UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION AND TRANSFER OF CHLORAMPHENICOL RESISTANCE GENE IN BACTERIA ISOLATED FROM AQUACULTURE PONDS

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MASTER OF SCIENCE

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By

NOOR AZRIN ABDUL RAHMAN

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Partial Fulfillment of the requirement for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Master of Science

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Noor Azrin Abdul Rahman

August 2011

Chairman : Prof. Dato' Mohamed Shariff Mohamed Din, PhD

Faculty : Veterinary Medicine

The presence of antibiotic residues and antibiotic resistant organisms in agriculture and aquaculture environments and products constitute a potential threat to the sustainable development and international trade in the industries. A total of 127 chloramphenicol resistance isolates obtained from selected aquaculture ponds in Selangor and Perak, Malaysia, were tested their susceptibility to common antibiotic used in aquaculture by using the disk diffusion method and minimal inhibitory concentration. Most of the chloramphenicol resistant isolates were also highly resistant to nitrofurantoin (80.3%), trimethoprim/ sulfamethoxazole (78%) and tetracycline (74.8%). The minimal inhibitory concentration (MIC) analysis showed that most of the isolates were resistant to chloramphenicol at MIC value > 1024 ppm. The chloramphenicol resistance genes detection in the isolates using multiplex PCR assay shown that, two chloramphenicol

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resistant genes (*cat* I and *cat* II) were successfully amplified from 51 out of 127 isolates. The assay determined that *cat* II was the dominant gene occurring in 47 out of 127 isolates. Forty one of 76 isolates that did not carry positive *cat* genes, gave positive changes in biochemical *cat* assay. Of the 51 *cat* gene positive isolates, 35 (69%) isolates harbored plasmids. Among the 35 isolates, 21 were observed to harbor plasmid and susceptible to streptomycin (> 6mm to STR10µg disk) and possibility of chloramphenicol resistance gene transfer from these positive isolates to naive bacteria via conjugation were tested by using plate mating method. However, the transfer of chloramphenicol resistant gene was not clearly detected in any isolate.

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Abstrak tesis diserahkan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk Ijazah Master Sains

PENCIRIAN DAN PEMINDAHAN GEN RINTANG TERHADAP KLOREMPINIKOL DI DALAM BAKTERIA YANG DIPENCILKAN DARIPADA KOLAM AKUAKULTUR

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Pengerusi : Prof. Dato' Mohammed Shariff Bin Mohammed Din, PhD

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Kehadiran sisa antibiotik dan organisma yang rintang terhadap antibiotik di dalam hasil pertanian dan akuakultur boleh menjejaskan kemampanan perkembangan industri ini. Sebanyak 127 isolat bakteria yang rintang terhadap klorempinikol telah diperolehi daripada kolam akuakultur di sekitar kawasan Selangor dan Perak, Malaysia telah diuji kepekaannya terhadap antibiotik yang kebiasaannya digunakan di bidang akuakultur dengan menggunakan kaedah peresapan cakera dan kepekatan perencatan minima. Kebanyakan isolat yang rintang terhadap klorempinikol juga menunjukkan tahap kerintangan yang tinggi terhadap nitrofurantoin (80.3%), trimitoprim/ sulfamethoxazole (78%) dan tetrasiklin (74.8%). Analisa kepekatan perencatan minima menunjukkan bahawa kebanyakan isolat ini rintang terhadap klorempinikol pada nilai melebihi 1024 bahagian per sejuta. Pengesanan kewujudan gen yang rintang terhadap klorempinikol menggunakan kaedah PCR multiplek menunjukkan dua gen (*cat* I dan *cat* II) telah berjaya diperbanyakkan pada 51 daripada 127 isolat. Daripada jumlah tersebut 47 isolat

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daripada 127 adalah positif terhadap *cat* II. Sebanyak 41 daripada 76 isolat didapati memberikan perubahan warna yang positif dalam cerakinan *cat* biokimia walaupun tidak mengandungi gen *cat*. Sejumlah 35 (69%) daripada 51 isolat yang mempunyai gen tersebut juga didapati mempunyai plasmid. Daripada 35 isolat, 21 isolat mengandungi plasmid dan rentan terhadap streptomisin (> 6mm terhadap dis STR 10µg) dan kebarangkalian pemindahan gen daripada isolat yang positif kepada isolat naïf melalui konjugasi diuji menggunakan kaedah pertemuan piring. Namun, pemindahan gen rintang terhadap klorempinikol tidak berlaku pada sebarang isolat.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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LIST OF ABBREVIATION

Ab	Antibiotic
Amp	Ampicilin
bp	Base pair
BS	Banting Farm
C	Celsius
cat	*Chloramphenicol acetyltransferases*
cm^2	Centimeter cubic
CHL	Chloramphenicol
CP	Chai Farm
DTNB	Dithiobis nitrobenzoic acid
DNA	Deoxyribonucleic acid
F	Nitrofurantoin
ISA	Isosensitest agar
ISB	Isosensitest broth
kg	Kilogram
LB	Luria- Bertani
MAR	Multiple antibiotic resistance
MDa	Mega Dalton
MIC	Minimal inhibitory concentration
ml	Milliliter
mm	Millimeter

