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From Medical Epidemiologist, Foodborne and Diarrheal Diseases Branch
Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases
Use of antimicrobial agents in aquaculture: potential for public health impact

To The Record

Antimicrobial agents have been widely used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish including *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella tarda Pasteurella piscicida, Vibrio anguillarum,* and *Yersinia ruckeri.* This memorandum summarizes evidence that aquaculture use of antimicrobial agents, as with other uses, selects for antimicrobial resistance in the exposed bacterial flora. Because antimicrobial agents used in aquaculture are administered by mixing them with feed which is dispersed in the water, use of antimicrobial agents in aquaculture directly doses the environment, which results in selective pressures in the exposed ecosystem (1,2). The emergence of antimicrobial resistance following use of antimicrobial agents in aquaculture has been identified in fish pathogens (1,2). For example, in several countries *A. salmonicida* is frequently resistant to multiple drugs including sulphonamides, tetracycline, amoxicillin, trimethoprim-sulfadimethoxine and quinolones (3_7), antimicrobial agents which are commonly used in aquaculture. The first isolation of *A. salmonicida* resistant to a specific antimicrobial agent has often been reported shortly after the introduction of the agent into aquaculture (3_6). Similar correlations between antimicrobial agents used in aquaculture and antimicrobial resistance are also reported among other fish pathogens (8,9).

Use of antimicrobial agents in aquaculture also selects for antimicrobial resistance among bacteria that are not fish pathogens. Several studies have assessed the impact of use of antimicrobial agents in aquaculture on the bacteria in the sediment and within fish in the local environment. For example, bacteria resistant to antimicrobial agents used on specific fish farms have been isolated from sediment beneath the fish "netpens"on those fish farms (10). In another study, bacteria resistant to antimicrobial agents used on specific fish farms were isolated from the intestinal contents of natural and commercial fish species captured on those fish farms; in contrast, no resistance was present among bacteria from the intestinal contents of fish from untreated areas (11).

Antimicrobial-resistant bacteria which result from use of antimicrobial agents in aquaculture can transfer these resistance determinants to other bacteria Many antibiotic resistance determinants in fish pathogens are frequently carried on transferable R plasmids (12_15). Horizontal spread of plasmids from fish pathogens may therefore transfer resistance genes to other bacteria including those that are pathogenic to humans (15). Horizontal transfer of resistance genes on plasmids has been demonstrated between bacteria in the water of fish ponds

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(15) and in marine sediments (16). Plasmids carrying resistance determinants have also been transferred *in vitro* from fish, pathogens to human pathogens including *Vibrio cholerae* (17), *Vibrio parahemolyticus* (18) and potential human pathogens including *Escherichia coli* (19,20). Furthermore, plasmids carrying multiple antimicrobial-resistance determinants have been transferred in simulated natural microenvironments between bacterial pathogens of fish, humans, and other animals, demonstrating that resistance determinants on plasmids can spread from fish pathogens to human pathogens (21). These studies indicate that dissemination of antimicrobial resistance determinants may be facilitated by the horizontal transfer of plasmids between related and diverse bacteria.

Bacteria, present in aquaculture settings may be transmitted to humans who come in contact with this ecosystem. For example, *Vibrio spp.* are part of the normal warm marine flora and cause wound infections in persons with open wounds or abrasions exposed to seawater or marine life (22). In 1991, an epidemic of *Vibrio cholerae* 01 infections affected Latin America; the epidemic strain in Latin America. was susceptible to the 12 antimicrobial agents tested except in coastal Ecuador where the epidemic strain became multidrug-resistant (23). The cholera epidemic in Ecuador began among persons working on shrimp farms. Multidrug-resistance was present in non-cholera *Vibrio* infections that were pathogenic to the shrimp. The resistance may have been transferred to *V. cholerae* 01 from other vibrios (23). Bacteria from the aquaculture ecosystem may also be transmitted directly to humans through handling of fish. Recently, the fish pathogen *Streptococcus iniae* has caused invasive infections in persons who handled store-bought aquacultured tilapia; *Streptococcus iniae* was isolated from the aquaculture ecosystem and on fish in grocery stores (24). Similarly, a new biotype of *Vibrio vulnificus* caused hundreds of serious infections among persons handling live tilapia produced by aquaculture in Israel (25). Bacteria on fish may also be transmitted to humans when the aquacultured fish are eaten, or when other foods, which have been cross-contaminated by bacteria from fish, are eaten. For example, *Vibrio parahaemolyticus* is a common foodborne disease in Japan where infections have been linked to the consumption of aquacultured fin fish (26), Furthermore, *Salmonella spp.*, a common cause of foodborne disease, has been isolated from aquacultured fish and shrimp ponds *(27,28).* These and other reports indicate that bacteria present in aquaculture ecosystems can be transmitted to humans.

