NAA & Antibiotic Use in Aquaculture:
Center for Disease Control Rebuttal

Memorandum

Date: December 20, 1999

From: NAA, Aquatic Animal Health Committee

Subject: Review of Dr. Fred Angulo's memo, October 18, 1999 titled: Use of antimicrobial agents in aquaculture: potential for public health impact.

This is a review of Dr. Fred Angulo's analysis (attached) of the scientific literature regarding the potential public health impact of the use of antimicrobial agents in aquaculture. Fred Angulo is a medical epidemiologist with the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). The National Aquaculture Association had challenged the lobbying efforts of Dr. Angulo in a letter dated September 24, 1999 and had requested the factual basis for his comments that certain US aquaculture practices posed a significant risk to public health.

The CDC analysis pieced together selected literature from peer reviewed journals in its attempt to substantiate their concerns. This was done presumably because there is no substantive evidence for aquaculture practices endangering public health. The CDC analysis made gross generalizations, assumptions and extrapolations in order to support their position. In at least one instance they have misread the scientific literature. CDC did not cite any evidence of problems potentially associated with US aquaculture practices but instead relied on isolated instances occurring in other countries. The CDC did not critically examine scientific literature that suggests factors other than antibiotics may account for aquatic bacteria that demonstrate antibiotic resistance. The CDC did not differentiate US aquaculture practices from those occurring elsewhere. There does not appear to be any recognition for the differences in the restrictive drug approval process in the US compared to other countries. There does not appear to be any recognition for differences in hygiene between countries and these differences create different potential to impact human health. There does not appear to be any recognition for the differences in husbandry practices in the US compared to other countries. All of these factors create a barrier to resistance transfer and could substantially impact relative risk.

The CDC analysis correctly identifies the potential for antimicrobial use under aquaculture conditions to select for bacteria resistant to an antibiotic. This is in agreement with widely documented scientific evidence that antimicrobial use of any extent in any environment is likely to select for antibiotic resistant bacteria in that environment (e.g. O'brien et al. 1987; Courcol et al. 1989; Inglis et al. 1993; Lewis 1995; Gaynes 1997; Levy 1997; Cristino 1999). Most scientific evidence highlights antibiotic resistance development in the human hospital setting although modifying factors in this environment have been identified (Gaynes and Monnet 1997). There are only a few reports in the international and US scientific literature describing the occurrence of resistant bacteria following use of antibiotics for therapeutic purposes in aquaculture (e.g. Tsoumas et al. 1989; Cooper et al. 1993; Starliper 1993). On the other hand, antibiotic resistance has also been observed in bacteria obtained from wild fish. Pettibone et al. (1996) isolated multiple antibiotic resistant *Aeromonas* spp. from wild brown bullhead (*Ictalurus nebulosus*) captured from the Buffalo River in New York. The assumption in this research was that extensive contamination of the Buffalo River with antibiotics from sewage outfalls led to the occurrence of antibiotic resistant aeromonads that could infect or colonize fish. Several others (e.g. Smith 1970; Grabow and Prozesky 1973; Baya et al. 1986; and Hirsch et al. 1999) have documented that antibiotics or antibiotic resistant bacteria can occur in sewage. Antibiotic resistant bacteria may also occur in the apparent absence of antibiotic use. Baya et al. (1986) found that nearly 24% of bacteria from clean water open ocean sites were resistant to penicillin and 14% were resistant to erythromycin. Spanggaard et al. (1993) noted 6% of bacterial isolates obtained from an unpolluted stream in Denmark were resistant to oxytetracycline. The factors responsible for the occurrence of antibiotic resistance in the absence of antibiotic use were not elucidated but deserve careful consideration. McPhearson et al. (1991), Kapetanaki et al. (1995), and Vaughan et al. (1996) report that other factors such as relatively high levels of nutrients can give rise to increases in the frequency of resistant bacteria in aquatic environments. These bacteria appear to be tolerant of antibiotic because of membrane mediated resistance (Smith et al. 1997) that is not plasmid mediated and occurs even in the absence of antibiotics. More recently Gilliver et al. (1999) report a significant level of antibiotic resistant *Enterobacter* from wild rodents living in an environment free of antibiotics attributed to human usage. The factors accounting for prevalence of this resistance are also unknown.