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NCCLS	National Committee for Clinical Laboratory Standards
nm	Nanometer
NOR	Norfloxacin
OD	Optical density
PCR	Polymerase chain reaction
ppm	Part per million
pps	Physiological-saline solution
rpm	Rotation per minute
sdH_2O	Sterile distilled water
S	Streptomycin
SP	Siong Farm
SXT	trimethoprim/sulfamethoxazole
TE	Tetracycline
µg	Microgram
µl	Micro liter
UPM	Universiti Putra Malaysia
°	Degree
±	Plus minus
%	Percentage
/	Per

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warna yang positif dalam cerakinan *cat* biokimia walaupun tidak mengandungi gen *cat*. Sejumlah 35 (69%) daripada 51 isolat yang mempunyai gen tersebut juga didapati mempunyai plasmid. Daripada 35 isolat, 21 isolat mengandungi plasmid dan rentan terhadap streptomisin (> 6mm terhadap dis STR 10µg) dan kebarangkalian pemindahan gen daripada isolat yang positif kepada isolat naïf melalui konjugasi diuji menggunakan kaedah pertemuan piring. Namun, pemindahan gen rintang terhadap klorempinikol tidak berlaku pada sebarang isolat.

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CHAPTER 1

INTRODUCTION

Malaysia's future total fish production can be boosted by the aquaculture sector that has a very good growth potential. It is undeniable that the fisheries sector comprising of fisheries and aquaculture plays an important role in the national economy in terms of employment, income, foreign exchange and particularly food production (Tan, 1998). Malaysia has the highest fish consumption with an estimated average per capita consumption of 49 kg per capita in year 2000. This is further increased to 53 kg per capita in the year 2005, and is expected to rise further to 56 kg per capita in the year 2010 (Othman, 2006).

Diseases are a major problem in these aquaculture industries. For example, vibriosis is a disease caused by *Vibrio* isolates which infect crustaceans and fishes. To overcome these problem, several antibiotics such chloramphenicol, tetracycline, ampicilin and streptomycin are usually used. The prolonged and overused of these antibiotics were developing antibiotic resistance in bacteria which has posed a challenge to the aquaculture industry (Bhattacharya *et al.*, 2000).

According to Tenover and Hughes, (1996), inappropriate and indiscriminate use of antibiotics in animal husbandry, agriculture and aquaculture are the reasons for increased antibiotic resistances in bacteria. The spread of these antibiotics resistant bacteria and resistant genes in the environment can be transferred back to animal and human through food chain. The aquaculture products which were contaminated with

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antibiotics residues combining with human bacterial pathogens are a public health hazards concerns (Cole *et al.,* 2003).

According to Rhodes *et al.,* (2000), interactive relationships exist between aquaculture bacteria and human bacteria in the environment. For example there were cases where antibiotic resistance bacteria isolated from fish hatcheries (*Escherichia coli* and *Aeromonas*) was found containing similar characteristics as bacteria isolated from patients in hospitals. These interactions not only lead to food- borne illness but also induced disease outbreaks in human since these bacteria can adapt rapidly in human body temperature. These conditions worsen if antibiotics that are typically used in aquaculture were also used in treating human disease and infection. For example, an antibiotic such as oxytetracycline that are used both in aquaculture and human treatment would decrease their therapeutic efficiency in aquaculture.

One option to overcome these problems is switching to other antibiotics such chloramphenicol which has broader spectrum activity (Benbrook, 2002). However, chloramphenicol are highly toxic to human even in low doses and it's already banned from use in aquaculture in many countries including Malaysia (DOF, 2009).

For this reason, this project was conducted with the objective to study the impact of chloramphenicol antibiotics as therapeutic, growth-promoting or prophylactic to the aquaculture industry. Studies by other researchers showed that the usage of these antibiotics contributed to resistance bacteria problems. These bacteria can transfer their

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resistant gene between non-pathogenic to pathogenic bacteria in natural habitat in aquaculture farm especially shrimp and fish and being a major health concern in term of food safety and foreign trade.

This research can also be a survey on level of contamination of chloramphenicol in aquaculture environment and action should be taken to prevent if the high occurrence and spread of multidrug- resistance bacteria in Malaysian aquaculture ponds.

Justifications of the study:

1. To assess the connection of hazards use of antibiotics in aquaculture farming and the impact to the aquatic organisms and public human health.
2. To improve the aquaculture farming system into a good practicing management system.
3. To determine whether the development of antibiotic resistance bacteria in Malaysian aquaculture environments is due to the introduction and spread of resistant genes or naturally exist.

