Human Health Consequences of Use of Antimicrobial Agents in Aquaculture

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Intensive use of antimicrobial agents in aquaculture provides a selective pressure creating reservoirs of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment. From these reservoirs, resistance genes may disseminate by horizontal gene transfer and reach human pathogens, or drug-resistant pathogens from the aquatic environment may reach humans directly. Horizontal gene transfer may occur in the aquaculture environment, in the food chain, or in the human intestinal tract. Among the antimicrobial agents commonly used in aquaculture, several are classified by the World Health Organisation as critically important for use in humans. Occurrence of resistance to these antimicrobial agents in human pathogens severely limits the therapeutic options in human infections. Considering the rapid growth and importance of aquaculture industry in many regions of the world and the widespread, intensive, and often unregulated use of antimicrobial agents in this area of animal production, efforts are needed to prevent development and spread of antimicrobial resistance in aquaculture to reduce the risk to human health.

Aquaculture is growing rapidly in many regions of the world, and aquaculture products constitute an important food supply with increasing economic importance. World aquaculture production more than doubled during the period 1994–2004, and countries in Asia accounted for 80%–90% of the total production. In 2004, the world aquaculture production of food fish amounted to 45.5 million tons, of which 30.6 million tons were produced in China alone, whereas India, Vietnam, Thailand, Indonesia, and Bangladesh together accounted for 6.8 million tons [1]. The industry covers a wide range of species and methods, from simple traditional systems, in which fish or other aquatic animals are reared in small ponds for domestic consumption, to intensive industrial scale production systems.

To control infectious diseases, similar strategies (eg, vaccination and use of antimicrobial agents) are employed in aquaculture as in other areas of animal production. Use of antimicrobial agents in aquaculture has resulted in the emergence of reservoirs of antimicrobial-resistant bacteria in fish and other aquatic animals, as well as in the aquatic environment [2–7].

The 2 most common routes of administration of antimicrobial agents in aquaculture are use of medicated feed and adding antimicrobial agents directly to the water (immersion therapy), and both of these methods imply flock treatment of the animals. These practices may result in heavy use of antimicrobial agents and convey a strong selective pressure not only in the animals, but also in the exposed environments [8]. Consequently, the use of antimicrobial agents in aquaculture results in a broad environmental application that impacts a wide variety of bacteria [9]. Effluents from terrestrial animals and humans may end up in the aquatic environment, whereby the reservoir in the aquatic environment may be influenced by resistance determinants and bacteria that have emerged in other environments [10, 11]. Sanitary barriers used in terrestrial food animal production are difficult to establish in aquaculture. These conditions, in combination with high population densities, poor water quality, or both, may lead to an increase in bacterial
Table 1. Antimicrobial Agents (and Classes) Used in Aquaculture and Their Importance in Human Medicine

<table>
<thead>
<tr>
<th>Antimicrobial agent (drug class)</th>
<th>Route of administration in aquaculture</th>
<th>Importance of antimicrobial class in human medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin (aminopenicillins)</td>
<td>Oral</td>
<td>Critically important</td>
</tr>
<tr>
<td>Ampicillin (aminopenicillins)</td>
<td>Oral</td>
<td>Critically important</td>
</tr>
<tr>
<td>Chloramphenicol (amphenicols)</td>
<td>Oral/bath/injection</td>
<td>Important</td>
</tr>
<tr>
<td>Florfenicol (amphenicols)</td>
<td>Oral</td>
<td>Important</td>
</tr>
<tr>
<td>Erythromycin (macrolides)</td>
<td>Oral/bath/injection</td>
<td>Critically important</td>
</tr>
<tr>
<td>Streptomycin, neomycin (aminoglycosides)</td>
<td>Bath</td>
<td>Critically important</td>
</tr>
<tr>
<td>Furazolidone (nitrofurans)</td>
<td>Oral/bath</td>
<td>Important</td>
</tr>
<tr>
<td>Nitrofurantoin (nitrofurans)</td>
<td>Oral</td>
<td>Important</td>
</tr>
<tr>
<td>Oxolinic acid (quinolones)</td>
<td>Oral</td>
<td>Critically important</td>
</tr>
<tr>
<td>Enrofloxacín (fluoroquinolones)</td>
<td>Oral, bath</td>
<td>Critically important</td>
</tr>
<tr>
<td>Flumequine (fluoroquinolone)</td>
<td>Oral</td>
<td>Critically important</td>
</tr>
<tr>
<td>Oxytetracycline, chlortetracycline, tetracycline (tetracyclines)</td>
<td>Oral/bath/injection</td>
<td>Highly important</td>
</tr>
<tr>
<td>Sulphonamides (sulphonamides)</td>
<td>Oral</td>
<td>Important</td>
</tr>
</tbody>
</table>