Newly available molecular characterizations of antimicrobial resistance determinants provides further evidence of the transmission of antimicrobial resistance between aquaculture ecosystems and humans. Some of the antimicrobial resistance determinants in *Salmonella* serotype Typhimurium definitive type 104 may have originated in aquaculture. *Salmonella* Typhimurium DTI04, which is typically resistant to ampicillin, chloramphenicol, florofenicol, streptomycin, sulfonamides, and tetracycline, was first isolated from an ill person in 1985 and emerged during the 1990s as a leading cause of human *Salmonella* infections. Tetracycline resistance in *S. Typhimurium* DT104 is due to a class G resistance gene (29). The class G resistance determinant is rare and has not previously been reported from *Salmonella* isolates. Class G was first identified in 1981 in tetracycline-resistant isolates of *Vibrio anguillarum*, a pathogen of fish (30). Furthermore, the recently described novel florofenicol resistance gene, *floR*, in *Salmonella* Typhimurium DTI04, which also confers resistance to chloramphenicol, is almost identical, by molecular sequence, to the florofenicol resistance gene first described in *Photobacterium damsela*, a bacteria found in fish; again, this resistance gene is rare and has not previously been reported from *Salmonella* isolates (31). Finally, all of the antimicrobial resistance

determinants in *S. Typhimurium* DT104 are grouped on the chromosome within two distinct integrons and an intervening plasmid-derived sequence. The Class G and *floR* determinants are located within the intervening plasmid-derived sequence. By molecular sequence, the plasmid-derived sequence is closely related (94% identity) to a plasmid identified in *Pasteurella piscicida*, a pathogen of fish (29, 32). These and other reports indicate that antimicrobial resistance determinants selected for in aquaculture ecosystems can be transmitted to bacteria that cause illness in humans, perhaps at a greater frequency than, previously suggested (33).

In summary, these data demonstrate that use of antimicrobial agents in aquaculture has selected for antimicrobial resistance among bacteria in the exposed ecosystems. This resistance can disseminate through the environment and can be transmitted to a variety of bacterial species, including bacteria that can infect humans.

Frederick Angulo, D.V.M., Ph.D.

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CROSS FILE SHEET

FILE NO: 98D-0969/C19

SEE FILE NO: 99D-2638/C21

Butler, Jennie C

From: Mitchell, Bert
Sent: Monday, February 28, 2000 1:36 PM
To: Butler, Jennie C
Cc: Beaulieu, Andrew J; Oeller, Margaret R; Gushee, Judith A; Haley, Carol J
Subject: FW: Antibiotic Resistance

Antiresistant Memorandum.doc

CDC comments.doc

Jenny, the attached documents should go on public display in Dockets No. 98D-0969 (antimicrobial resistance)and 99D-2638 (extra-label use of medicated feeds for minor species).

-----Original Message-----
From: Randy MacMillan [mailto:randy@clearsprings.com]
Sent: Monday, February 28, 2000 11:36 AM
To: gmitchel@cvm.fda.gov
Subject: Antibiotic Resistance

Bert- attached are the two documents we discussed last week. One document is the CDC (Fred Angulo) response to an NAA request for documentation and the second is the NAA review of the CDC response. You requested these to perhaps introduce them as public comment. Thanks.
Randy
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