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Objectives of the study:

Therefore, the objectives of this study are:

1. To determine the susceptibility of chloramphenicol resistance isolates to antibiotics commonly used in Malaysia aquaculture industry.
2. To detect the presence of chloramphenicol resistant genes in the isolates.
3. To detect the presence of plasmids in chloramphenicol resistance isolates.
4. To investigate the possibility of chloramphenicol resistant gene transfer from positive to naive bacteria via conjugation assay.

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REFERENCES

Aalbaek, B., Rasmussen, J., Nielsen, B. and Olsen, J.E. 1991. Prevalence of antibiotic-resistant *Escherichia coli* in Danish pigs and cattle. *Acta Pathologica, Microbiologica et Immunologica Scandinavica.* 99, 1103-1110.

Agersø, Y., Bruun, M.S., Dalsgaard, I., Larsen, J.L. 2007 The tetracycline resistance gene *tet(E)* is frequently occurring and present on large horizontally transferable plasmids in *Aeromonas* spp. from fish farms. *Aquaculture*. 266, 47-52.

Akhtas, Zerrin., Martin Day, Cigdem Bal Kayacan, Sukufe Diren and E. John Threlfall. 2007. Molecular characterization of *Salmonella Typhimurium* and *Salmonella Enteritidis* by plasmid analysis and pulsed-field gel electrophoresis. *International Journal of Antimibrobial Agents*. 30, 541-545.

Alderman, D. J. and Hastings, T.S. 1998. Antibiotic use in aquaculture: Development of antibiotic resistance-potential for consumer health risks. *International Journal Food Science Technology*. 33, 139-155.

Alderman, D. and Smith, P. 2001. Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish disease. *Aquaculture*. 196, 211-243.

Alton, N.K. and Vapnek, D. 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon *Tn9*. *Nature*. 282, 864-869.

Ammor, M.S., Gueimonde, M., Danielsen, M., Zagorec, M., van Hoek, A.H., de Los Reyes-Gavilán, C.G., Mayo, B., Margolles, A., 2008. Two different tetracycline resistance mechanisms, plasmid-carried tet(L) and chromosomally located transposon-associated tet(M), coexist in Lactobacillus sakei Rits 9. *Applied and Environmental Microbiology*. 74, 1394-1401.

© COPYRIGHT UPM

Andreeva, A. E., Borissova, B. E., Mironova, R., Glykos, N.M., Kotsifaki, D., Ivanov, I., Krysteva, M and Kokkinidis, M. 2000. Crystallization of type I chloramphenicol acetyltransferase: an approach based on the concept of ionic strength reducers. *Acta Crystallographica*. 56, 101-103.

Andrews, J. M. 2001. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*. 48, 5-16.

Avorn, J.l., Barrett, J.F., Davey, P.G., McEwen, S.A., O'Brien, T.F and Levy, S.B. 2001. Antibiotic resistance: synthesis of recommendations by expert policy groups. Alliance for the prudent use of antibiotics. World Health Organization.

Azemun, P., Stull, T., Roberts, M., and Smith, A.L. 1981. Rapid detection of Chloramphenicol Resistance in *Haemophilus influenza. Antimicrobial Agents and Chemotherapy.* 20, 168-170.

Bager, F., Madsen, M., Christensen, J., Aarestrup, F.M. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* in Danish poultry and pig farms. *Preview Veterinary Medicine.* 31, 95-112.

Baquero, F., Martìnez, J.L. and Cantòn, R. 2008. Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*. 19, 260-265.

Barnes A.C., Hasting T.S., Amyes G.B. 1994. Amoxicillin resistance in Scottish isolates of *Aeromonas salmonicida*. *Journal of Fish Diseases*. 17, 357-363.

Bartlett, J. M. S and Stirling, D. 2003. A short history of the polymerase chain reaction. *Methods in Molecular Biology*. 226, 3-6.

© COPYRIGHT UPM

Bauer, A. W., Kirby, W. M. M., Sherris, J. C and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 45, 493- 496.

Belogurov, A. A., Delver, E. P., and Rodzevich, O. V. 1993. Plasmid pKM101 encodes two nonhomologous antirestriction proteins (*ArdA* and *ArdB*) whose expression is controlled by homologous regulatory sequences. *Journal of Bacteriology*. 175, 4843-4850.

Benbrook, C. M. 2002. Antibiotic Drug Use in U.S. Aquaculture. The Northwest Science and Environmental Policy Center Sandpoint, Idaho. *www.epa.gov/ppcp/links.html*. Accessed on 23 October 2010.

Bennet, P.M. 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*. 153, 347-357.

Bhattacharya, M., Choudhury, P. and Kumar, R. 2000. Antibiotics and metal-resistant strains of *Vibrio parahaemolyticus* isolated from shrimps. *Microbiology of Drug Resistant*. 6, 171-172.

