. The rate of relative percent survival of bivalent vaccines was significantly higher than the monovalent vaccines in all experimental groups.

18.0 IMMUNOLOGICAL STUDIES

The Blood cells play an important role in cellular defense mechanisms. The blood parameter in fingerlings reflects its healthy state. Immunological parameters of fingerlings blood are useful tools for diagnosis of diseases. Moreover, Leukocytes are one of the factors that can influence the immunity of the fingerlings and leucocyte numbers or the variation in the proportion of different cell types has been used as indicators of health status in aquatic animals. Agglutinating antibody titre can be used to measure the amount of antibodies that are present in the sample collected from the fingerlings which are vaccinated with already prepared particulate antigen.

18.1 BLOOD LEUKOCYTE COUNT

The leukocytes or white blood cells are the defense cells of the organism and can be used to assess the immune system. In this study, 30 and 60 days post vaccinated fingerlings blood was analysed for the leukocyte count by using Neubauer's counting chamber of haemocytometer in oil immersion microscope. The microscopic observation results were given below.

The *Edwardsiella tarda* monovalent vaccination results showed that the leukocyte counts after 30dpv was increased in OMP vaccines than the WC vaccines. Immunoadjuvant enhanced the leucocyte count in OMP vaccines with adjuvanated group and WC vaccines with adjuvanated group respectively. In Control group,
leukocyte count was low when compared with blank group and other experimental groups. The microscopic observations are presented in Fig-81.

In the case of *Pseudomonas fluorescens*, monovalent vaccination results in *Cirrhinus mrigala* fingerlings revealed that the leukocyte counts after 30dpv was recorded as increased in OMP vaccines than WC vaccines. Adjuvant enhanced the leukocyte counts in OMP vaccines and WC vaccines. In the Control group, leukocyte count was very less when compared with blank group and other experimental groups. The microscopic observations are presented in Fig-82.

In the present investigation, it was observed that the leukocyte counts in bivalent/mixed vaccination results in *C. mrigala* fingerlings after 30dpv was found higher in OMP vaccines than WC vaccines. In adjuvanated vaccine group, the results revealed that OMP vaccines with adjuvant was higher than that of WC vaccines with adjuvant. In the Control group, leukocyte count was found to be low when compared to the blank and other experimental groups. The microscopic observations are presented in Fig-83.

From the present study, *Edwardsiella tarda* monovalent vaccination results in *Cirrhinus mrigala* fingerlings showed that the leukocyte counts after 60dpv (Booster vaccination) was increased in the OMP vaccines than that of WC vaccines. Vaccine plus adjuvant enhanced the leukocyte counts in *Cirrhinus mrigala* fingerlings, the leukocyte count was higher in OMP vaccines with adjuvant and WC vaccines with adjuvant when compared with vaccine without adjuvanated experimental groups. In the Control group, leukocyte count was found to be decreased when compared with blank group and other experimental groups. The microscopic observations are presented in Fig-84.
In the present study, *Pseudomonas fluorescens* monovalent vaccination in *Cirrhinus mrigala* fingerlings results showed that the leukocyte counts after 60dpv (Booster vaccination) was recorded as increased in OMP vaccines and WC vaccines. In adjuvant vaccinated group results revealed that OMP vaccines with adjuvant was higher than that of WC vaccines with adjuvant. In the Control group, leukocyte count was reduced when compared with blank group and other experimental groups. The microscopic observations are presented in Fig-85.

The Bivalent/mixed vaccination results in *Cirrhinus mrigala* fingerlings showed that the leukocyte counts after 60dpv (Booster vaccination) was recorded as increased in OMP vaccines and WC vaccines. In immunoadjuvant vaccinated group, results revealed that OMP vaccines with adjuvant was higher than that of WC vaccines with adjuvant. In the Control group, leukocyte count was reduced when compared with blank group and other experimental groups. The microscopic observations are presented in Fig-86.

