ISSN: 2347-5129
IJFAS 2015; 2(3): 114-117

www.fisheriesjournal.com
Received: 28-10-2014
Accepted: 29-11-2014

Md. Ibrahim Kholil
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Md. Mer Mosharraf Hossain
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Md. Sharif Neowajh
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Md. Sherajul Islam
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Mitheela Kabir
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Correspondence:
Md. Mer Mosharraf Hossain
Department of Fisheries and Marine Bioscience, Jessore University of Science and Technology Jessore- 7408, Bangladesh.

Comparative efficiency of some commercial antibiotics against *Pseudomonas* infection in fish

Md. Ibrahim Kholil, Md. Mer Mosharraf Hossain, Md. Sharif Neowajh, Md. Sherajul Islam, Mitheela Kabir

Abstract
Pseudomonas sp is a gram-negative, rod shape bacterium belonging to the family Pseudomonadaceae. The species is highly adaptable opportunistic pathogen, capable of surviving in a variety of environment, including aquaculture environment. Antibiotics are used in the aquaculture environment, and their improper usage poses a risk of potential transfer of resistance from aquaculture bacteria to human and animal pathogens. This study was conducted to isolate *Pseudomonas* sp from infected fish and their antibiotic susceptibility pattern also investigated by using different antibiotics. All of the isolates tested were sensitive to applied antibiotics at different doses. In bactericidal activity, the oxytetracycline showed lowest number of colonies (1×10^{-4} c.f.u./ml), tetracycline showed moderate number of colonies (2.5×10^{-4} c.f.u./ml) and ciprofloxacin showed highest number of colonies (4.5×10^{-4} c.f.u./ml). In antibiotic susceptibility testing the zone of inhibition was found for oxytetracycline was 24mm, tetracycline was 21 mm and ciprofloxacin was 18 mm. In agglutination titer, oxytetracycline showed better result than tetracycline and ciprofloxacin any kind of different doses. The highest Relative percent survival **(**RPS) value found for Oxytetracyline was 72.2%, RPS value for tetracycline was 50% and lowest RPS value found for ciprofloxacin was 33.33%. Based on the results of this study, it can be said that oxytetracycline can be more effective antibiotics than tetracycline & ciprofloxacin for the treatment of *Pseudomonas* infection in fish.

Keywords: *Pseudomonas,* efficiency, antibiotics, oxytetracycline.

1. Introduction
Pseudomonas infection has been incriminated as the most common bacterial infection among fish and appear to be stress related disease of freshwater fish especially under culture conditions. The green fluorescent species, *P. fluorescens* and *P. putida* are the genus of fresh and brackish water fish. *P. fluorescens* participated in causing freshwater Eel disease), Red spot disease of pike and Red spot disease of Cyprinids, Percids and Coracoids. *Pseudomonas fluorescens* affects freshwater and salt-water fish throughout the world and causes severe economic losses and decrease fish farms efficiencies. (Khalil *et al.,* 2010). Objectives of the present study were-The objective of this study is to study and identify *Pseudomonas* from infected fish and to evaluate the efficiency of some commercial antibiotics against *Pseudomonas* infection in fish.

2. Materials and method
2.1. Bacteriological examination
The infected fishes were taken out from the fish farm in chancra, Jessore. Then sterilized inoculating loop was rubbed on the infected region (blood, skin, liver, spleen and kidneys) of fishes. Inoculating loop was then streaked on nutrient agar and BHI agar mixed plate and incubated at 37 °C for 24 hours. After incubation, the isolated colony was enriched in nutrient broth at 37 °C for 24 hours. The etiological agent for the lesions were identified by microscopic examination, oxidize test and physiological observation (Amlacher *et al.,* 1970).

2.2. Preparation of antibiotics
Antibiotics were taken from pharmaceutical store for veterinary use. Then stored in the cool and dry place. In the experiment these antibiotics (oxytetracycline, tetracycline & ciprofloxacin) were crushed for obtaining powder form and mixed with PBS solution by

magnetic stirrer for 15 minutes. To ensure actual dose different weight were taken by electric balance.