Birnboim, H.C. and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Research*. 7, 1513-1523.

Bischoff, K. M., White, D.G., Hume, M. E., Poole, T. L., Nisbet D. 2005. The chloramphenicol resistance gene cmlA is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS Microbiology Letters*. 243, 285-291.

Bischoff, K.M., White, D.G., McDermott, P.F., Zhao, S., Maurer, J.J., Gaines, S. and Nisbet, D.J. 2002. Characterization of chloramphenicol resistance in b-hemolytic *Escherichia coli* associated with diarrhea in neonatal swine. *Journal of Clinical Microbiology*. 40, 389-394.

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Boerlin, P., Wissing, A., Aarestrup, F.M., Frey, J., Nicolet, J. 2001. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in *enterococci* from pigs. *Journal Clinical Microbiology*. 39, 4193-4195.

Brown, K. D., Kulis, J., Thomson, B., Chapman, T. H., Mawhinney, D. B. 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. *Science of the Total Environment.* 366, 772-783.

Brunel, F. and Davison, J. 1979. Restriction insensitivity in bacteriophage T5 III: Characterization of *Eco*R1 sensitive mutants by restriction analysis. *Journal Molecular Biology*. 128, 527-543.

Bundock, P. and Hooykaas, P. J. 1996. Integration of *Agrobacterium tumefaciens* T-DNA in the *Saccharomyces cerevisiae* genome by illegitimate recombination. *Proceeding of National Academy of Scienc, USA.* 93, 15272-15275.

Catry, B., Laevens, H., Devriese, L.A., Opsomer, G and De Kruif, A. 2003. Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology Therapy*. 26, 81-93.

Charles, I.G., Keyte, J.W. and Shaw, W.V. 1985. Nucleotide sequence analysis of the *cat* gene of Proteus mirabilis, comparison with the type I (*Tn9*) *cat* gene. *Journal of Bacteriology*. 164, 123-129.

Chilley, P. M. and Wilkins, B. M. 1995. Distribution of the *ardA* family of antirestriction genes on conjugative plasmids. *Microbiology.* 141, 2157-2164.

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews.* 65, 232-260.

© COPYRIGHT UPM

Clewell, D.B., Flannagan, S.E. and Jaworski, D.D. 1995. Unconstrained bacterial promiscuity: The Tn*916*-Tn1*545* family of conjugative transposons. *Trends Microbiology*. 3, 229–236.

Cole, E.C., Addison, R.M., Rubino, J.R., Leese, K.E., Dulaney, P.D., Newell, M.S., Wilkins, J., Gaber, D.J., Wineinger, T and Criger, D.A. 2003. Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *Journal of Applied Microbiology*. 95, 664-676.

Couturier, M., Bex, F., Bergquist, P.L. and Maas, W.K. 1988. Identification and classification of bacterial plasmids. *Microbiology Review*. 52, 375-95.

Dalsgaard, I., Nielsen, B. and Larsen, J.L. 1994. Characterization of *Aeromonas salmonicida* subsp. *salmonicida*: a comparative study of strains of different geographic origin. *Journal of Applied Bacteriology*. 77, 21-30.

Dang, H., Zhang, X., Song, L., Chang, Y., Yang, G., 2007. Molecular determination of oxytetracycline-resistant bacteria and their resistance genes from mariculture environments of China. *Journal of Applied Microbiology*.103, 2580-2592.

Dang, H., Zhao, J., Song, L., Chen, M. and Chang, Y. 2009. Molecular characterizations of chloramphenicol and oxytetracycline-resistant bacteria and resistance genes in mariculture waters of China. *Marine Pollution Bulletin*. 58, 987-994.

Davies, J. 1996. Origins and evolution of antibiotic resistance. *Microbiologia*. 12, 9-16.

Davison, J.1999. Genetic exchange between bacteria in the environment. *Plasmid*. 42, 73-91.

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de la Cruz, F and Davies J. 2000. Horizontal gene transfer and the origin of species: Lessons from bacteria. *Trends in Microbiology*. 8, 128-133.

De Souza, M. J., Nair, s., Loka bharathi, P.A. and Chandramohan, D. 2006. Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic Marine waters. *Exotoxicol*. 15, 379-384.

Dib, J. R., Weiss, A., Neumann, A., Ordoñez, O., Estévez, M.C. and Farías, M. A. 2009. Isolation of bacteria from Remote High Altitude Andean Lakes able to grow in the presence of antibiotics. *Recent Patents on Anti-Infective Drug Discovery*. 4, 66-76.

DOF. 2009. Peraturan-peraturan perikanan (kawalan kualiti ikan bagi eksport ke kesatuan eropah) 2009. Department of Fisheries. *Ministry of Agriculture and Agro-based Industry Malaysia.* Accessed on 13 Dec 2010.

DOF. 2011. In press. Restaurants and hotels to offer 'green menu'. http://www.fis.com/fis Accessed on 17 February 2011.