The CDC did not critically evaluate differences in resistance breakpoints amongst the scientific literature they cited. While this may have been beyond the scope of their assignment, the issue merits considerable examination. The danger of assuming all literature published is relevant and indicative of real resistance was recently illustrated by Ewert (1998). Ewert (1998) compared the resistance breakpoints used by the United Kingdom’s Laboratory of Enteric Pathogens (LEP) to the breakpoints used by the US National Committee for Clinical Laboratory Standards (NCCLS) for *Salmonella typhimurium* and ciprofloxacin. The LEP breakpoint is 0.25 µg/ml while the NCCLS breakpoint is 4 µg/ml. Clearly, international standardization of resistance criteria is essential if decisions about public health risk are to be made. Such analysis of the CDC literature cited was also beyond the scope of our analysis.

The CDC analysis correctly identifies that resistance can occur among aquatic bacteria that are not fish pathogens. The evidence cited above supports this. However, the CDC analysis suggests resistance will not occur in areas not subjected to antibiotic use. This is incorrect as identified above (e.g. Baya et al. 1986; Spanggaard et al. 1993: and Gilliver et al. 1999). The CDC analysis of the literature cited (Ervik et al. 1994) is in error. Ervik et al. (1994) did not examine the prevalence of antibiotic resistant bacteria in fin fish from untreated areas as suggested by CDC. Only blue mussels (*Mytilus edulis*) were in fact examined up to 500 m away (a reference station) from a marine net pen fish farm. A greater prevalence of resistant bacteria were isolated from the blue mussels collected next to the farm but resistance was still detectable even at the reference station. Factors accounting for resistance distant from the fish farm were not explored but could be related to factors independent of antibiotic use (McPhearson et al. 1991; Kapetanaki et al. 1995; Vaughn et al. 1996; and Smith et al. 1997). Ervik et al. (1994) did examine the concentration of oxolinic acid and flumequine antibiotics in blue mussel and wild fish tissue near the net pen. Highly variable concentrations were detected ranging from 0.95 to 4.89 µg $g.^{-1}$ In a similar but more extensive examination of blue mussels, Coyne et al. (1997) examined the concentration of oxytetracycline. Oxytetracycline was not detected in mussels collected 20 m from the net pen. Coyne et al. (1997) observed significant concentrations of oxytetracycline in mussels but the presence was extremely transient and affected mussels were confined to the immediate proximity of the cages. These authors suggested that the most prudent action to limit any potential human health risk was to educate farm personnel as to the risks. The implication is to refrain from eating mussels obtained from the immediate vicinity of the net pens if there was concern by the employee.

The CDC correctly states that antimicrobial resistant bacteria occurring in aquaculture environments could transfer resistance factors to other bacteria but how frequently this occurs in the environment is not known. We believe a similar transfer can occur from other bacteria to those occurring in the aquaculture environment. Thus, under artificial environments, Kruse and Sørum (1994) demonstrated that resistance plasmids could be transferred from the human pathogen *Vibrio cholerae* to the fish pathogen *Aeromonas salmonicida* and from a bovine *E. coli* isolate to *A. salmonicida*. What is not clear is how successful such transfer would be under real conditions. Kruse and Sørum (1994) used simulated natural environments and controlled laboratory conditions attempting to answer this question. Factors that may influence the transfer probability include probability of sharing the same environment, bacterial abundance and temperature. Clearly additional study is required if the significance of such transfer potential from fish pathogens or aquatic bacteria to human pathogenic bacteria is to be properly determined. The CDC analysis did not address the probability of this occurring. At lease some of the antibiotic resistance detected in aquatic bacteria is not transferable (Wood et al. 1986; Griffiths and Lynch 1989; Piddock et al. 1989; and Barnes et al. 1990).