From all the above results recorded from studies on leukocyte count with all the experimental groups, we could infer that the leukocyte counts showed a gradual increase in vaccinated groups (Monovalent and Bivalent) at 30 and 60 days post vaccination when compared to unvaccinated blank groups or control groups. Booster vaccination helped to increase the leukocyte counts when compared with primer dose. Bivalent/mixed vaccination results in *Cirrhinus mrigala* fingerlings showed that the leukocyte counts after 60dpv (Booster vaccination) was recorded as maximum in OMP vaccines and WC vaccines when compared with monovalent vaccines. The immunoadjuvant helps to improve the leukocyte counts when combined with OMP and WC vaccines in all experimental periods.
18.1.1 STATISTICAL ANALYSIS - BLOOD LEUCOCYTE COUNT

Statistical analysis was carried out in SPSS software by using one-way ANOVA analysis that was used to compare the leukocyte count of the experimental group 30 dpv as shown in the Table-17. Other Statistical test such as Duncan Multiple Range Test (DMRT) was also used for determining the significant difference among the experimental group (vaccinated group). Based on DMRT, variations were clearly seen in the mean values of Blood leukocyte count after 30dpv in various experimental groups. There was a gradual increase in leukocyte count in the case of Edwardsiella tarda experimental group. The mean value of Blank was found to be 183 when compared with the Control which showed a decrease in mean value up to 150. The mean values of leukocyte count in WC, WC+A, OMP and OMP+A of Edwardsiella tarda showed significant increase as 230, 267, 358 and 486 respectively (Table-17 and Fig-87).

The leukocyte studies with P.fluorescens experiment also revealed a gradual increase in mean values as follows: Blank (183), Control (153), WC (219), WC+A (263), OMP (341) and OMP+A (386) respectively. The leukocyte studies with Mixed bivalent vaccine result showed that the mean value of blank was 183, Control was 139, WC was 287, WC+A was321, OMP was 443 and OMP+A was 670.

When the mean values of leukocyte count of all experimental groups were compared, the results revealed that WC of E. tarda, P. fluorescens and mixed vaccine did not show much variation. In the case of WC+A experimental groups, bivalent vaccine showed significant increase when compared with the other two bacterial groups. The leukocyte studies with OMP groups also showed significant increase to the level of 443 with bivalent vaccine when compared with other two
groups. Lukocyte studies with OMP+A experimental group showed interesting results where in monovalent OMP+A of *E. tarda* was higher than OMP+A of *P. fluorescens* and the bivalent vaccine showed significant increase as to the level of 670 (Table-17 and Fig-87).

One way ANOVA was carried out to find the significant differences among the bacteria (*E. tarda, P. fluorescens*, and Mixed /Bivalent vaccine) of leukocyte count values of different experimental groups after 30 dpv (Table-18). Since (*P*<0.001), there is significant difference among bacteria of leukocyte count values in 30dpv of different types of vaccines. Based on DMRT Test bivalent (*Edwardsiella tarda* and *Pseudomonas fluorescens*) differed with monovalent *Edwardsiella tarda* and monovalent *Pseudomonas fluorescens* at 5% level in control. Moreover, all the other bacteria were different from each other in WC vaccines, WC vaccines with adjuvant, OMP vaccines and OMP vaccines with adjuvant.

One way ANOVA was carried out to find the significant differences among the experimental groups after 60 dpv as shown in Table-19. Since (*P*<0.001) there is significant differences among experimental groups of leukocyte count values in 60dpv of different bacterial vaccines. Based on DMRT, variations were clearly seen in the mean values of Blood leukocyte count after 60dpv in various experimental groups. There was a gradual increase in leukocyte count in the case of *Edwardsiella tarda* experimental group. The mean value of Blank was found to be 183 when compared with the Control which showed a decrease in mean value up to 150. The mean values of leukocyte count in WC, WC+A, OMP and OMP+A of *Edwardsiella tarda* showed significant increase, 280, 389, 430 and 620 respectively (Table-19 and Fig-88). OMP adjuvanated group showed higher leukocyte counts than the other experimental groups in *Edwardsiella tarda*. 
The leukocyte studies with *P. fluorescens* experiment also revealed a gradual increase in mean values as follows: Blank (182), Control (153), WC (230), WC+A (284), OMP (321) and OMP+A (427) respectively. The leukocyte studies with Mixed bivalent vaccine result showed that the mean value of blank was 182, Control was 138, WC was 318, WC+A was 384, OMP was 529 and OMP+A was 779. The results of OMP of *E. tarda* and OMP adjuvanated group of *P. fluorescens* were more or less similar, 430 and 427 respectively.