Ehrlich, J. Q., Bartz, Q. R., Smith, R. M., Joslyn, D. A. and Burkholder, P. R. 1947. Chloromycetin a new antibiotic from a soil actinomycete. *Science.* 106, 417.

Fabiansson, S., Nilsson, T., Backstrom, J. 1976. Tissues concentrations of chloramphenicol after intramuscular injection in pigs. *Journal of Science Food Agriculture*. 27, 1156–1162.

Frederick, A. 1999. Use of antimicrobial agents in aquaculture: potential for public health impact. A report from Medical Epidemiologist, Foodborne and Diarrheal Diseases Branch Division of Bacterial and Mycotic Diseases, National Center for Infectious Disease. *National Aquaculture Association.* 1-5.

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Goldburg, R., Rebecca, J. and Triplett, T. 1997. Murky Waters: Environmental Effects of Aquaculture in the United States, New York: *Environmental Defense Fund*.

Gorman, C.M., Moffat, L.F. and Howard, B.H. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Molecular Cellular Biology*. 2, 1044-1051.

Grisez, L. 2005.Alternative Options to Antibiotics. Workshop on: Antibiotic Resistance in Asian Aquaculture environments. *www.medinfo.dist.unige.it/Asiaresist/ workshop.* Accessed on 10 April 2007.

Hanekamp, J. C. 2002. Chloramphenicol in shrimp. *Global Aquaculture Advocate October*, 64-68.

Harwood, C. R., Williams, D. M. and Lovett, P. S. 1983. Nucleotide sequence of *Bacillus pumilus* gene specifying *chloramphenicol acetyltransferase*. *Gene*. 24, 163-169.

Heinemann, J. A. and Sprague, G. F. Jr. 1989. Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature.* 340, 205–209.

Heppell, J and Davis, H.L. 2000. Application of DNA vaccine technology to aquaculture. *Advanced Drug Delivery Reviews*. 43, 29-43.

Ho, S. P., Hsu, T. Y. and Wang, W. S. 2000. Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. *Journal Veterinary Medicine Science.* 62, 479 - 485.

Huggins, A. S., Bannam, T. L. and Rood, J. I. 1992. Comparative sequence analysis of the *cat* B gene from Clostridium butyricum. *Antimicrobial Agents Chemotherapy*. 36, 2548-2551.

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Hummel, A., Holzapfel, W. H. and Franz, C. M. A. P. 2007. Characterisation and transfer of antibiotic resistance genes from *enterococci* isolated from food. *Systematic and Applied Microbiology.* 30, 1-7.

Junaidi, C. A. 2010. The aquaculture prospect in Malaysia. In Malaysia-Norway Aquaculture Seminar towards High Technology and Sustainable Aquaculture Industry in Malaysia. Department of Fisheries Malaysia. www.dof.gov.my**.** Accessed on 1 Dec 2010.

Kingsman, A. and Willetts, N. J. 1978. The requirements for conjugal DNA synthesis in the donor strain during flac transfer. *Journal of Molecular Biology.* 122, 287-300.

Kümmerer, K. 2004. Resistance in the environment. *Journal of Antimicrobial Chemotherapy*. 54, 311-320.

Lawrence, J. G. 1997. Selfish operons and speciation by gene transfer. *Trends Microbiology.* 5, 355-359.

Leslie, A. G. W. 1990. Refined crystal structure of type III chloramphenicol acetyltransferase at 1.75 Å resolution. *Journal of Molecular Biology.* 213, 167-186.

Li, J., Yie, J., Foo, W.T., Ling, Julia, M.L., Huaishu, X. and Norman, Y.S 2003. Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured silver sea bream, *Sparus sarba. Marine Pollution Bulletin*. 39, 45-49.

Lipps, G. editor.2008. Plasmids: Current Research and Future Trends. *Caister Academic Press.*

Lyon, B. R., Skurray, R. A. 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiological Reviews.* 51, 88-134.

© COPYRIGHT UPM

Macrina, F.L., Kopecko, D.J., Jones, K.R., Ayers, D.J. and McCowen, S. M. 1978. A multiple-plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. *Plasmid.* 1, 417-420.

Maiti, N. K., Mandal, A. S., Mohanty and Mandal, R.N. 2009. Phenotypic and genetic characterization of *Edwardsiella tarda* isolated from pond sediments. *Comparative Immunology, Microbiology and Infectious Diseases*. 32, 1-8.

Marks, H., Van Duijse, J.J.A., Zuidema, D., Van Hulten, M.C.W., Vlak, J.M. 2005.Fitness and virulence of an ancestral White Spot Syndrome Virus isolate from shrimp. *Virus Research*.110, 9-20.

Marqueaz, B. 2005. Bacterial efflux systems and efflux pumps inhibitors. *Biochimie.* 87, 1137-1147.