The CDC analysis correctly identified a **possibility** of the transmission of bacteria present under aquaculture conditions to humans however the CDC did not address the **probability** of such transfer. Aquaculture is unique in that zoonotic bacteria are rare in most aquatic animals. The CDC also did not address the factors accounting for such transfer when it did occur, which is rare. Only rarely have people become infected with bacteria present in or on fish and these are usually associated with conditions of poor hygiene or consumption of raw products. Fish are poikilothermic animals such that few bacterial pathogens of farmed fish in temperate climates are capable of infecting humans (Alderman and Hastings 1998). Rarely can even warm water fish pathogens or aquatic bacteria occur in humans. Thus, there are isolated reports of various bacteria infecting both fish and humans; for example *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pleisiomonas shigelloides*, and *Streptococcus iniae* (Smith et al. 1994; Weinstein et al. 1997). While the relative frequency of such infections in humans appears low, they can occur. Infection with *S. iniae* occurred in personnel involved in the processing of *Tilapia* but has not been reported since its initial detection. CDC describes a scenario they believe suggests that human infection with antibiotic resistant *Vibrio cholera* occurred because of the transfer of resistance factors from non-cholera *Vibrio* present in shrimp from Ecuador shrimp farms. While such a transfer scenario is plausible, the probability of this occurring is unknown and likely very small. More probable alternative explanations can be presented. An epidemiologic case-control study of the cholera epidemic in Ecuador identified several risk factors for infection that included drinking unboiled contaminated water, drinking a beverage from a street vendor, eating raw seafood, and eating cooked crab (Weber et al. 1994). These risk factors suggest poor hygiene was a significant factor in the Ecuador cholera epidemic. Another possibility, for example, is that delivery and use of antibiotics in Ecuador are not well regulated for shrimp or for humans. Unregulated human use of antibiotics could promote occurrence of antibiotic resistant *V. cholera* in human carriers, fecal matter and sewage. Subsequent disposal of sewage could contaminate shrimp farms and farm workers. Antibiotics used in Ecuador for humans or for shrimp may also be substandard (Arya 1999). Substandard drugs, whose activity is greatly diminished, could increase the probability of developing bacterial antibiotic resistance. The CDC analysis also cites the occurrence of *Vibrio vulnificus* infections amongst individuals handling live *Tilapia* produced in Israel (Bisharat and Raz 1996). In the Israeli incident, *Tilapia* were uncharacteristically marketed alive rather than euthanized. Subsequent handling of the live fish led to various penetrating wounds from the spines of the *Tilapia*. The source of the bacteria associated with the infections may have been bacteria carried by live *Tilapia*. Proper food handling is a well-known preventive for minimizing food borne illness. In Israel, when the fish were no longer marketed live, human *Vibrio* infections ceased to occur (Bisharat and Raz 1996). Various *Vibrio* species are normal bacterial flora in the marine environment (Baumann et al. 1984) so it is to be expected that fish obtained from that environment would have these bacteria present. It is probably appropriate to assume infections could occur again if live fish are not handled carefully. Proper handling may have a significant impact on the prevalence of food borne pathogens. Parsonnet and Kass (1987) found very low prevalence of infection with antibiotic resistant *E. coli* among female poultry abattoir workers exposed to considerable amounts of antibiotic resistant bacteria. While not absolute protection, it seems reasonable to believe that washing hands minimizes infections from poultry borne *Salmonella* and *Campylobacter* as does attention to good processing practices and proper cooking. CDC further cites reference to food borne disease from *V. parahemolyticus* infections in Japan which were linked to farm raised finfish. *V. parahemolyticus* infections may occur as the result of consumption of raw, improperly cooked or recontaminated seafood (Oliver and Kaper 1997). Consumption of raw seafood in the United States is relatively rare (with the exception of raw molluscs) but does occur. CDC also cites isolation of *Salmonella* from farm raised fish and shrimp ponds. Salmonella are ubiquitous in the natural environment (D'Aoust 1997) but whether all species or serovars can cause disease is unknown. CDC implies but does not state that the *Salmonella* from farm raised fish environments could cause human disease. Certain genes are required for full virulence. For example, it is believed that the invasion gene operon, *invA*, is essential in *Salmonella* for full virulence (Galan and Curtiss 1989). Salmonella virulence is also associated with a virulence plasmid *spvC* (Gulig et al. 1993) which is not present is all salmonella. Swamy et al. (1996) demonstrate that non-typhimurium *Salmonella* **infrequently** have the *spvC* plasmid. Björkman et al. (1998) found that most *S. typhimurium* mutants resistant to streptomycin, rifampicin, and nalidixic acid were avirulent in mice suggesting they would also be avirulent in humans. It remains to be demonstrated that *Salmonella* spp from farm raised fish or shrimp ponds might cause human disease and if so under what conditions.