When the mean values of leukocyte count of all experimental groups were compared, the results revealed that WC of *E. tarda*, *P. fluorescens* and Mixed/Bivalent vaccine did not show much variation. In the case of WC+A experimental groups, bivalent vaccine showed significant increase when compared with the other two bacterial groups. The leukocyte studies with OMP groups also showed significant increase to the level of 529 with bivalent vaccine when compared with other two groups. Leukocyte studies with OMP+A experimental group showed interesting results where in monovalent OMP+A of *E. tarda* was higher than OMP+A of *P. fluorescens* and the Bivalent vaccine showed significant increase as to the level of 779 (Table-19 and Fig-88). The above results revealed that the bivalent vaccine was more efficient in eliciting the immune response when compared to other experimental groups.

One-way ANOVA analysis was carried out to find the differences among the bacteria (*E. tarda*, *P. fluorescens*, and Mixed/Bivalent vaccine) of leukocyte count of various experimental groups after 60 dpv (Table-20). Since (P<0.001), there is significant difference among bacterial leukocyte count values in 60dpv of various bacterial vaccines. Based on DMRT bivalent vaccine (*Edwardsiella tarda* and *Pseudomonas fluorescens*) was found to be significantly different when compared with monovalent *Edwardsiella tarda* and monovalent
*Pseudomonas fluorescens* at 5% level in control group. Also *P. fluorescens* showed variations with *Edwardsiella tarda* in the case of bivalent vaccines produced from WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant.

T-test showed significant differences between 30 and 60dpv of Blood leukocyte count values \((10^4 \text{ MM}^3)\) of the experimental groups. The WC vaccine prepared from *E. tarda* showed standared deviation of 10 in both 30 and 60dpv, WC with adjuvant showed a deviation from 10.79 to 12.77 and the P value showed significant results (<0.001). The OMP vaccine of *E. tarda* showed much difference in standared deviation 20.13 and 10.06 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen, which is revealed from the significant difference in values observed in standared deviation as 6 and 20 at 30 and 60dpv respectively (Table-21).

The WC vaccine prepared from *P. fluorescens* showed standared deviation of 7.93 and 7.02 in 30 and 60dpv, WC with adjuvant showed a deviation from 20.03 to 10.59 and the P value showed significant results(<0.001). The Bivalent OMP vaccine showed much difference in standared deviation 47.51 and 13.61 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen and the significantly different values observed in standared deviation was 8.32 and 24.7 at 30 and 60dpv respectively (Table-21).

The WC vaccine prepared from Bivalent vaccine (*E. tarda* and *P. fluorescens*) showed standared deviation of 6.11 and 5.29 in 30 and 60dpv, WC with adjuvant showed a deviation from 3.05 and 8 and the P value showed significant results (<0.001). The OMP vaccine of *P. fluorescens* showed much
difference in standard deviation 4.16 and 32.02 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen and the significantly different values, it has been observed that standard deviation was 8.7 and 28.3 at 30 and 60dpv respectively (Table-21). The WC of *P. fluorescens*, WC of mixed vaccine and OMP+A of mixed vaccine did not show significant result while other groups denoted significance at 1% level.

T-test was used to assess the differences between 30 and 60dpv of leukocyte count of experimental groups of different bacteria (*E. tarda*, *P. fluorescens*, and mixed vaccine) as shown in Table-21. Since (P<0.001), there is significant differences observed between 30 and 60dpv in control, WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of *Edwardsiella tarda* vaccine. WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of *Pseudomonas fluorescens* and WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of mixed bacterial pathogens. The final result revealed that booster vaccinated group (60dpv) had higher leukocyte counts than primer dose (30dpv). Since P <0.05, there is significant difference between 30dpv and 60dpv in control group. There is no significant difference between 30dpv and 60dpv in blank group, since P>0.05.

### 18.2 SERUM ANTIBODY TITRE (AGGLUTINATION TEST)

The assessment of agglutinating antibody titre is an easy approach to measure circulating antibodies in serum samples collected from fish previously immunized with particulate antigen preparations. The agglutination is a clumping reaction between specific antibodies and a particulate antigen. So the present study was aimed to standardize and apply the agglutination assay to measure the serum
agglutinating antibody titre production after immunization with different vaccines prepared from OMP and WC against *E. tarda* and *P. fluorescens* followed by the administration of the immunomodulator, *Asparagus racemosus*.