NOTE. Data are adapted from World Health Organization Expert Consultations on “Critically Important Antimicrobials for Human Medicine” that took place in Canberra, Australia, in 2005 [15] and in Copenhagen, Denmark, in 2007 [16]. Note that the use of certain antimicrobial agents in aquaculture (eg, chloramphenicol and nitrofurans) is regulated or banned in most countries.

Because resistance is class-wide, all antimicrobial agents in these classes were similarly classified in the Canberra and Copenhagen reports [15, 16], even though these specific antimicrobial agents are not used in humans.

Infections and contribute to increased use of antimicrobial agents, thereby increasing the selective pressure on bacteria in the aquatic environment.

In general, aquatic bacteria are not different from other bacteria in their responses to exposure to antimicrobial agents, and they are capable of transferring antimicrobial resistance genes to other bacteria [12, 13]. The apparent overlap between various ecological environments, including aquaculture and the human environment, means that bacteria and the drug-resistance genes that they contain may be exchanged between these environments, implying a risk that drug resistance genes may be transferred to humans from the reservoir in aquatic bacteria. This constitutes a potential human health hazard that has received relatively little attention, because the human health consequences of use of antimicrobial agents in animals have been regarded mainly in relation to terrestrial farm animals. This review focuses on the human health consequences of the use of antimicrobial agents in aquaculture.

DEVELOPMENT AND SPREAD OF ANTIMICROBIAL RESISTANCE

Development and spread of antimicrobial resistance has become a global public health problem influenced by the use of antimicrobial agents in both humans and animals. It is generally acknowledged that the use of antimicrobial agents drives the emergence of antimicrobial-resistant microorganisms and further promotes the dissemination of drug-resistant bacteria and resistance genes [14]. Spread of antimicrobial resistance is not necessarily restricted by phylogenetic, geographic, or ecological borders. Thus, use of antimicrobial agents in one ecological niche, such as in aquaculture, may impact the occurrence of antimicrobial resistance in other ecological niches, including the human environment.

Many antimicrobial agents used in human medicine are also used in aquaculture. Table 1 summarizes the main antimicrobial agents used in aquaculture worldwide and their importance in human medicine as identified during World Health Organization (WHO) Expert Consultations on “Critically Important Antimicrobials for Human Medicine” in Canberra, Australia, in 2005 [15] and in Copenhagen, Denmark, in 2007 [16]. Thus, among the antimicrobial agents commonly used in aquaculture, several are classified by the WHO as critically important for use in humans. Occurrence of resistance to these antimicrobials in human pathogens severely limits the therapeutic options in human infections, and therefore, the use of these antimicrobial agents in animals should be controlled or avoided to prevent the spread of drug resistance.

Antimicrobial agents are commonly used in aquaculture to prevent or treat disease outbreaks, but there is little published documentation giving details of usage patterns. A study conducted in 2003 [17] showed that a large proportion of shrimp farmers along the Thai coast used antimicrobial agents in their...
farms. Seventy-four percent of the farmers used antimicrobial agents in the production of shrimp, and at least 13 different antimicrobial agents were used. Some farmers reported treating their shrimp with antimicrobial agents for prophylaxis on a daily basis. The authors concluded that a more restrictive use of antimicrobial agents could have positive effects for the individual farm and, simultaneously, decrease impacts on regional human medicine and adjacent coastal ecosystems.

Few countries monitor the quantity of antimicrobial agents used in animals, and data on the quantity of antimicrobial agents used in aquaculture are scarce. In some countries (eg, countries in North America and in Europe), licensing and regulation of the use of antimicrobial agents in aquaculture is strictly enforced, and the use of antimicrobial agents in aquaculture is frequently guided by veterinary professionals. However, a large proportion of the global aquaculture production takes place in countries with few regulations and limited enforcement for the authorization of antimicrobial agents used in animals [18].