2.3. Bactericidal activity
Antibiotic susceptibility tests were carried out against the isolated bacterial strains with three antibiotics by disc diffusion method (Cuppuccino and Sherman *et al.,* 1998). Antibiotics disc of Oxytetracycline (30 µg), tetracycline (30 µg) and ciprofloxacin (30 µg) were used for the study (Diayanithi *et al*., 2012). NA and BHI agar were poured into the plates and allowed to solidify. The standard inoculum (10^6 cfu/ml) was swabbed over the surface of the media using sterile cotton swab to ensure the confluent growth. Finally the inoculated plates were incubated at 37 ^{0}C for 24 hours and. After days the bacteria were counted.

2.4. Antimicrobial susceptibility test
The antibiotic susceptibility test was performed by the isolates of *Pseudomonas* sp from the infected region of fish using the disc diffusion method (Cuppuccino and Sherman *et al.,* 1998), the following antibiotic test discs (tetracycline 30 µg, oxytetracycline 30 µg, ciprofloxacin 30 µg) were used in the present study. The antibiotics discs were placed on Mueller Hinton agar plates previously seeded with 18 h broth culture of *Pseudomonas* sp. The plates were incubated at 37 ^{0}C for 24 h, after which zones of inhibition were measured interpreted accordingly.

2.5. Bacterial agglutination test
In sterile acrylic micro plates with 96 wells, 30 µL of PBS were distributed in the wells using a multichannel pipette. Subsequently, 30 µL of antibiotics was placed in the first column, and from this solution the antibiotics was two-fold serial diluted in PBS buffer of the following well until the penultimate well since the last one was the negative control, containing only 30 µL PBS buffer to maintain 30 µL final volume per well and the following serum dilutions: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048. Then the bacterial strain 30 µl (*Pseudomonas* sp) were given of 96 wells. Lastly, responses on antibody production are dependent on the dose and time of administration. (Yildirim *et al.,* 2003).

2.6. Challenge test
Eighty fish were brought from fish pond and stored in four aquariums. Each of the aquarium was contained 20 fish. Then the fishes were fed with oxytetracycline, tetracycline and ciprofloxacin antibiotics enriched diet at 40 µg/mg. No antibiotic were given in the aquarium which are kept in control group. The susceptibility of the fish fed with different antibiotics and challenge with *Pseudomonas sp.* was examined for 31 days. The cumulative mortality and Relative percent survival (RPS) was calculated by the following formula: (Amend *et al.,* 1981).

$$RPS = 1 - \left(\frac{percent\ mortality\ in\ treated\ group}{percent\ mortality\ in\ control\ group}\right) \times 100$$

2.7. Statistical analysis
To evaluate the significance of the differences in immune response and protection between vaccinated and unvaccinated fish two statistical analysis were performed. One way analysis of variance (ANOVA) was used to analyze the data and the efficiency of antibiotics in regarding dose and treatment of fish by MS Excels. Chi square (X^2) test is used to compare RPS values. There was significant difference exist between treatment and control of fishes regarding cure of disease. Values were considered significantly different at *P* values <0.05 (Zar *et al.,* 1984).

3. Results
3.1. Bacteriological examination
Results from the culture characteristics indicated that the bacteria isolate from the infected fishes are exhibiting the typical pseudomonad morphological and physiological characteristics, such as creamy, rounded, gram negative and motile. So these bacteria are *Pseudomonas* sp.

3.2. Preparation of antibiotic solution
To test the bactericidal activity, antibiotic susceptibility test, agglutination titer, and challenge test antibiotic solution were prepared with PBS. To get the 100 ml antibiotic solution of 10 µg to 45 µg of the selected antibiotics were mixed with PBS solution.

3.3. Bactericidal activity
The lowest number of bacterial colonies indicated the efficiency of antibiotics to kill the pathogen. With ciprofloxacin, tetracycline, oxytetracycline treatment, the oxytetracycline showed lowest number of colony forming unit (c.f.u.), i.e. 1×10^{-4} c.f.u./ml , developing on nutrient agar and BHI agar 30 µg/ml dose, with highly significant differences to the control which showed number of colony forming unit (c.f.u.), i.e. 10×10^{-4} c.f.u./ml. With tetracycline the number of colonies, i.e. 2.5×10^{-4} c.f.u./ml, developing on nutrient agar and BHI agar 30 µg/ml dose and in ciprofloxacin 30 µg/ml dose found highest number of colony forming unit (c.f.u) , i.e. 4.5×10^{-4} c.f.u./ml. (Fig. 1).