The immune response or antibody titre values were measured using agglutination test after 30 and 60 days of post vaccination. Microscopic observation of the bacterial isolate and the diluted serum collected from the vaccinated fishes showed clumps of antigen-antibody molecules confirming bacterial agglutination. In the center of the well, if a button like structure is present it indicates the value of agglutination and the value of the serum are considered as the reciprocal of the last dilution showing an agglutination that is visible. The microscopic observation results were given below.

*Edwardsiella tarda* monovalent vaccination results showed increased values of antibody titre after 30dpv in OMP vaccines (antibody titre values) than the WC vaccines (antibody titre values). Immunoadjuvant should enhanced the antibody titre values in OMP vaccines with adjuvanted group and WC vaccines with adjuvanted group respectively. In Control group antibody titre values was lower when compared with other experimental groups. In blank group no visible agglutination was observed.

In the case of *Pseudomonas fluorescens*, monovalent vaccination in *Cirrhinus mrigala* fingerlings revealed that the antibody titre values after 30dpv showed increased levels of protection with OMP vaccines(antibody titre values) than WC vaccines(antibody titre values). Adjuvant enhanced the antibody titre values in OMP vaccines with adjuvant and WC vaccines with adjuvant respectively. In the blank group and control group no antibody titre value was observed.
In bivalent/mixed vaccination in *Cirrhinus mrigala* fingerlings the results observed after 30dpv showed an antibody titre values which was higher in OMP vaccines than antibody titre values in WC vaccines. In adjuvant plus vaccine group, results revealed that OMP vaccines with adjuvant showed higher antibody titres values than antibody titre values of WC vaccines with adjuvant. In the Control group antibody titre values was low when compared with other experimental groups.

*Edwardsiella tarda* monovalent vaccination in *Cirrhinus mrigala* fingerlings showed that the antibody titre values after 60dpv (Booster vaccination) was increased in the OMP vaccines (antibody titre values) than that of WC vaccines (antibody titre values). Vaccine plus adjuvant enhanced the antibody titre values in *Cirrhinus mrigala* fingerlings. The antibody titre values were higher in OMP vaccine with adjuvant and WC vaccine with adjuvant when compared with vaccine without adjuvant experimental groups. In the Control group low level of antibody titre values were observed when compared with other experimental groups.

In case of *Pseudomonas fluorescens* monovalent vaccination in *C. mrigala* fingerlings results showed that the antibody titre values after 60dpv (Booster vaccination) was recorded as increased in OMP vaccines (antibody titre values) than that of WC vaccines (antibody titre values). In adjuvanated vaccine group results revealed that antibody titre values in OMP vaccines with adjuvant was higher than that of WC vaccines with adjuvant. In the blank group and control group no antibody titre values was observed in *Pseudomonas fluorescens* monovalent vaccinated groups.

Furthermore, the antibody titre values were higher in fish vaccinated with Bivalent/mixed OMP vaccines (Booster vaccination) than in fish vaccinated with WC vaccines. The immunoadjuvant plus vaccine group results revealed that
antibody titre values in Bivalent/mixed OMP vaccines with adjuvant was higher than that of Bivalent/mixed WC vaccines with adjuvant. In Control group antibody titre values were observed as minimum level when compared with other experimental groups.

The antibody titre values of vaccinated groups (Monovalent and Bivalent) showed enhanced titre values than unvaccinated groups in 30 and 60 days of post vaccination and this indicate that the antibody titres values were directly proportional to the RPS values (level of protection). The immunoadjuvant helped to enhance the immunological activities along with the vaccines.

From the above results, it can be inferred that the immunization with Edwardsiella tarda and Pseudomonas fluorescens along with administration of Asparagus racemosus has enhanced the agglutination antibody titre against both bacterial pathogens in all experimental groups. But the control group displayed Zero titre since there was no agglutination reaction observed.