**THE RISKS ASSOCIATED WITH ANTIMICROBIAL-RESISTANT BACTERIA IN AQUACULTURE**

The use of antimicrobial agents in aquaculture presents a risk to public health because of the development of acquired antimicrobial resistance in fish pathogens and other aquatic bacteria; such drug-resistant bacteria can act as reservoirs of resistance genes, from which genes can disseminate to human pathogens (eg, the spread of resistance genes from *Aeromonas* species to *Escherichia coli*). This can be viewed as an indirect spread of antimicrobial resistance from aquatic environments to humans by horizontal gene transfer. Furthermore, some groups of aquatic bacteria (eg, some *Vibrio* species) are regarded as human pathogens, and other bacterial species can be opportunistic pathogens in humans. Infection in humans caused by antimicrobial-resistant bacteria from these groups can be viewed as direct spread of antimicrobial resistance from the aquatic environment.

**Indirect spread of antimicrobial resistance by horizontal gene transfer.** Development and spread of antimicrobial resistance as a consequence of exposure to antimicrobial agents is well documented in both human medicine and veterinary medicine. It is also well documented that fish pathogens and other aquatic bacteria can develop resistance as a consequence of exposure to antimicrobial agents [19]. Examples include *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Citrobacter freundii*, *Yersinia ruckeri*, *Photobacterium damselae* subspecies *piscicida*, *Vibrio anguillarum*, *Vibrio salmonicida*, *Flavobacterium psychrophilum*, and *Pseudomonas fluorescens*.

Acquired sulfonamide resistance in *A. salmonicida*, which causes disease in fish that inhabit temperate and cold climates, was reported in 1955 in the United States, and in the 1960s, multidrug-resistant strains were observed in Japan. Since that time, multidrug-resistant *A. salmonicida* have been described from many countries in various parts of the world, and transferable resistance plasmids are commonly detected in these strains [19].

The use of quinolones in aquaculture has resulted in the development of quinolone resistance in strains of *A. salmonicida*. This resistance was mainly mediated by mutation in the gyrase A gene (gyrA) [19]. Development of resistance in shrimp pathogens, such as *Vibrio harveyi*, because of exposure to antimicrobials has also been reported [20]. Resistance to norfloxacin, oxolinic acid, trimethoprim, and sulphamethoxasole was found to be high among bacteria in mud samples from shrimp farming locations in Vietnam, and *Bacillus* and *Vibrio* species were predominant among bacteria that were resistant to antimicrobials [6]. A high prevalence of resistance to sulphonamides in bacteria from shrimp hatcheries in India has been reported [21].

The fact that some bacteria that cause infections in fish belong to the same genera as bacteria causing infections in humans is likely to increase the probability of spread of antimicrobial resistance from aquaculture to humans. Studies have demonstrated that plasmids that harbor resistance determinants are transferable from fish pathogens and aquatic bacteria, not only to other bacteria within the same genus, but also to *E. coli* [12, 13]. Plasmids that carry multidrug-resistant determinants have been shown to be transferable to *E. coli* from *A. salmonicida*, *A. hydrophila*, *E. tarda*, *Citrobacter freundii*, *P. damselae* subspecies *piscicida*, *V. anguillarum*, and *V. salmonicida* [19]. A large plasmid carrying resistance to 6 antimicrobial agents was shown to be transferable from *Vibrio cholerae* O1 to *A. salmonicida*, *A. hydrophila*, *Vibrio parahaemolyticus*, *V. cholerae*, *V. anguillarum*, *Shigella* species, *Salmonella* species, and *E. coli* [22]. Plasmids with varying resistance genes have been transferred in vitro from fish pathogens to human pathogens, including *V. cholerae* and *V. parahaemolyticus* [23]. A 21-kb plasmid coding for resistance to cephalothin that could be transferred to *E. coli* was isolated from *Vibrio* strains from shrimp ponds [24]. Genes coding for tetracycline resistance in fish farm bacteria and human clinical isolates in Japan showed high similarity, suggesting that they were derived from the same source [25]. Furthermore, in laboratory experiments, transfer of tetracycline resistance from marine strains of *Photobacterium* species, *Vibrio* species, *Aeromonas* species, and *Pseudomonas* species could be transferred to *E. coli* by conjugation, suggesting that transfer of resistance from marine bacteria to bacteria associated with the human gut is possible.