In the United States, the US Food and Drug Administration monitors the occurrence of *Salmonella* in seafood. In the 1998 *Salmonella* in Seafood Assignment (DOEP 98-12) Summary, *Salmonella spp* were detected in 11 of 405 samples tested (2.7%). Of these eleven, eight were from aquaculture operations. All of these had been further processed to some extent so the origin of the *Salmonella* spp. could not be ascertained. The seafood with the highest abundance of *Salmonella* was from wild captured shrimp from India. Hatha and Lakshmanaperumalsamy (1995) sampled the prevalence of *Salmonella* among fish and crustaceans from four major seafood retail outlets in India. A total of 240 *Salmonella* strains were isolated from 1,006 samples (24%). Of these, 9% were resistant to bacitracin, 7% to chloramphenicol and 46% to oxytetracycline. The authors conclude that the high prevalence of resistance was due to the use of human wastewater in aquaculture industries of third world countries. Post-harvest contamination of products might also arise from processing under poor sanitary conditions according to these researchers.

The CDC states that "these and other reports indicate that bacteria present in aquaculture ecosystems can be transmitted to humans." The implication of this statement is that there is something unique about aquaculture ecosystems that promote the occurrence of potentially pathogenic bacteria. While there are indeed reports documenting the occurrence of human pathogens in aquaculture ecosystems, the occurrence of actual human disease associated with these environments is rare. No reports documenting that fish farm workers have a greater prevalence of bacterial disease than those working in other environments could be discovered.

Review of CDC Water Related Disease Reports (St. Louis 1988; Levine et al. 1990) did not document any disease outbreaks associated with US fish farms. The CDC does not currently have a monitoring program directed specifically at aquaculture facilities or areas. Alderman and Hastings (1997) argue that the probability of pathogens from fish farms affecting humans is very low.

CDC cites evidence suggesting that *Salmonella* serotype *typhimurium* definitive type 104 (DT 104) that are tetracycline resistant may have originated in aquaculture. The evidence for this is ascribed to the unique class G resistance gene that was first described in isolates of *V. anguillarum*, a pathogen of fish. CDC further speculates that the novel florfenicol resistance gene (*florR*) in *S. typhimurium* DT 104 came from *Photobacterium (Vibrio?) damsela*, a bacteria found on or in marine fish and in marine environments. These arguments are highly speculative. The detection of a unique class of resistance gene in a fish pathogen for the first time may merely be due to a fortuitous examination of a particular isolate. Bolton et al. (1999) state that florfenicol-resistant *E. coli* are also flo_{st} (which is the same as *florR*) positive and that florfenicol resistance is likely to be more commonly found in other bacterial species if testing is done. Bolton et al. (1999) also suggest that it has not been the use of florfenciol in cattle that accounts for the occurrence and perhaps selection of the flo_{st} geneotype but rather other factors. The CDC argument is further discredited by the recent detection of the class G tetracycline resistance gene in *Pseudomonas* isolates collected from Michigan apple orchards with no or limited history of oxytetracycline useage (Schnabel and Jones 1999). CDC stretches credibility in speculating that the origin of the *S. typhimurium* DT 104 *florR* was from *P. damsela*. The *P. damsela* reported by CDC (but not specifically referenced in their memo) was probably cultured from wild fish not in those raised under aquaculture conditions (Love et al. 1981). In contrast to CDC, Briggs and Fratamico (1999) cite evidence that DT 104 commonly occurs in cattle and has been contracted by humans exposed to cattle. Wall et al. (1995) provide evidence for the transmission of *S. typhimurium* from cattle to man. Reports documenting the transmission of *S. typhimurium* from fish to man could not be found.