18.2.1 STATISTICAL ANALYSIS - SERUM ANTIBODY TITRE

(AGGLUTINATION TEST)

One way ANOVA were carried out to find the significant differences among the antibody titre values of experimental groups after 30 dpv (Table-22). Moreover, Duncan Multiple Range Test (DMRT) was used for determining the significant differences among vaccinated groups. Based on DMRT, variations were clearly seen in the mean values of antibody titre values after 30dpv in various experimental groups. There was a gradual increase antibody titre values in the case of Edwardsiella tarda experimental group. The mean value of Control was found to be 1 when compared with the Blank which showed a decrease in mean value and it was 0. The mean values of antibody titre values in WC, WC+A, OMP and OMP+A
of \textit{Edwardsiella tarda} showed significant increase 2, 3, 4.6 and 6 respectively (Table-22 and Fig-89).

The antibody titre values with \textit{P. fluorescens} experiment also revealed a gradual increase in mean antibody titre values as follows: Blank (0), Control (0), WC (1.3), WC+A (2.3), OMP (3.3) and OMP+A (5) respectively. The antibody titre values with Mixed bivalent vaccine result showed that the mean antibody titre values of blank was 0, Control was 1, WC was 4, WC+A was 6, OMP was 7 and OMP+A was 8.

When the mean values of antibody titre values of all experimental groups were compared, the results revealed that WC of \textit{E. tarda}, \textit{P. fluorescens} and mixed vaccine did not show much variation. In the case of WC+A experimental groups, bivalent vaccine showed significant increase when compared with the other two bacterial groups. The antibody titre values with OMP groups also showed significant increase to the level of 7 with bivalent vaccine when compared with other two groups. Antibody titre values with OMP+A experimental group showed interesting results wherein OMP+A of \textit{P. fluorescens} was 5, OMP+A of \textit{E. tarda} was 6 and OMP+A of bivalent vaccine was 8. The antibody titre value of WC+A of \textit{E. tarda} was similar to WC+A of bivalent vaccines which means that the immunogenic property of \textit{E. tarda} vaccine in the bivalent vaccine dominated with respect to the WC+A of \textit{P. fluorescens}. This result could be confirmed with the result presented in monovalent WC+A values which was only 1.3. Monovalent OMP+A of \textit{E. tarda} was higher than OMP+A of \textit{P. fluorescens} and the bivalent vaccine showed significant increase as to the level of 8 (Table-22 and Fig-89).

One way ANOVA was used to analyze the significant differences among the bacteria (\textit{Edwardsiella tarda}, \textit{Pseudomonas fluorescens} and mixed/bivalent
Vaccine) of antibody titre values of various experimental groups after 30 dpv (Table-23). Since (P<0.001), there is significant difference among bacteria antibody titre values in 30dpv of various bacterial vaccines. Based on DMRT bivalent (Edwardsiella tarda and Pseudomonas fluorescens) was found to be significantly different when compared with monovalent Edwardsiella tarda and monovalent Pseudomonas fluorescens at 5% control level. Moreover, all the other bacteria were different from each other in WC vaccines, WC vaccines with adjuvant, OMP vaccines and OMP vaccines with adjuvant.

One way ANOVA was used to find the significant differences among the experimental groups after 60 dpv (Table -24). Since (P<0.001) there is significant differences among experimental groups of antibody titre values in 60dpv of various bacterial vaccines. Based on DMRT, variations were clearly seen in the mean values of antibody titre values after 60dpv in various experimental groups. There was a gradual increase in antibody titre values in the case of Edwardsiella tarda experimental group. The mean value of Control was found to be 1 when compared with the Blank which showed a decrease in mean value to be 0. The mean values of antibody titre values in WC, WC+A, OMP and OMP+A of E. tarda showed significant increase 3.66, 4.66, 5.33 and 7 respectively (Table-24 and Fig-90).

The antibody titre values with P. fluorescens experiment also revealed a gradual increase in mean values as follows: Blank (0), Control (0), WC (3), WC+A (4), OMP (4.66) and OMP+A (5.66) respectively. The antibody titre values with bivalent vaccine result showed that the mean value of blank was 0, Control was 1.33, WC was 5.66, WC+A was 7.33, OMP was 8.66 and OMP+A was 10. From the above results obtained from ANOVA analysis it was confirmed that similar antibody titre values obtained in most of the experimental groups. The result of adjuvanated monovalent WC vaccine group of E. tarda was similar to monovalent OMP vaccine.
of *P. fluorescens* which was 4.66 in both cases. Similarly, the results of adjuvanated monovalent OMP vaccine of *P. fluorescens* were similar to WC bivalent vaccine group which was 5.66 in both groups.

