

WORKSHOP ON:
Antibiotic Resistance in Asian Aquaculture Environments

Antibiotic Resistance Problem in Thailand

J. Tangtrongpiros

Abstract- Antibiotic resistance has been occurred due to many factors such as drug misuse, over using of antibiotic and production of resistance factor of bacteria. Food and drug authority of Thailand permit to use Oxytetracycline, Sulfadimethoxine/or metroprim, Enrofloxacin, Sulfamethoxazole/ trimetroprim and Neomycin for aquatic animal. Drug sensitivity test is recommended to perform before using antibiotic. Organic food is the aim of animal production.

Keywords - Antibiotic, Resistance, Thailand

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Antimicrobial Resistance in Bacteria Isolated from Aquaculture Environments in the Philippines

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Antibiotics have been used in aquaculture as feed additives to promote growth and added in water to prevent disease occurrence. Therapeutic doses are used to treat diseases. Long-term use and misuse of antibiotics may result to drug resistant bacterial strains and accumulation of unwanted residues in the cultured commodity upon slaughter and harvest. Some of the antibiotics that have been used in shrimp hatcheries and grow-out ponds are tetracycline, rifampicin, chloramphenicol, nitrofurans, erythromycin, oxolinic acid and furazolidone. Oxytetracycline, furanace, terramycin, Ektecin, chloramphenicol and sulfa drugs have been used to treat fish bacterial infections. Antibiotic resistant bacteria have been isolated from shrimp rearing water, natural bodies of water that received effluents from aquaculture, and from cultured species in the Philippines.

Resistance to oxytetracycline and furaltadon were reported in bacteria isolated from crab and nearshore sediments. Bacteria from shrimp ponds have been reported to be resistant to oxytetracycline, furazolidone, oxolinic acid and chloramphenicol. Luminous vibrios from shrimp larvae and ponds were resistant to erythromycin, kanamycin, oxytetracycline, Penicillin, streptomycin, sulfadiazine and triple sulfa. *Aeromonas* sp. from fish, shrimp, and their rearing water were resistant to streptomycin, oxytetracycline and trimethoprim/sulphamethoxazole. Multiple antibiotic resistance has also been reported in bacteria isolated from shrimp, shrimp ponds, luminous bacteria from shrimp post larvae, and *Aeromonas hydrophila* from fish. Despite the recorded usage, antibiotic residues in cultured food fish have not yet been a problem of the Philippine aquaculture industry as far as trade is concerned.

However, chloramphenicol and nitrofurazolidone residues have been detected in shrimp for export using the enzyme link immunoassay (ELISA) technique.

To minimize the use of antibiotics, different alternative methods to prevent diseases in aquaculture have evolved. Government agencies also issued regulations on the use of antibiotics.

Keywords- Antibiotic, resistance, Philippines, aquaculture

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Current status of aquatic veterinary drugs usage for Aquaculture in Vietnam

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Aquaculture develops fast in Vietnam during last 10 years leading aquatic veterinary drugs are widely used. The paper presents status of aquatic veterinary drugs usage for aquaculture in Vietnam based on available information. There are 1,893 products in which at least there are 476 antibiotic products available in the market. Base on the purpose of use, 9 groups are categorized and the usage of different groups as well as management strategies also presented.

Keywords-Aquaculture; Aquatic veterinary drugs.

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Bacterial Resistance to Antimicrobials Use in Shrimp and Fish Farms in the Mekong Delta, Vietnam

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Antimicrobial resistance is an accelerating and accumulating problem. Spread of resistance amongst microorganisms may affect the ability to treat infectious diseases in cultured animals and presents a major threat to public health. However, there is very little information on antimicrobials use in aquaculture in the Mekong River Delta of Vietnam. Result from a survey on antimicrobials use in aquaculture conducted in 2004 showed that a large proportion (90%) of interviewed farms in the Delta applied antimicrobials for prophylactic and therapeutic treatment. There were 122 antibiotic products used in shrimp farming, in which 77 products contain antibiotics belong to the quinolon group, 34 products have aminosid antibiotics and 31 products comprise polypeptid and sulfamid compounds. Only 29 products were reported in fish farming including b-lactamin group (4 products), quinolon and sulfamid (3 products) and a mixture of different groups of antibiotics (17 products). Some banned or limited use antibiotics in aquaculture were still found during the survey.