Mazel, D. and Davies, J. 1999. Antibiotic resistance in microbes. *Cell Molecular Science*. 56, 742-754.

McArthur, J. V. and Tuckfield, R.C. 2000. Spatial patterns in antibiotic resistance among stream bacteria: effects of industrial pollution. *Applied Environmental Microbiology*. 66, 3722-3726.

McManus, M. C. 1997. Mechanisms of bacterial resistance to antimicrobial agents. *American Journal of Health System Pharmacy*. 54,1420-33.

Meyer, L.W. 1988. Use of plasmid profiles in epidemiologic surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance. *Clinical Microbiology Review*. 1, 228-243.

Moffatt, B. A. and Studier, F. W. 1988. Entry of bacteriophage T7 DNA into the cell and escape from host restriction. *Journal of Bacteriology*. 170, 2095-2105.

© COPYRIGHT UPM

Molina-Aja, A., Garcia-Gasca, A., Abreu-Grobois, A., Bolan-Mejia, C., Roque, A. and Gomez-Gill, B. 2002. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured *penaied* shrimp. *Microbiology Letters.* 213, 7-12.

MPEDA, 2002. Marine Products Export Development Authority Notification No. MAS/12/80-2002/QC dt. 20.05.2002. Ministry of Commerce and Industry, Government of India, Chennai.

Munns, R.K., Holland, D.C., Roybal, J.E., Storey, J.M. and Long, A.R. 1994. Gas chromatographic determination of chloramphenicol residues in shrimp. Interlaboratory study. *Journal of AOAC International*. 77, 596-601.

Murray, I.A. and Shaw, W.V. 1997. *O*-acetyltransferases for chloramphenicol and other natural products. *Antimicrobial Agents Chemotherapy*. 41, 1-6.

Murray, I.A., Gil, J.A., Hopwood, D.A. and Shaw, W.V. 1989. Nucleotide sequence of the chloramphenicol acetyltransferase gene of Streptomyces acrimycini. *Gene*. 85, 283-291.

Murray, I.A., Martinez-Suarez, J.V., Close, T.J. and Shaw, W.V. 1990. Nucleotide sequences of genes encoding the type *II chloramphenicol acetyltransferases* of *Escherichia coli* and *Haemophilus influenzae*, which are sensitive to inhibition by thiol reactive reagents. *Biochemistry Journal*. 272, 505-510.

National Committee for Clinical Laboratory Standards. 1993. Performance Standards for antimicrobial Disc Susceptibility Tests: tentative Standards, 13, 24 NCCLS document M2–A5. National Committee for Clinical Laboratory Standards, Vallinova, PA.

Neu, H. C. 1992. The crisis in antibiotic resistance. *Science*. 257, 1064-1073.

© COPYRIGHT UPM

Nikaido, H. 2001. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. *Seminars in Cell and Developmental Biology*. 12, 215-223.

O'Brien, T. F. 2002. Emergence, spread and environmental effect of antimicrobial resistance: how use of antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clinical Infectious Disease*. 34, 78-84.

O'Reilly, A. and Smith, P. 1999. Development of methods for predicting the minimum concentrations of oxytetracycline capable of exerting a selection for resistance to this agent. *Aquaculture.* 180, 1-11.

Okwumabua, O., O'Connor, M. and Shull, E. 2003. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. *FEMS Microbiology Letters*. 218, 79-84.

Othman, M. F. 2006. Recent report on coastal marine aquaculture status in Malaysia. Department of Fisheries Malaysia. *Ministry of Agriculture and Agro-based Industry Malaysia. www.dof.gov.my.* Accessed on 1 Jan 2010.

Padilla, C., Lobos, O., Brevis, P., Abaca, P., Hubert, E., 2004. In vitro antibacterial activity of the peptide PsVP-10 against antimicrobial-resistant *Enterococcus faecalis* isolated from clinical samples. *Journal of Antimicrobial Chemotherapy*. 53, 390-392.

Parkhill, J., Wren, B.W., Thomson, N.R., Titball, R.W., Holden, M.T.G., Prentice, M.B., Sebaihia, M., James, K.D., Churcher, C., Mungall, K.L., Baker, S., Basham, D., Bentley, S.D., Brooks, K., Cerdeno-Tarraga, A.M., Chillingworth, T., Cronin, A., Davies, R.M., Davis, P., Dougan, G., Feltwell, T., Hamlin, N., Holroyd, S., Jagels, K., Leather, S., Karlyshev, A.V., Moule, S., Oyston, P.C.F., Quail, M., Rutherford, K., Simmonds, M., Skelton, J., Stevens, K., Whitehead, S. and Barrell, B.G. 2001. Genome sequence of Yersinia pestis, the causative agent of plague. *Nature*. 413, 523–527.

© COPYRIGHT UPM

Petersen, A., Andersen, J.S., Kaewmak, T., Somsiri, T., Dalsgaard, A. 2002. Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Applied Environmental Microbiology.* 68, 6036-6042.