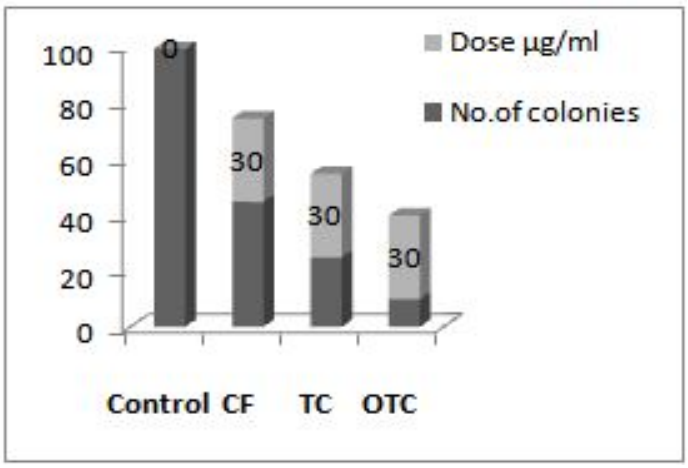

Fig 1: Bactericidal activity of *Pseudomonas* sp. against different antibiotics.

3.4. Antibiotic susceptibility test
In antibiotic susceptibility assays, *Pseudomonas* sp were susceptible against oxytetracycline, tetracycline and ciprofloxacin. The zone of inhibition for oxytetracycline is 24mm, for tetracycline is 21 mm and for ciprofloxacin is 18 mm were found by treatment. In the antibiotic susceptibility testing result indicated that oxytetracycline exhibited maximum activity than that of tetracycline and ciprofloxacin. Ciprofloxacin exhibited lowest activity than tetracycline and oxytetracycline. (Table. 1).

Table 1: Antibiotic susceptibility assay with three antibiotics.

Antibiotics	Zone of inhibition (mm)
Ciprofloxacin	18
Oxytetracycline	24
Tetracycline	21

3.5. Bacterial agglutination test
In agglutination titre Oxytetracycline showed better result than tetracycline and ciprofloxacin at different doses.

3.6. Challenge test
The highest survivability were 75% and 55% for Oxytetracycline and Tetracycline for 40 µg/mg dose. The lowest survivability of 10% was noted in the control while 40% survivability was observed for Ciprofloxacin 40 µg/mg dose application. (Fig. 2).

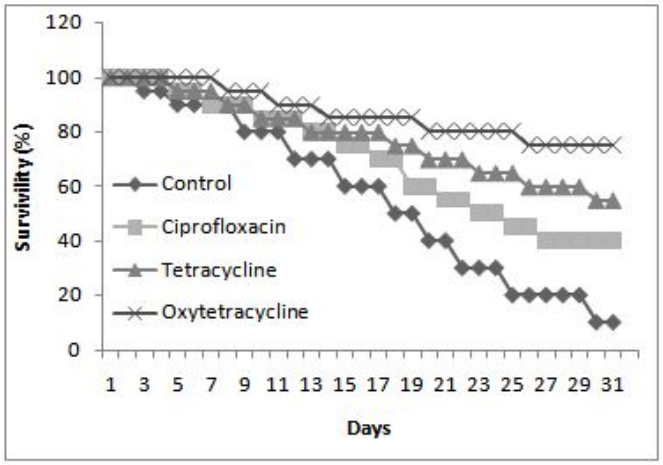

Fig 2: Survival rate of fish against different antibiotics.

The lowest cumulative mortality was 25% and 45% for Oxytetracycline and Tetracycline 40 µg/mg dose. The highest mortality of 90% was noted in the control while 60% mortality was observed for Ciprofloxacin 40 µg/mg dose application. (Fig. 3).