Susceptibility to common antibiotics used in aquaculture was performed using disk diffusion method. Fifty *Vibrio* bacterial isolates from diseased and healthy prawn larval *Macrobrachium rosenbergii* were tested with 14 antibiotics. The result showed that a large number of strains were resistant to penicillin (98%), vancomycin (90%) and polymyxinB (64%). Sensitivity test of 32 luminous *Vibrio* bacterial isolates from shrimp postlarvae *Penaeus monodon* to five antimicrobial agents revealed that all isolates were resistant to ampiciline and only 1-3 tested isolates were resistant to oxinilic acid, streptomycin, tetracillin, oxytetracylin and chloramphenicol. Bacterial isolates from diseased catfish *Pangasius hypothamus* including *Edwardsiella ictaluri* (13 isolates), *Aeromonas hydrophila* (3 isolates) and *Aeromonas sobria* (5 isolates) were tested with 11 antimicrobials. All *E. ictaluri* isolates were resistant to oxytetracylin, oxolinic acid and sulphonamid, whereas, all tested *Aeromonad* isolates were resistant to sulphonamid. Besides, *Aeromonas hydrophila* isolates were also resistant to amoxicillin. Antibiotic resistance also occurred in bacterial isolates from aquaculture environment. Of 123 isolates, which were resistant to chloramphenicol, 9.8% were resistant to tetracycline, 11% were resistant to trimethoprim/sulfadiazine, 24% resistant to ampicillin, 35% were resistant to nitrofurantoin and 33% were resistant to norfloxacin.

The research findings indicated that a more restrictive use of antibiotics could be warning and antibiotic residues monitoring in aquaculture should be implemented.

Keywords - antibiotic resistance, aquaculture, fish, shrimp, environment.

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Antibiotic Susceptibility of *Vibrio* spp. Isolated from Diseased Shrimp, Pond water and Water sources in Sri Lanka: A Preliminary Study

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Shrimp industry in Sri Lanka is experiencing disease outbreaks continuously, both viral and bacterial. The hatcheries and farmers use various remedial measures including antibiotics to overcome the problem. Most commonly used antibiotics are erythromycin and tetracyclines and these are used in an ad hoc manner. The antimicrobial susceptibility of bacterial flora of shrimp and brackish water environments has not been studied systematically in Sri Lanka.

This communication presents results of an in vitro antibiotic susceptibility study of 104 isolates of five different *Vibrio* species namely *Vibrio harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum* and *V. vulnificus* originated from haemolymph of diseased shrimp (*Penaeus monodon*) and water. The susceptibility tests were performed according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS) using Mueller Hinton agar with 1% NaCl.

The antibiotics employed were erythromycin (15µg), sulphamethoxazole (25µg), chloramphenicol (30µg) and tetracycline (30µg). Chloramphenicol was used as a marker to assess the degree of resistance. Of the isolates tested, 94.8%, 89.9% and 78.3% showed sensitivity to chloramphenicol, sulphamethoxazole and tetracycline respectively. None of the isolates were sensitive to erythromycin. The results are suggestive of emergence of antibiotic resistance among bacteria originated from diseased shrimp and their environment.

Key words- Shrimp, *Vibrio*, antibiotic, susceptibility, resistance

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In Vitro Minimum Inhibitory Concentrations of Florfenicol and Chloramphenicol against Clinical *Vibrio* Isolates from Black Tiger Shrimp (*Penaeus monodon*)

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Florfenicol and Chloramphenicol were tested for their in vitro antimicrobial activity against 102 clinical *Vibrio* isolates. *Vibrios* were isolated from the hepatopancreas of the diseased shrimp collected from different culture areas of Thailand, and identified by conventional tube media or API-20 system (Biomérieux, France). The agar dilution method as described by the National Committee of Clinical Laboratory Standards (NCCLS) was used to determine Minimum Inhibitory Concentrations (MICs) of each chemical against the isolated *Vibrios*. The effect of components in seawater on antimicrobial activity of each chemical was also observed by a comparison between MIC values tested on Mueller Hinton Agar (MHA) dissolved in distilled water with added 1% NaCl and on agar dissolved in 5‰ seawater. MIC values for both antimicrobials ($\leq 8 \mu\text{g/ml}$) compared to the NCCLS interpretive standard of Chloramphenicol against *Vibrio cholerae* suggested that all of the tested isolates were susceptible to both antimicrobials. The observed MIC range of Florfenicol (0.5-4.0 $\mu\text{g/ml}$) was more potent than Chloramphenicol (0.5-8.0 $\mu\text{g/ml}$). The activity of both antimicrobials were not substantially influenced by an addition of 5‰ seawater to the test system. The study concluded that, with respect to MIC testing and any observed implication of seawater effect, Florfenicol is a prospective antimicrobial for shrimp *Vibrio* pathogens.