CDC speculates that antimicrobial resistance determinants resulting from aquaculture's use of antibiotics are transferred to human pathogens at a frequency greater than previously suggested. In contrast to the CDC speculation, recent reviews (Smith et.al. 1994; Alderman and Hastings 1997) provide evidence that the probability of antimicrobial resistance occurring in human pathogens as a result of the use of antibiotics in aquaculture is low. These authors state that the probability of resistance transfer in the US is even less than in other countries because of the very restrictive approval process and the conditions of use in the US. We add that animal production regimes and management conditions practiced in the US further reduce probability of resistance gene transfer. Most of the aquaculture practiced in the US are freshwater based and most have relatively good control over the effluent through settling ponds that capture solids.

The CDC review is highly speculative and does not address probable risk. The CDC review does not differentiate between aquaculture in the US or in other countries. Aquaculture practices differ dramatically from country to country particularly regarding the use of antibiotics. In Japan for example, 29 antibiotics or combinations may be used (Okamoto 1992) and in Chile 16-17 drugs may be used. In contrast, only two antibacterial drugs are approved, available and used in the US aquaculture food fish industry. Antibiotics are not used in US aquaculture as growth promoters and are only applied for the treatment of certain bacterial diseases. Considerable difference also occurs in the type of water used to cultivate aquatic animals. In China and India for example, waters from human sewage may be used. Kontara and Maswardi (1999) report integrated aquaculture is common in Indonesia. Integrated aquaculture occurs when fecal matter from a poultry operation is deposited in a fish pond to fertilize the pond stimulating algal growth for fish consumption. In the US, this practice does not occur nor is sewage used as a source of aquaculture water. Processing standards are also substantially different. FDA recently instituted a mandatory seafood processor Hazard Analysis Critical Control Point program which provides increased assurance that only approved antibiotics are used and not misused, and processed fish are not likely to contain human pathogens.

CDC has not addressed the issue of whether reduction in the use of antibiotics in US aquaculture would make a significant difference in the prevalence of antibiotic resistant human pathogens.

This is an important question because its answer would have great bearing on how best to address the use of antibiotics in US aquaculture. Conflicting reports, even in hospital settings where success is most likely, make such a judgement difficult. One report (Cristino 1999) suggests reduction in use of macrolides in Danish hospitals has led to a decrease in the prevalence of erthromycin resistant *Staphylococcus pyogenes*. While this requires further exploration and does not address animal agriculture or aquaculture uses, other reports fail to substantiate this observation. Two recent reports (Schragg and Perrot 1996; and Levin et al. 1997) suggest reduction in antibiotic use will have little impact on the prevalence of resistant bacteria. A differential impact between hospitals and other environments might be expected because of differences in dynamics. Over prescription of antibiotic in hospitals is one potential factor. Hospitals are also subject to considerable bacterial migration as patients enter and leave. Such migration and replacement of bacterial populations could occur in an accelerated fashion because of routine sanitation that destroys resident microflora. In contrast, outside the hospital, such sanitation practices are unlikely to occur and change in microbial flora could be slower. Other factors likely to affect resistance gene transfer probability are cell density and donor-recipient compatibility. Considerably more information must be available before informed decisions can be made.