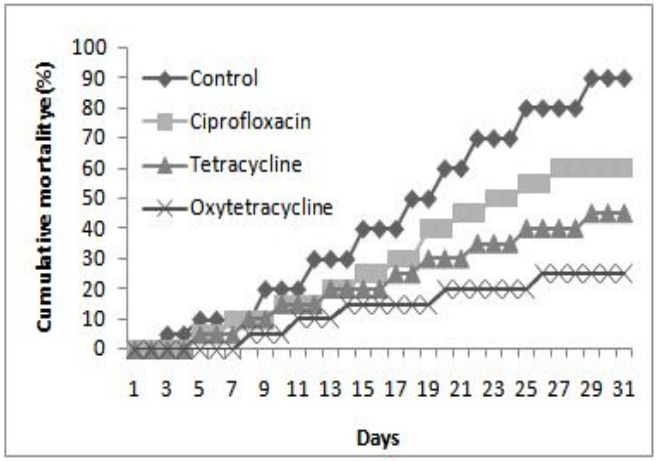

Fig 3: Cumulative mortality rate of fish against different antibiotics.

The highest RPS value was found for Oxytetracyline i.e. 72.2%.Rps value for tetracycline was 50% and lowest RPS value for ciprofloxacin, i.e.33.33%.

3.7. Statistical analysis
The calculated value of chi square (x^2) is 13.18 which is greater than the tabulated value (10.83) at 0.1% level of significance and 1 degree of freedom. So value of x^2 is highly significant and the null hypothesis is rejected, i.e. there is difference exist between treatment and control of fishes regarding cure of disease. The calculated value of (Analysis of variance) P was less than 0.05. So there is difference exist among the efficiency of antibiotics in regarding dose and treatment of fish.

4. Discussion
Bacterial diseases are major constrain for aquaculture industry throughout the world. The appearance and development of a fish disease is the result of the interaction of pathogen, host and environment. *Pseudomonas* sp is one most serious disease in fishes caused by different species of *Pseudomonas* species leading heavy loss to fish farmers.

In the present study *Pseudomonas* were isolated from fishes suffering with *Pseudomonas* sp. All the characters shown by bacteria are similar to as described by Buchnan and Gibbons *et al.,* (1974) and Austin and Austin *et al.* (1993). The similar finding were also reported by Khalil *et al.* (2010) who isolated *P. fluorescens* from *Pseudomonas septicemia* affected fish, *Oreochromis niloticus. Pseudomonas* sp.

Under the present study three antibiotics namely Ciprofloxacin, Oxytetracycline, tetracycline were tested in *vitro* against *Pseudomonas* species isolated from *Pseudomonas* septicemia affected fishes. It has been observed that all the bacteria were susceptible to Oxytetracycline, tetracycline and Ciprofloxacin. Oxytetracycline is highly effective against *Pseudomonas* species Ciprofloxacin is less effective against *Pseudomonous* sp. while tetracycline showed moderate effective against *Pseudomonous* species. More or less similar studies were done by Khalil *et al.* (2010) and Pervez *et al.* (2007).

The similar finding were also reported by Sova patra *et al.* (2009) who showed that two antibiotics (imipenem 10 µg/disc & tetracycline 10 µg/disc) were more effective than other antibiotics such as ciprofloxacin 10 µg/disc, colistin 10 µg/disc, amikacin 30 µg/disc, gentamicin 10 µg/disc, netilmicin 10 µg/disc, tobramycin 10 µg/disc, ticarcilin 10 µg/disc, amikacin 30 µg/disc, gentamicin 10 µg/disc, netilmicin 10 µg /disc, tobramycin 10 µg/disc,) against *Pseudomonas* sp.

The less similar finding were also reported by Khalil *et al.* (2010) who showed that antibiotics namely, gentamycin and amikin were effective antibiotics against *Pseudomonas* sp. While it was resistance to a wide range of recent antibiotics. In the present study, Oxytetracycline is highly effective against Pseudomonas species, Ciprofloxacin is less effective against *Pseudomonous* sp. and while tetracycline shown moderate effective against *Pseudomonous* species.