Keywords - Black tiger shrimp, Minimum Inhibitory Concentration, Florfenicol, Chloramphenicol.

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Qualitative and Quantitative Comparison of Bacterial Flora Associated with Hatchery-reared and Wild-caught Shrimp Postlarvae

Celia R. Lavilla-Pitogo, Leobert D. de la Peña and Milagros R. Paner

Because of high mortality recorded in pond-reared shrimps due to luminescent vibriosis infection, a study was conducted to determine if postlarvae (PLs) could be major sources of luminescent bacteria (LB). Batches of hatchery-reared (PL12 to 18) and wild-caught *Penaeus monodon* PLs were examined to determine their bacterial load. Results show that although all PLs have associated *Vibrio* spp., not all of them harbored detectable levels of LB. Fifty eight percent of wild-caught postlarval batches did not have associated LB compared with only 23-44% of hatchery-reared postlarvae. A significant difference in quantitative LB load was noted between hatchery reared and wild-caught PLs with the former harboring up to 3.0×10^5 cfu LB/postlarva. Wild-caught PLs had only up to 3.5×10^2 cfu LB/postlarva.

Antimicrobial sensitivity tests using disc diffusion method show significant resistance to Chloramphenicol and Oxytetracycline among isolates from hatchery-reared PLs (33 and 44%) compared with bacteria from wild-caught PLs (3 and 6%) and near shore seawater (0 and 12%). The differences between the quantitative and qualitative bacterial flora of hatchery-reared and wild-caught PLs may have contributed to the occurrence of luminescent vibriosis in grow-out ponds, which generally make use of hatchery-reared postlarvae.

Keywords – bacterial flora, *Penaeus monodon*, postlarvae, antibiotic resistance

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How to maintain the microbial biodiversity

J. Swings and V. Storms

Different collections or BRC's are in different financial and environmental conditions and do not necessarily have the same challenges and opportunities. In general however, three important cornerstones can be distinguished. As a first cornerstone, BRCs organize the professional conservation and distribution of biological materials and the data related to them. Key values are authenticity, genetic integrity and validity of the information provided. There is a need for global standards to assure top quality of biological materials and data through appropriate Total Quality Management. The second cornerstone is the linking of BRC's to scientific centres in taxonomy, molecular genetics, biochemistry, cellular biology, biochemistry or genomics. The third cornerstone, common for all BRCs is the valuation and thus demonstrable utilization of its holdings and its data. The bioeconomic logic of industry, healthcare and the research related to it is here clearly the driving force of a mainly economic reality.

Keywords - Biodiversity, bioeconomics, Culture Collections

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Alternative Options to Antibiotics

L. Grisez

In theory, antibiotics are to be used in order to treat existing bacterial infections and thereby helping the host to overcome an infection or a disease state. In practice however, antibiotics are used when diseases occur, irrespective of the nature of the disease or even as a prophylactic treatment against possible infections later.

The correct application of antibiotics requires knowledge of the etiology of the disease and knowledge on the required dose and the required application strategy. Whereas in terrestrial animals dosage and application strategy for most antibiotics is well documented this is not so for aquatic animals.

Furthermore, the delivery of antibiotics to fish usually involves a top-dressed-feed application in which the leaching of the active compound from the feed and loss of appetite because of the disease are major obstacles.

The extensive use of antibiotics in Asian aquaculture is easily condemned from an environmental safety and consumer safety view point. However, the survival of the fish represents the farmer' livelihood and without more safe means to fight diseases the farmer has little choice then to protect his investment and survival.

This paper presents alternative options to farmers in Asia to decrease disease occurrence as well as to decrease the use of antibiotics.

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Probiotics as Alternative to Antimicrobials: Limitations and Potential

O. Decamp and D. J. W. Moriarty

Probiotics are used in aquaculture as a means of disease control, supplementing or replacing the use of antimicrobial compounds. Their possible modes of actions include competitive exclusion, alteration of microbial metabolism, and the stimulation of host immunity and/or bioremediation. Their potential and their limitations will be discussed.