Literature Cited

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

Arya, S.C. 1999. Antibiotic resistance in the two Americas. ASM News 65: 585.

Barnes, A.C., C.S. Lewin, T.S. Hastings and S.G.B. Amyes. 1990. Cross resistance between oxytetracycline and oxolinic acid in *Aeromonas salmonicida* associated with outer membrane changes. Microbiol. Lett., 72: 337-340.

Baumann, P., A.L. Furniss, and J.V. Lee. 1984. Genus 1. Vibrio Pacini 1854, 411. In: Bergey's Manual of Systematic Bacteriology. N.R. Kreig (ed). Williams and Wilkins. Baltimore 518-545.

Baya, A.M., P.R. Brayton, V.L. Brown, D.J. Grimes, E. Russek-Cohen and R.R. Colwell. 1986. Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples. Appl. Environ. Micro. 51: 1285-1292.

Bisharat, N. and R. Raz. 1996. *Vibrio* infection in Israel due to changes in fish marketing. Lancet 384: 1585-1586.

Björkman, J., D. Hughes and D.I. Anderson. 1998. Virulence of antibiotic-resistant *Salmonella typhimurium*. Proc. Natl. Acad. Sci. 95:3949-3953.

Bolton, L.F., L.C. Kelley, M.D. Lee, P. Fedorka-Cray, and J.J. Maurer. 1999. Detection of mutlidrug-resistant *Salmonella enterica* serotype *typhimurium* DT 104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J. Clinical Microb. 37: 1348-1351.

Briggs, C. and P.M. Fratamico. 1999. Molecular characterization of an antibiotic resistance gene cluster of Salmonella typhimurium DT 104. Antimicrob. Agents and Chemo. 43: 846-849.

Cooper, R.K., C. E. Starliper, E.B. Shotts, Jr., and P.W. Taylor. 1993. Comparision of plasmids isolated from Romet-30-resistant Edwardsiella ictaluri and tribrissen-resistant Escherichia coli. J. Aquat. An. Health 5: 9-15.

Courcol, R.J., M. Pinkas, and G.R. Martin. 1989. A seven year survey of antibiotic susceptibility and its relationship with use. J. Antimicrob. Chemother. 23: 441-445.

Coyne, R., M. Hiney and P. Smith. 1997. Transient presence of oxytetracycline in blue mussels (*Mytilus edulis*) following its therapeutic use at a massive Atlantic salmon farm. Aquaculture. 149:175-181.

Cristino, J.M. 1999. Correlation between consumption of antimicrobials in humans and development of resistance in bacteria. Int. J. Antimicrobial Agents 12: 199-202.

D'Aoust, J.Y. 1997. Salmonella species. In: Food Microbiology: Fundamentals and Fronteirs. M.P. Doyle, L.R. Beuchat and T.J. Montville (eds.). ASM Press, Washington, D.C. 129-158.

Ervik, A., B. Thorsen, V. Eriksen, B.T. Lanestead and D.B. Samuelsen. 1994. Impact of administering antibacterial agents on wildfish.. Dis. Aquatic Org. 18:45-51.

Ewert, K. 1998. Emerging fluoroquinolone resistance in food-borne pathogens. Proc. Acad. Vet. Consultants 26: 28-40.

Galan, J.E. and R. Curtiss. 1989. Cloning and molecular characterization of genes whose products allow *S. typhimurium* to penetrate tissue culture cells. Proc. Natl. Acad. Sci. USA 86: 6383- 6387.

Gaynes, R. 1997. The impact of antimicrobial use on the emergence of antibiotic resistance in hospitals. Ciba Foundation Symposium 207: 47-56.

Gaynes, R. and D. Monnet. 1997. The contribution of antibiotic use on the frequency of antibiotic resistance in hospitals. In: Antibiotic resistance: origins, evolution, selection and spread. Wiley, Chichester (Ciba Foundation Symposium 207). p 47-60.