The transfer of plasmids containing resistance genes between fish pathogens and other aquatic bacteria illustrates that these
bacteria can act as reservoirs of resistance genes that can be further disseminated. Ultimately, resistance genes in the aquatic environment may reach human pathogens and thereby add to the burden of antimicrobial resistance in human medicine. Molecular characterization shows that some of the antimicrobial resistance determinants in multidrug-resistant *Salmonella* Typhimurium DT104, such as tet(G) causing tetracycline resistance and flo-like gene that confers resistance to both chloramphenicol and florfenicol, are also present in some fish pathogenic bacteria [26, 27]. Furthermore, it has been recently demonstrated that *Vibrio* species found in aquatic environments harbour *qnr*-like quinolone resistance determinants that resemble the *qnr* genes found in human pathogens, indicating that the aquatic environment may serve as a reservoir of quinolone resistance determinants [28, 29]. The results of these molecular characterizations indicate that resistance genes can be exchanged between fish pathogens and human bacteria.

**Direct spread of antimicrobial resistance.** Aquatic environments can be a source of drug-resistant bacteria that can be directly transmitted to and cause infections in humans, and because of resistance traits, antimicrobial treatment of infections caused by these bacteria may result in treatment failures in humans. The spread to humans may be through direct contact with water or aquatic organisms, through drinking water, or through the handling or consumption of aquaculture products. Direct spread from aquatic environments to humans can involve human pathogens, such as *V. cholerae*, *V. parahaemolyticus*, *Vibrio vulnificus*, *Shigella* species, and *Salmonella* species, or opportunistic pathogens, such as *A. hydrophila*, *Plesiomonas shigelloides*, *E. tarda*, *Streptococcus iniae*, and *E. coli*. The occurrence of antimicrobial-resistant *Salmonella* species in aquatic environments is most likely attributable to contamination from human, animal, or agricultural environments [30].

In a study of ready-to-eat shrimp, 13 brands from 4 countries were obtained from local grocery stores [31]. A total of 1564 isolates representing 162 bacterial species were isolated and tested for resistance to 10 antimicrobial agents. Forty-two percent of the isolates and 81% of the species were resistant to antimicrobial agents. Numerous antimicrobial-resistant human pathogens were isolated, including *E. coli*, *Enterococcus* species, *Salmonella* species, *Shigella flexneri*, *Staphylococcus* species, and *Vibrio* species. Because ready-to-eat shrimp are not cooked before they are eaten, the authors suggested that widespread trade of this product provides an avenue for international dissemination of antimicrobial-resistant pathogens.

**CONSEQUENCES FOR HUMANS OF TRANSFER OF ANTImICROBIAL-RESISTANT BACTERIA FROM AQUACULTURE**

The consequences of antimicrobial resistance in bacteria causing infections in human include (1) an increased number of infections and (2) an increased frequency of treatment failures and increased severity of infection [13].

**Increased number of infections.** Antimicrobial agents may disturb the microflora of the human intestinal tract and place treated individuals at increased risk for certain infections. Individuals taking an antimicrobial agent for any reason are therefore at increased risk for infection due to pathogens that are resistant to the antimicrobial agent. This effect has been demonstrated in case-control studies involving persons infected with antimicrobial-resistant *Salmonella* species, in which persons who are exposed to antimicrobial agents for unrelated reasons, such as treatment of an upper respiratory tract infection, are at increased risk for infection due to *Salmonella* species that are resistant to the antimicrobial agent [32]. This increased risk can be expressed in the form of an attributable fraction; for example, the proportion of *Salmonella* infections that occurred as a result of the *Salmonella* species being resistant to the antimicrobial agent (ie, infections occurring as a result of the person taking the antimicrobial agent for an unrelated reason). Although there is no current data from aquaculture-related studies, it is reasonable to assume that the same phenomenon that has been demonstrated for *Salmonella* species can occur with other drug-resistant human pathogens for which resistance may have originated in aquaculture and that antimicrobial treatment (for unrelated reasons) may put the patient at risk for infection due to such drug-resistant pathogens.

**Increased frequency of treatment failure and increased severity of infection.** Increased frequency of treatment failure and increased severity of infection as a result of antimicrobial resistance may result in prolonged duration of illness, increased frequency of bloodstream infection, increased hospitalization, or increased mortality [13]. Prolonged duration of illness has been demonstrated in case-control studies of fluoroquinolone-resistant *Campylobacter* [33, 34], and for infections due to quinolone-resistant *Salmonella* Typhimurium, an increased severity of infection was demonstrated [35]. Also for antimicrobial-resistant non-Typhi *Salmonella* serotypes and *Campylobacter*, increased morbidity or mortality has been demonstrated [36]. It is reasonable to assume that the same phenomenon that has been demonstrated for *Salmonella* and *Campylobacter* species can occur with other drug-resistant human pathogens, for which resistance may originate in aquaculture.

