

BACTERIOLOGICAL STUDY IN ASSOCIATION WITH ANTIBIOTIC  
AND HEAVY METAL RESISTANCE PATTERN OF  
WATER SAMPLE FROM FRESHWATER  
GIANT PRAWN HATCHERY

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FACULTY OF AGROTECHNOLOGY AND FOOD SCIENCE  
UNIVERSITI MALAYSIA PERANGANI  
2009

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HEAVY METAL RESISTANCE PATTERN OF WATER SAMPLE FROM  
FRESHWATER GIANT PRAWN HATCHERY**

**By**

**Khabibah Binti Md Amin**

**Research report submitted in partial fulfillment of  
the requirements for the degree of  
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**Department Of Fisheries Science and Aquaculture  
FACULTY OF AGROTECNOLOGY AND FOOD SCIENCE  
UNIVERSITY MALAYSIA TERENGGANU  
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**FAKULTI AGROTEKNOLOGI DAN SAINS MAKANAN  
UNIVERSITI MALAYSIA TERENGGANU**

**PENGAKUAN DAN PENGESAHAN LAPORAN  
PROJEK ILMIAH I DAN II**

Adalah ini diakui dan disahkan bahawa laporan ilmiah bertajuk:

Bacteriological study in association with antibiotic and heavy metal resistance pattern of water sample from freshwater giant prawn (*Macrobrachium rosenbergii*) hatchery.

oleh..... Khabibah Binti Md Amin....., No.Matrik UK 14699 telah diperiksa dan semua pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Perikanan dan Akuakultur..... sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains Agroteknologi (Akuakultur)....., Fakulti Agroteknologi dan Sains Makanan, Universiti Malaysia Terengganu.

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## DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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Date : 16 May 2009

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## ABSTRACT

The total bacterial flora, antibiotic resistance and metal resistance the diseases associated with water sample of giant freshwater prawns post larvae, *Macrobrachium rosenbergii* were carried out. A study was undertaken to investigate the presence and the total plate count of bacteria isolates by using non selective agar medium such as Trypticase Soy Agar (TSA) and selective agar such as Thiosulfate Citrate Bile Sucrose Agar (TCBS), Pseudomonas Aeromonas Selective Agar Base (GSP), Mac Conkey Agar, Eosin Methylene Blue Agar (EMB) and Xylose Lysine Deoxycholate Agar (XLD). The total bacterial load on TSA, Mac Conkey and GSP agar varied on  $10^2$  cfu ml<sup>-2</sup> with higher counts seen in GSP agar that was  $6.1 \times 10^2$  bacteria per ml. But the total bacteriological analysis on EMB, TCBS, and XLD agar to few to determine the count. 29 bacterial isolates were identified as *Vibrio spp*, *Escherichia coli*, *Pseudomonas spp*, *Salmonella* and *Aeromonas spp*. Heavy metal test with different level concentration such as cadmium dichloride anhydrous (cd) (25, 5.0,10,20 and 40mg/L), cuprum (Cu) (1.5, 300, 600, 1200 and 2400mg/L), Mercuric dichloride (Hg) (2.5, 5, 10, 20, 40µg) and potassium dichromate (Cr) (25, 50, 100, 200 and 400mg/l). The results showed toxicities in the order of Cd=Hg=Cr>Cu. In this pilot study, all bacterial isolates were examined for their resistance to different 15 antibiotics. These antibiotic included: Flumequine (50µg), Nitrofurantoin (50µg), Florfenicol(30µg), Amoxycillin(25µg), Doxycycline (30µg), Oleandomycin (15µg), Tetracycline (30µg), Ampicillin (10µg), Lincomycin (15µg), Colistin Sulphate (25µg), Oxolinic Acid (2µg), Novobiocin (30µg), Spiramycin (100µg), Erythromycin (15µg) And Fosfomycin (50µg). Bacterial isolates were indicated that hundred percent resistance to Doxycycline, Oleandomycin, Linomycin, Novobiocin, Spiramycin, Fosfomycin and Flumequine. The lowest incidence of resistance was found in Nitrofurantoin, Florfenicol, Amoxicillin, Tetracyclin, Ampicillin, Colistin Sulphate, Oxolinic Acid, and Erythromycin. MAR index shown that >0.2 for isolate bacteria indicated that environment was more polluted. Knowledge of the qualitative and quantitative aspects of bacterial flora in the hatchery would help to understand disturbances, if any, brought about during disease outbreaks to *M. rosenbergii*.