Gilliver, MA., M. Bennett, M. Begon, S.M. Hazel, and C.A. Hart. 1999. Enterobacteria-Antibiotic resistance found in wild rodents. Nature. 401 (6750): 233-234.

Grabow, W.O.K. and O.W. Prozesky. 1973. Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob. Agents and Chemo. 3: 175-180.

Griffiths, S.G. and W.H. Lynch. 1989. Characterisation of *Aeromonas salmonicida* mutants with low level resistance to multiple antibiotics. Antimicrob. Ag. Chemother., 33: 19-26.

Gulig, P.H., H. Dunbara, D.G. Guiney, A.J. Lax, F. Norel, and M. Rhen. 1993. Molecular analysis of *spv* virulence genes of the *Salmonella* virulence plasmids. Mol. Microbiol. 7: 823-830.

Hatha, A.A.. and P. Lakshmanaperumalsamy. 1995. Antibiotic resistance of *Salmonella* strains isolated from fish and crustaceans. Letters in Appl. Micro. 21: 47-49.

Hirsch, R., T. Ternes, K. Haberer and K.L. Kratz. 1999. Occurrence of antibiotics in the aquatic environment. The Science of the Total Environment. 225: 109-118.

Inglis, V, S.D. Miller, and R.H. Richards. 1993. Resistance of *Aeromonas salmoncida* to amoxicillin. J. Fish Dis. 16: 389-395.

Kapetanaki, M., J. Kerry, M. Hiney, C. O'Brien, R. Coyne and P. Smith. 1995. Emergence in oxytetracycline-free marine mesocosms, of microorganisms capable of colony formation on oxytetracycline-containing media. Aquaculture 134: 227-236.

Kontara, E.K. and A. Maswardi. 1999. Present status of common carp farming in Indonesia. World Aquaculture 30: 14-62.

Kruse, H. and H. Sørum, 1994. Transfer of multiple drug resistant plasmids between bacteria of diverse origins in natural microenvironments. Applied and Environmental Microbiology. 60:4015-4021.

Levin, B.R., M. Lipsitch, V. Perrot, S. Schrag, R. Antia, L. Simonsen, N. Moore Walker and F.M. Stewart. 1997. The population genetics of antibiotic resistance. Clinical Inf. Dis. 24(suppl 1): S9-16.

Levine, W.C., W.T. Stephenson and G.F. Craun. 1990. Waterborne disease outbreaks, 1986-1988. CDC MMWR March 01, 1990/39(SS-1): 1-9.

Levy, S.B. 1997. Antibiotic resistance: an ecological imbalance. In *1997 Antibiotic resistance: origins, evolution, selection and spread*. Wiley, Chichester (Ciba Foundation Symposium 207). P 1-14.

Lewis, R. 1995. The rise of antibiotic-resistant infections. FDA Consumer Magazine. 29(7).

Love, M., D. Teebken-Fisher, J.E. Hose, J.J. Farmer III, and G.R. Fanning. 1981. *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish, *Chromis punctipinnis*. Science 214: 1139-1140.

McPhearson, R.M., A. DePaola, S.R. Motes, Jr., and A.M. Guarino. 1991. Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. Aquaculture 99: 203-211.

O'Brien, C. and the Members of Task Force 2. 1987. Resistance of bacteria to antibacterial agents: report of Task Force 2. Rev. Infectious Dis. 9(S3): S244-S260.

Okamoto, A. 1992. Restrictions on the use of drugs in aquaculture in Japan. In: Chemotherapy in Aquaculture: from theory to reality (edited by C. Michel and D.J. Alderman). pp 109-114. Office International de Epizooties, Paris.

Oliver, J.D. and J.B. Kaper. 1997. Vibrio species. In: Food Microbiology: fundamentals and frontiers. (M.P. Doyle, L.B. Benchat and T.J. Montville eds. ASM Press, Washington, DC. 768 pp.