Phuong, N.T., Oanh D.T.H., Dung T.T. and Sinh L.X. 2005. Bacterial Resistance to Antimicrobials uses in and fish farms in the Mekong Delta, Vietnam. Workshop on: Antibiotic Resistance in Asian Aquaculture Environments. *www.medinfo.dist.unige.it/Asiaresist/ workshop.* Accessed on 10 April 2007.

Rhodes, G., Huys, G., Swings, J., Mcgann, P., Hiney,M., Smith, P., and Roger W. Pickup, W. R. 2000. "Distribution of Oxytetracycline Resistance Plasmids between *Aeromonads* in Hospital and Aquaculture Environments: Implications of TN1 721 in Dissemination of the Tetracycline Resistance Determinant Tet A." *Applied Environmental Microbiology*. 66, 3883-3890.

Roberts, M., Corney, A. and Shaw, W.V. 1982. Molecular characterization of three chloramphenicol acetyltransferases isolated from *Hemophilus influenzae*. *Journal of Bacteriology*. **151,** 737-741.

Salyers, A. A., and Shoemaker, N. B. 1996. Resistance gene transfer in anaerobes: New insights, new problems. *Clinical Infectious Disease.* 23, 36-43.

Sambamurty, A.V.S.S. 2008. Plasmids. Molecular Biology. *Alpha Science International Ltd*. 35.1- 35.14.

Samuelsen, O.B., Hjeltnes, B. and Glette, J. 1998. Efficacy of orally administered florfenicol in the treatment of furunculosis in Atlantic salmon. *Journal of Aquaculture Animal Health.* 10, 55-61.

© COPYRIGHT UPM

Samuelsen, O.B., and Bergh, Ø. 2004. Efficacy of orally administered florfenicol and oxolinic acid for the treatment of vibriosis in cod (*Gadus morhua*). *Aquaculture*. 235, 27-35.

Schroder, G., Krause, S., Zechner, E.L., Traxler, B., Yeo, H.J., Lurz,R., Waksman, G. and Lanka, E. 2002. TraG-like proteins of DNAtransfer systems and of the Helicobacter pylori type IV secretion system: inner membrane gate for exported substrates. *Journal of Bacteriology*. 184, 2767-2779.

Schwarz, S. and Chaslus-Dancla, E. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary Research*. 32, 201-225.

Schwarz, S. and Cardoso, M. 1991. Nucleotide sequence and phylogeny of a chloramphenicol acetyltransferase encoded by the plasmid pSCS7 from *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy*. 35**,** 1551-1556.

Schwarz, S., Kehrenberg, C., Doublet, B., Cloeckaert, A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews*. 28, 519-542.

Shakila, R. J., Vyla, S. P., Kumar, R. S., Jeyasekaran, G., Jasmine, G. I. 2006. Stability of chloramphenicol residues in shrimp subjected to heat processing treatments. *Food Microbiology*. 23, 47-51.

Shaw, W.V. 1983. Chloramphenicol acetyltransferase, enzymology and molecular biology. *Critical Reviews in Biochemestry*. 14, 1-46.

Simon, C. and Stille, W. 2000. Chloramphenicol. In: Antibiotika- Therapie in Klinik und Praxis. 172-175.

© COPYRIGHT UPM

Son, R., Zulkifli, Y., Alitheen, N.B., Raha, A.R., Yeap, S. K., Marlina and Nishibuchi, M. 2009. Antibiotic resistance and plasmid profiling of *Vibrio arahaemolyticus* isolated from cockles in Padang, Indonesia. *International Food Research Journal.* 16, 53-58.

Son, R., Nasreldin, E.H., Zaiton, H., Samuel, L., Rusul, G. and Nimita, F. 1998. Characterisation of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. *FEMS Microbiology Letters*. 165, 139-143.

Spratt, B. G. 1994. Resistance to antibiotics mediated by target alterations. *Science*. 264, 375-82.

Stanisich, V.A. 1988. Identification and analysis of plasmids at the genetic level. Methods in Microbiology. *Academic Press London.* 21, 11-47.

Stentiford, G.D., Bonami, J.R. and Alday-Sanz, V. 2009. A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European legislation. *Aquaculture*. 291, 1-17.

Sun, K., Wang, H. L., Zhang, M., Xiao, Z. Z. and Sun, L. 2009. Genetic mechanisms of multi-antimicrobial resistance in a pathogenic *Edwardsiella tarda* strain. *Aquaculture.* 289, 134-139.

Syvanen, M. 1994. Horizontal gene transfer: Evidence and possible consequences. *Annual Review Genetic.* 28, 237-261.

Szpirer, C.Y., Faelen, M. and Couturier, M. 2000. Interaction between the RP4 coupling protein TraG and the pBHR1 mobilization protein Mob. *Journal of Molecular Microbiology*. 37, 1283- 1292.