## ABSTRAK

Jumlah bakteria flora, rintangan antibiotik dan rintangan logam bagi kepelbagaian penyakit dalam sampel air selepas larva udang galah air tawar, *Macrobrachium rosenbergii* telah diketahui. Kajian mengkaji kehadiran dan jumlah pengiraan bakteria yang telah dipencilkan di atas piring agar menggunakan media bukan pilihan seperti Trypticase Soy Agar (TSA) dan agar pilihan iaitu Thiosulfate Citrate Bile Sucrose Agar (TCBS), Pseudomonas Aeromonas Selective Agar Base (GSP), Mac Conkey Agar, Eosin Methylene Blue Agar (EMB) dan Xylose Lysine Deoxycholate Agar (XLD). Jumlah koloni bakteria di atas media agar TSA, Mac Conkey dan GSP ialah  $10^2$  cfu ml<sup>-2</sup> dengan bacaan tertinggi pada agar GSP iaitu  $6.1 \times 10^2$  bakteria per ml tetapi jumlah analisis bakteria pada media agar EMB, TCBS, dan XLD adalah terlalu sedikit untuk diambil keputusannya. Sebanyak 29 bakteria telah berjaya dipencilkan dan dikenalpasti iaitu *Vibrio spp*, *Escherichia coli*, *Pseudomonas spp*, *Salmonella* dan *Aeromonas spp*. Ujian logam berat dengan menggunakan kepekatan yang berbeza seperti cadmium dichloride anhydrous (Cd) (25, 5.0, 10, 20 and 40mg/L), cuprum (Cu) (1.5, 300, 600, 1200 and 2400mg/L), mercuric dichloride (Hg) (2.5, 5, 10, 20, 40 $\mu$ g) dan potassium dichromate (Cr) (25, 50, 100, 200 and 400mg/l). Keputusan menunjukkan tahap ketoksikan dengan susunan Cd=Hg=Cr>Cu. Dalam kajian ini, semua bakteria yang dipencilkan telah dilakukan kajian untuk menentukan tahap kerintangan terhadap 15 antibiotik. Antibiotik tersebut termasuklah: Flumequine (50 $\mu$ g), Nitrofurantoin (50 $\mu$ g), Florfenicol(30 $\mu$ g), Amoxicillin(25 $\mu$ g), Doxycycline (30 $\mu$ g), Oleandomycin (15 $\mu$ g), Tetracycline (30 $\mu$ g), Ampicillin (10 $\mu$ g), Lincomycin (15 $\mu$ g), Colistin Sulphate (25 $\mu$ g), Oxolinic Acid (2 $\mu$ g), Novobiocin (30 $\mu$ g), Spiramycin (100 $\mu$ g), Erythromycin (15 $\mu$ g) dan Fosfomycin (50 $\mu$ g). Pemencilan bakteria menunjukkan bahawa seratus peratus rintang kepada Doxycycline, Oleandomycin, Linomycin, Novobiocin, Spiramycin, Fosfomycin dan Flumequine. Bacaan Indeks MAR bakteria yang dipencilkan >0.2 membuktikan bahawa persekitaran telah mengalami pencemaran yang tinggi. Pengetahuan berkaitan aspek kualiti dan kuantiti ke atas bakteria flora dalam tangki penetasan membantu kepada pemahaman yang tidak jelas, yang membawa penyakit maut kepada *M. rosenbergii*.