This finding were also reported by Abdullahi *et al.* **(**2013) *P. aeruginosa* were resistant towards 8 different types of antibiotics, chloramphenicol (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), gentamycin (30 µg), ampicillin (10 µg), erythromycin (15 µg), norfloxacin (10 µg), and carbenicillin (100 µg). The results showed 4 different patterns of antibiotics resistance. Gentamicin, norfloxacin and nalidixic acid can be effective agents for the treatment of *P. aeruginosa* in aquaculture.

The similar finding were also reported by Mesaros *et al.* (2007) Ciprofloxacin was more effective antibiotics against *Pseudomonas* sp than other antibiotics such as (tobramycin, amikacin, imipenem gentamycin).

The similar finding were also reported by Mastan *et al. (*2013) *Pseudomonas* sp were sensitive to Chloramphenicol, Oxytetracycline Kanamycin, and Ciprofloxacin. Chloramphenicol and Oxytetracycline showed highly effective against all the four bacteria (*Pseudomonas anguilliseptica*, *P. fluorescens, P. aeruginosa,* and *Pseudomonas* sp). Ciprofloxacin is highly effective against *P. anguilliseptica*, while Kanamycin, Ciprofloxacin and Vancomycin shown moderate effect against four species. Erythromycin has less effect on three bacterial species. Amikacin and Ampicillin did not show any effect against all bacteria. In the present study, Oxytetracycline is highly effective against *Pseudomonas* species. Ciprofloxacin is less effective against *Pseudomonous sp.* while tetracycline shown moderate effective against *Pseudomonous* species.

5. Conclusion

At the turn of the third millennium, *Pseudomonas sp* clearly represents one of the most challenging pathogenic bacteria. Different antibiotics are used to treatment *Pseudomonas sp* from fishes at different doses. Based on the results of this study, it could be concluded that Oxytetracycline is highly effective against *Pseudomonas* species, Ciprofloxacin is less effective against *Pseudomonous sp.* while tetracycline were shown moderate effective against *Pseudomonous* species.

6. References

1. Abdullahi R, Lihan S, Carlos BS, Bilung ML, Mikal MK, Collick F. Detection of *opr*L gene and antibiotic resistance of *Pseudomonas aeruginosa* from aquaculture environment. Pelagia Research Library European Journal of Experimental Biology 2013; 3(6):148-152.
2. Amend DF. Potency testing of fish vaccines. Developments in Biological standardizati 1981; (49):47454.
3. Amlacher E. Textbook of fish diseases water proof. Edition by D.A.Conroy and R.L. Herman, 1970.
4. Austin B, Austin D. Bacterial fish pathogens, Second Edition *Pseudomonadaceae* representatives, 1993, 253.
5. Buchanan RE, Gibbons NE. Bergey's Manual of Determinative Bacteriology, 8 Edn, Williams and Willkins Co., Baltimore, 1974, 1246.
6. Cappuccino G, Sherman N. Microbiology: a laboratory manual. Benjamin/Cumming Science Publishing, California, 1998, 254.
7. Dhayanithi NB, Ajith Kumar TT, Kathiresan K. Effect of neem extract against the bacteria isolated from marine fish. Journal of Environmental Biology 2010; 31:409-412.
8. Khalil SA, Khalil RH, Saad TT, Safaa MH. Studies on *Pseudomonas Septicemia* among cultured *Oreochromis niloticus.* Journal of the Arabian Aquaculture Society 2010; 5(1).
9. Mastan SA. *Pseudomonas septicemia* in *Labeo rohita* and *Cyprinus carpio* in Andhra Pradesh natural occurrence and artificial challenge. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5.
10. Mesaros N, Nordmann P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem R *et al. Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. International Journal of Pharmacy and Pharmaceutical Sciences, 2007.
11. Pervez N. Biochemical and Serological characterization of bacteria in various fish diseases, PhD, thesis submitted to Barkatullah Univ., Bhopal, 2007.
12. Yildirim M, Lim C, Wan P, Klesius PH. Growth performance and immune response of channel catfish (*Ictalurus punctatus*) fed diets containing graded levels of gossypol-acetic acid. Aquaculture 2003; 219:751-768.
13. Zar JH. Production. In: Biostatistical Analysis (ed. By J. H. Zar), Prentice-Hall, Englewood Cliffs, NJ, USA, 1984, 293-305.