Key words - Probiotics, Bacillus, Vibrio, biocides, antimicrobials, probiotics

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Monitory on Drug Resistance of *Vibrio* Bacteria in Shrimp Farm

L. Ruangpan and T. Chaweepack

To monitor drug resistant bacteria in shrimp farm against the common drugs used in Thailand, total number of 395 vibrio strains were isolated from moribund shrimp rearing in Samutsakhon, Chachengsoa, Rayong, and Chanthaburi Provinces during 2001-2004. Minimal inhibitory concentration (MIC) using agar method was performed for determine bacterial susceptibility to oxolinic acid (OA), oxytetracycline (OTC), trimethoprim (TM) , and sulfadiazine (SD). MIC breakpoints (ug/ml) of drug resistance were obtained based on NCCLS interpretative guidelines. Results of the study show that during 2001-2003, high number of vibrio strains in all studied areas resisted continuously to SD (100 %) and TM (80-100%). Whereas, an average percent of 53.75 and 17.67 strains resisted to OTC and OA, respectively. The average percent of OTC resistant strains gradually declined year by year from 2001 to 2003, and rapidly declined in 2004. No OA resistant strain was detected in all area in 2004, while in 2003, only 6% of OA resistant strains which obtained from Chachengsoa were observed. It should be noted that most of the resistant strains detected between 2001 to 2003 belong to keyword, shrimp from multiple drug resistance but, only monomer and double types drug resistance were detected in 2004. Our results provide necessary information on the impact of drug usage which may caused by misuse and noncompliance among the users. This study was partly supported by Japanese Trust Fund, SEAFDEC.

Keyword - drug resistance, vibrio bacteria ,shrimp farm

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Mechanism of action of two newly discovered antibiotics

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Using an innovative high-throughput test devised to recognize selective inhibitors of bacterial translation, upon screening approximately 25000 products of microbial secondary metabolism, we have identified two peptide antibiotics. The two compounds, denominated GE81112 and GE82832 were purified and characterized chemically and functionally.

The target of both antibiotics was found to be the 30S ribosomal subunit while the functions affected by GE81112 and GE82832 were the activity of the P-site and the translocation, respectively.

Keywords - Highthroughput screening, antibacterial agents, translational inhibitors, translocation, initiation complex formation, 30S ribosomal subunit

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Nucleotide Sequences of *gyrA*, *gyrB*, *parC* and *parE* of *Vibrio anguillarum* and Application for Detection of Their Mutations in the Different Levels of Quinolone-Resistant *Vibrio anguillarum* Strains Isolated from Diseased Fish in Japan

Channarong Rodkhum, Takashi Maki, Ikuo Hirono, and Takashi Aoki

The nucleotide sequences which were homologue to known *gyrA*, *gyrB*, *parC* and *parE* were cloned and sequenced from *Vibrio anguillarum*. Oxolinic acid-resistant mutants of *V. anguillarum* were obtained from in vitro-induced mutation and *V. anguillarum* strains isolated from diseased fish in Japan. The minimal inhibitory concentration (MIC) ranges of the mutants were 8-250 µg/ml. Quinolone resistance-determining region (QRDRs) of *gyrA*, *gyrB*, *parC* and *parE* genes were amplified from oxolinic acid-resistant *V. anguillarum* by PCR based on specific primers for *gyrA*, *gyrB*, *parC* and *parE* QRDRs. PCR products were cloned, sequenced and analyzed for the mutation. The mutations detected in GyrA QRDRs were amino acid residue position 83 counted from N-terminus of amino acid sequence of *V. anguillarum* GyrA, yielding a Ser-83 changed to Ile. The mutations detected in ParC QRDRs were amino acid residue position 85 counted from N-terminus of amino acid sequence of *V. anguillarum* ParC, yielding a Ser-85 changed to Leu. No amino acid alterations were discovered in GyrB and ParE QRDRs. One point mutation only in the GyrA and ParC QRDRs were detected in all resistant strains.

The results suggest that *gyrA* and *parC* were the targets of quinolone resistance in *V. anguillarum* as in other gram-negative bacteria, while *gyrB* and *parE* were not the targets of quinolone resistance in *V. anguillarum*.