Parsonnet, K.C. and E.H. Kass. 1987. Does prolonged exposure to antibiotic-resistant bacteria increase the rate of antibiotic-resistant infection? Antimicrob. Agents and Chemotherapy 31: 911-914.

Piddock, L., M. Hall, D.J. Giggs, and R. Wise. 1989. Selection and phenotypic characterization of the mechanism of resistance of Enterobacteriaceae to quinolones. Rev. Infect. Dis. 2(S5): S977-S978.

Pettibone, G.W., J.P. Mear and B.M. Sampsell. 1996. Incidence of antibiotic and metal resistance and plasmid carriage in Aeromonas isolated from brown bullhead (*Ictalurus nebulosus*). Letters in Applied Microbiology. 23:234-240.

Schragg, S.J. and V. Perrot. 1996. Reducing antibiotic resistance. Nature 381: 120-121.

Schnabel, E.L. and A.L. Jones. 1999. Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. Appl. Environmental Microb. 65: 4898-4907.

Smith, H.W. 1970. Incidence in river water of *Escherichia coli* containing R factors. Nature 228: 1286-1288.

Smith, P., M.P. Hiney, and O.B. Samuelsen. 1994. Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Ann. Rev. Fish Dis. 4: 273-313.

Smith, P., L. Brogan, N. Brophy, M. Frawley, and D. Collins. 1997. Further observation on the emergence of resistant bacteria in the absence of overt selection. Abstracts of the VIIIth European Assoc. Fish Pathologists. Edinburgh, p117.

Spanggaard, B., F. Jørgensen, L. Gram and H.H. Huss. 1993. Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. Aquaculture 115: 195-207.

Starliper, C.E., R.K. Cooper, E.B. Shotts, Jr., and P.W. Taylor. 1993. Plasmid-mediated Romet resistance of *Edwardsiella ictaluri*. J. Aquat. An. Health 5: 1-8.

St. Louis, M.E. 1988. Water-related disease outbreaks, 1995. CDC MMWR June 01, 1988/37(SS-2): 15-24.

Swamy, S.C., H.M. Barnhart, M.D. Lee and D.W. Dreesen. 1996. Virulence determinants *invA* and *spvC* in Salmonellae isolated from poultry products, wastewater, and human sources. Appl. Environ. Microbiol. 62: 3368-3771.

Tsoumas, A., D.J. Alderman and C.J. Rodgers. 1989. *Aeromonas salmonicida*: development of resistance to 4- quinolones antimicrobials. J. Fish Dis. 12: 493-507.

Vaughn, S., R. Coyne, and P. Smith. 1996. The critical importance of sample site in the determination of the frequency of oxytetracycline resistance in the effluent microflora of a freshwater fish farm. Aquaculture 139: 47-54.

Wall, P.G., D. Morgan, K. Lamden, M. Griffin, E.J. Threlfall, L.R. Ward and B. Rowe. 1995. Transmission of multi-resistant *Salmonella typhimurium* from cattle to man. Vet. Rec. 136: 591-592.

Weber, J.T., E.D. Mintz, R. Canizares, A. Semiglia, I. Gomez, R. Sempertegui, A. Davila, K.D. Greene, N.D. Puhr, D.N. Cameron, F.C. Tenover, T.J. Barrett, N.H. Bean, C. Ivey, R.V. Tauxe, and P.A. Blake. 1994. Epidemic cholera in Ecuador: multidrug-resistance and transmission by water and seafood. Epidemiol. Infect. 112: 1-11.

Weinstein, M.R., M. Litt, D.A. Kertesg, P. Wyper, D. Ross, M. Coulter, A. McGreen, R. Facklam, C. Ostach, B.M. Willey, A. Borezyk, D.E. Low and the Investigative Team. 1997. Invasive infection due to a fish pathogen: *Streptococcus iniae*. New England Journal of Medicine. 337:589-594.

Wood, S., R. McCashion, and W. Lynch. 1986. Multiple low-level resistance in *Aeromonas salmonicida*. Antimicrob. Ag. Chemother., 29: 992-996.