**RESIDUES OF ANTImICROBIAL AGENTS**

In parallel to human health risk from antimicrobial-resistant bacteria in aquaculture, the presence of residues of antimicrobial agents in aquaculture products also presents a risk to humans, exemplified by allergy, toxicity, alterations of the intestinal flora, and selection for antimicrobial-resistant bacteria [37]. The risk depends on the type and quantity of the antimicrobial agent encountered or consumed, and in general,
lower exposure means lower risk. In a Food and Agriculture Organisation of the United Nations (FAO)/Organization International des Epizooties (OIE; World Organization for Animal Health)/WHO consultation on scientific issues related to nonhuman use of antimicrobial agents that was held in Geneva, Switzerland, in December 2003, it was concluded that toxicological effects following intake of residues of antimicrobial agents in foods, under present regulatory regimes, represents a significantly less important human health risk than does the risk related to antimicrobial-resistant bacteria in food [13].

**RISK MANAGEMENT OPTIONS**

The most effective means to prevent and control the development and spread of antimicrobial resistance is to reduce use of antimicrobial agents by reducing the need for antimicrobial treatment [38]. To arrive at effective prevention and control of use of antimicrobial agents in aquaculture, similar elements are needed in aquaculture as in other areas of animal production. A regulatory framework at the national level is needed for registration, approval, and control of use of antimicrobial agents in all countries in which antimicrobial agents are used in aquatic animals. Production management should include stocking programs and management practices to avoid the introduction of pathogens and to prevent disease outbreaks and should include control measures to be implemented if disease occurs.

An important measure in relation to disease prevention is the introduction of vaccines, which can substantially reduce the need for antimicrobial agents. The rapid growth of the salmon industry in Norway from the beginning of the 1980s was accompanied by a marked increase in antimicrobial consumption. Because of effective vaccine strategies and improved health management, the use of antimicrobial agents in Norwegian aquaculture was reduced by 99% from 1987 through 2007, despite a substantial increase in production (Figure 1). In 1992, ~210 mg of antimicrobial agents were used per kg of fish produced in Norwegian aquaculture. By 1994, only 6 mg were used per kg of fish [39].

An important component in the management of antimicrobial resistance in general is monitoring of the quantity of antimicrobial agents used and of antimicrobial resistance. This also applies to aquaculture. Monitoring data constitutes the basis for risk assessment and risk management, including interventions and evaluation of the impact of interventions and compliance with regulations or guidelines on prudent use of antimicrobial agents. Furthermore, monitoring data provide the basis for focused and targeted research.

**CONCLUSIONS**

Use of antimicrobial agents in aquaculture provides a selective pressure that creates reservoirs of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment. Characterization of antibiotic resistance genes from various ecological environments has demonstrated their promiscuous nature and their ability to cross phylogenetic, geographic, and ecological borders. From the reservoir in the aquaculture environment, some drug-resistant pathogenic bacteria can be transferred to humans, but more importantly, resistance genes from bacteria in the aquatic environment can disseminate by horizontal gene transfer and reach human pathogens. Among the antimicrobial agents commonly used in aquaculture, several are classified by the WHO as critically important for use in humans. Occurrence of resistance to these antimicrobial agents in human pathogens severely limits the therapeutic options in human infections. The risk of horizontal gene transfer from fish pathogens and other bacteria in the aquatic environment to human pathogens has not been fully investigated, but it is likely to be significant.

Considering the rapid growth and importance of the aquaculture industry in many regions of the world and the widespread, intensive, and often unregulated use of antimicrobial agents in this area of animal production, efforts are needed to prevent the development and spread of antimicrobial resistance in aquaculture. These efforts should be focused on improvement of management routines, regulatory control of the use of antimicrobial agents, implementation of prudent use guidelines, and monitoring of the use of antimicrobial agents and antimicrobial resistance. Furthermore, international cooperation is needed to support and assist developing countries in capacity building and implementation of preventive measures. In this effort, the leadership of international organizations, such as the FAO, OIE, and WHO, is crucial.

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