Keywords - *V. anguillarum*, *gyrA*, *gyrB*, *parC*, *parE*, Quinolone resistance

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Molecular Detection and Confirmation of Fluoroquinolone-resistant mutation in *GyrA* and *ParC* QRDRs from Fluoroquinolone-resistant *Vibrio parahaemolyticus* strains isolated from Diseased Black Tiger Shrimps (*Penaeus monodon*) and Their Cultural Environments in Thailand

Channarong Rodkhum, Supawadee Na-pompet, Anan Jongthaleong, Ikuo Hirono, and Takashi Aoki

Quinolone resistance-determining regions (QRDRs) in the *gyrA* and *parC* genes were amplified by singlestep colony PCR in 38 isolates of *Vibrio parahaemolyticus* strains isolated from black tiger shrimp (*Penaeus monodon*) and their cultural environments in Thailand. The minimal inhibitory concentration (MIC) ranges were 0.064-8 µg/ml. The specific primers for *GyrA* and *ParC* QRDRs obtained from reported sequences of *V. parahaemolyticus gyrA* and *parC*. PCR products were directly sequenced and analyzed for the mutation. The mutations detected in *GyrA* QRDRs were amino acid residue position 83 of *V. parahaemolyticus GyrA*, yielding a Ser-83 changed to Ile. The mutations detected in *ParC* QRDRs were amino acid residue position 85 of *V. parahaemolyticus ParC*, yielding a Ser-85 changed to Phe. Nevertheless, the MIC range which were caused of the mutation in *gyrA* and *parC* QRDRs of the strains from this study were not corresponded to previous study by Okuda et al. which have done in laboratoryinduced ciprofloxacin-resistant *V. parahaemolyticus*. Additionally, the MICs were not corresponded with the point mutation detected from sequence analysis. We can detect the point mutation in *gyrA* and *parC* QRDR genes of *V. parahaemolyticus*, while MIC value of that strains not show the significant level of resistant to fluoroquinolone. The results suggest that the detection of mutation in *gyrA* and *parC* QRDRs has higher accuracy for detection of fluoroquinolone resistant *V. parahaemolyticus* than the determination of MIC.

Keywords - *V. parahaemolyticus*, Shrimp, Fluoroquinolone-resistance, QRDRs

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Surveillance of Antimicrobial Resistance in Food – borne Vibrios from Black Tiger Shrimp *Penaeus monodon*

Nutcharnart Tipmongkolsilp, Yanin Limpanon, Monton Lertworapreecha, Janenuj Wongtavatchai

The primary surveillance of antimicrobial resistance in food – borne vibrios from black tiger shrimp *P.monodon* was studied using the values of Minimum Inhibitory Concentration (MICs) of different antimicrobials against clinical vibrio isolates. The standard, in vitro, MICs of 10 antimicrobials; amoxicillin, chloramphenicol, ciprofloxacin, enrofloxacin, erythromycin, kanamycin, nalidixic acid, norfloxacin, tetracycline and trimethoprim/sulfamethoxazole were evaluated against vibrios isolated from diseased black tiger shrimps *Penaeus monodon*. One hundred and ninety-seven pathogenic vibrio isolates obtained from different shrimp culture areas in Thailand during January 2001 – January 2002 were *V. fluvialis* 50 isolates (25.38%), *V. damsela* 41 isolates (20.81%), *V. vulnificus* 39 isolates (19.80%), *V. parahaemolyticus* 28 isolates (14.21%), *V. cholerae* 25 isolates (12.69%) and *V.alginolyticus* 14 isolates (7.11%). MICs were examined for all the tested antimicrobials and the degree of susceptibility of shrimp vibrio pathogens to each compound was analysed by determining their MICs.

The investigation revealed that most of the tested isolates were susceptible to kanamycin, enrofloxacin, chloramphenicol, norfloxacin, trimethoprim/sulfamethoxazole and ciprofloxacin. Approximately 60% of the tested isolates was susceptible to tetracycline, while less than 50% were susceptible to nalidixic acid, amoxicillin and erythromycin.

Keywords - Black tiger shrimp, Minimum Inhibitory Concentration, Florfenicol, Chloramphenicol

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Two methods to investigate antibiotic resistance of bacteria from shrimp ponds

Eleonor A. Tendencia

The incidence of resistance to oxolinic acid and oxytetracycline of bacteria from shrimp ponds were investigated using two different methods. One, by plating serially diluted samples onto agar plates with and without antibiotics; and the other, by disc agar diffusion method. This paper will compare the two methods based on the protocols and results obtained. The advantages and disadvantages of the use of these methods will also be discussed.

Keywords - methods, antibiotic resistance, shrimp ponds