When the mean values of antibody titre values of all experimental groups were compared, the results revealed that WC of *E. tarda, P. fluorescens* and Mixed/Bivalent vaccine did not show much variation. In the case of WC+A experimental groups, bivalent vaccine showed significant increase when compared with the other two bacterial groups. The antibody titre values with OMP groups also showed significant increase to the level of 8.66 with bivalent vaccine when compared with other two groups. Antibody titre values with OMP+A experimental group showed interesting results where in monovalent OMP+A of *E. tarda* was higher than OMP+A of *P. fluorescens* and the bivalent vaccine showed significant increase as to the level of 10 (Table-24 and Fig-90).

One way ANOVA was used to find if there are any significant differences among the bacteria (*E. tarda, P. fluorescens* and Mixed/Bivalent Vaccine) of antibody titre values of various experimental groups after 60 dpv (Table-25). Since (*P*<0.001), Significant difference was observed in 60dpv of different vaccine groups. Based on DMR Test *Edwardsiella tarda* was differing with monovalent *Pseudomonas fluorescens* and bivalent vaccine in WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant. Also, *P. fluorescens* vaccine group was differing with *Edwardsiella tarda* and bivalent vaccines. Bivalent vaccine (*Edwardsiella tarda* and *Pseudomonas fluorescens*) was differing with monovalent *Edwardsiella tarda* and monovalent *Pseudomonas fluorescens* at 5% level in control group.
T-test showed significant differences between 30 and 60dpv of serum antibody titre values of the experimental groups. The WC vaccine prepared from *E. tarda* showed standard deviation of 0 and 0.57 in 30 and 60dpv, WC with adjuvant showed a deviation from 0 to 0.57 and the P value showed significant results(<0.001). The OMP vaccine of *E.tarda* showed difference in standard deviation 0.57 and 0.57 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen. From the significant different values observed in standard deviation was 0 and 0 at 30 and 60dpv respectively (Table-26).

The WC vaccine prepared from *P. fluorescens* showed standard deviation of 0.57 and 0 in 30 and 60dpv, WC with adjuvant showed a deviation from 0.57 and 0 and the P value showed significant results(<0.001). The OMP vaccine of *P. fluorescens* showed similarly in standard deviation of 0.57 and 0.57 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen. From the significant different values it has been observed that standard deviation was 0 and 0.57 at 30 and 60dpv respectively (Table-26).

The WC vaccine prepared from Bivalent vaccine (*E.tarda* and *P. fluorescens*) showed standard deviation of 0 and 0.57 in 30 and 60dpv, WC with adjuvant showed a deviation from 0 to 0.57 and the P value showed significant results(<0.001). The bivalent OMP vaccine showed much difference in standard deviation 0 and 0.57 at 30 and 60dpv respectively and the P value was <0.001. The OMP with adjuvant also showed marked increase in protection from the pathogen. From the significantly different values standard deviation was observed as 0 and 0 at 30 and 60dpv respectively (Table-26).
T test was carried out to find the significant differences between 30 and 60dpv of antibody titre of experimental groups of various bacteria (E. tarda, P. fluorescens and Mixed Vaccine; shown in Table-26). Since (P<0.001), there was a significant differences in antibody titre values between 30 and 60dpv in control, WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of Edwardsiella tarda. Control, WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of Pseudomonas fluorescens and Control, WC vaccine, WC vaccine with adjuvant, OMP vaccine and OMP vaccine with adjuvant of mixed bacteria. The final result was booster vaccinated group (60dpv) which showed higher antibody titre values than primer dose (30dpv). Since P <0.05, there is significant difference between 30dpv and 60dpv in control group. There is no significant difference between 30dpv and 60dpv in blank group, since P>0.05.

The immunization with Edwardsiella tarda and Pseudomonas fluorescens in this study has been effective in inducing a benefic response such as increase in leukocyte count and increase in antibody titre, since this may protect fish against bacterial disease outbreak. The immunogenic compound used in the vaccine is not pathogenic, but may have been derived from pathogenic microorganisms.

In aquaculture, immunization can be an alternative to antibiotics administration and can be a reliable method to confer protection against bacterial diseases prompted by the pathogens E. tarda and P. fluorescens in addition to the fact that led to the indiscriminate use of antibiotics which has increased resistance of bacteria to antibiotics worldwide.