© COPYRIGHT UPM

Tan, C. K. 1998. Overview of aquaculture in Malaysia. In Gopinath, N., and Tarlochan S. editors. Aquaculture practice in Malaysia. *Malaysian Fisheries Society*. 1-13.

Tendencia E.A. and Lavilla-Pitogo C.R. 2005. Antimicrobial R Resistance in Bacteria Isolated from Aquaculture Environments in the Philippines. Workshop on*:* Antibiotic Resistance in Asian Aquaculture Environments. *www.medinfo.dist.unige.it/Asiaresist/ workshop.* Accessed on 10 April 2007.

Tenover, F.C. 2006. Mechanisms of antimicrobial resistance in bacteria. *American Journal Infectious Control.* 34, S3-S10.

Tenover, F.C. and Hughes, J.M. 1996. Development and spread of multiply-resistant bacteria pathogens. *The Journal of the American Medical Association*. 275, 300-304.

Thomas, M. and File, Jr. 1999. Overview of Resistance in the 1990s. *CHEST***.** 115, 3-8.

Tiainen, T., Pedersen, K., Larsen, J.L. 1995. Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar *O*2 and *Vibrio ordalii*. *Journal of Applied Bacteriology*. 79, 384-392.

Trieu-Cuot, P., de Cespedes, G. and Horaud, T. 1992. Nucleotide sequence of the chloramphenicol resistance determinant of the streptococcal plasmid pIP501. *Plasmid*. 28, 272- 276.

Van den Bogaard, A.E.J.M. and Stobberingh, E.E. 2000. Epidemiology of resistance to antibiotics links between animals and humans. *International Journal of Antimicrobial Agents*. 14, 327-335.

© COPYRIGHT UPM

Van der Meer, J. R., de Vos, W. M., Harayama, S., and Zhender, A. J. B. 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. *Microbiology Review*. 56, 677-694.

Van, T. T. H., Moutafis, G., Tran, L. T. and Coloe, J. P. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Applied and Environmental Microbiology*. 73, 7906-7911.

Verner- Jeffreys, D.W., Algoet, M., Pond, M. J., Virdee, H. K., Bagwell, N.J., Roberts, E.G.2007. Furunculosis in Atlantic salmon (*Salmo salar* L.) is not readily controllable by bacteriophage therapy. *Aquaculture*. 270,475- 484.

Waksman, S. A. 1947. What is an antibiotic or an antibitic substance. *Mycologia* . 39, 565- 569.

Walton, J. R. 1992. Use of antibiotics in veterinary practice. *Journal of Medical Microbiology*. 36, 69- 70.

Waters, V.L. 1999. Conjugative transfer in the dissemination of beta-lactam and aminoglycoside resistance. *Frontiers in Bioscience*. 4, 416-439.

Wegener, H.C., Aarestrup, F.M., Jensen, L.B., Hammerum, A.M., Bager, F., 1999. Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emergence Infectious Disease*. 5, 329-335.

Wilkins, B. M., Chilley, P. M., Thomas, A. T., and Pocklington, M. J. 1996. Distribution of restriction enzyme recognition sequences on broad host range plasmid RP4: Molecular and evolutionary implications. *Journal of Molecular Biology*. 258, 447-456.

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Willetts, N. and Wilkins, B. 1984. Processing of plasmid DNA during bacterial conjugation. *Microbiology Review.* 48, 24-41.

Yao, J. D. C. and Moellering Jr. R.C. 1999. Manual of Clinical Microbiology. In Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., and Yolken, R.H. editors. ASM *Press, Washington, D.C.* 1474-505.

Yoo, M.H., Huh, M.D., Kim, E.H., Lee, H.H. and Jeong, H.D. 2003. Characterization of *chloramphenicol acetyltransferase* gene by multiplex polymerase chain reaction in multidrug-resistant strains isolated from aquatic environments. *Aquaculture*. 217, 11-21.

Zaidenzaig, Y. and Shaw, W.V. 1976. Affinity and hydrophobic chromatography of three variants of chloramphenicol acetyltransferase specified by R factors in *Escherichia coli*. *FEBS Letters.* 62, 266-271.

Zechner, E.L., de la Cruz, F., Eisenbrandt, R., Grahn, A.M., Koraimann, G., Lanka, E. 2000. Conjugative- DNA Transfer Processes. In Thomas, C.M. editor. *The Horizontal Gene Pool. Harwood Academic Press*. 87-173.

Zhao, J. and Aoki, T. 1992. Cloning and nucleotide sequence analysis of a chloramphenicol acetyltransferase gene from *Vibrio anguillarum*. *Microbiology Immunology*. 36, 695-705.

Zupan, J. R., and Zambryski, P. 1995. Transfer of T-DNA from *Agrobacterium* to the plant cell. *Plant Physiology.* 107, 1041-1047.

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