

Minireview

Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health

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Summary

The worldwide growth of aquaculture has been accompanied by a rapid increase in therapeutic and prophylactic usage of antimicrobials including those important in human therapeutics. Approximately 80% of antimicrobials used in aquaculture enter the environment with their activity intact where they select for bacteria whose resistance arises from mutations or more importantly, from mobile genetic elements containing multiple resistance determinants transmissible to other bacteria. Such selection alters biodiversity in aquatic environments and the normal flora of fish and shellfish. The commonality of the mobilome (the total of all mobile genetic elements in a genome) between aquatic and terrestrial bacteria together with the presence of residual antimicrobials, biofilms, and high concentrations of bacteriophages where the aquatic environment may also be contaminated with pathogens of human and animal origin can stimulate exchange of genetic information between aquatic and terrestrial bacteria. Several recently found genetic elements and resistance determinants for quinolones, tetracyclines, and β -lactamases are

shared between aquatic bacteria, fish pathogens, and human pathogens, and appear to have originated in aquatic bacteria. Excessive use of antimicrobials in aquaculture can thus potentially negatively impact animal and human health as well as the aquatic environment and should be better assessed and regulated.

Introduction

Even though much of the rapid growth of aquaculture over the past quarter century has taken place in Asia (Arthur *et al.*, 2000; Costa-Pierce, 2003; 2010; Naylor and Burke, 2005; Asche *et al.*, 2008; Cole *et al.*, 2009; Diana, 2009), development and application of intensive methods of salmon farming in Norway and Chile have resulted in their being among the top 12 aquacultural producers of animal protein in the world (Chopin *et al.*, 2008; FAO, 2010). This widespread growth of aquaculture has been accompanied by an increased use of a wide range of chemicals including antimicrobials (Haya *et al.*, 2001; Armstrong *et al.*, 2005; Cabello, 2006; Buschmann *et al.*, 2009; Cole *et al.*, 2009; Asche *et al.*, 2010; Burrridge *et al.*, 2010; Millanao *et al.*, 2011). Increases in aquacultural antimicrobial use have been difficult to assess because of the large size and geographical extent of the industry, the various modalities employed (i.e. extensive, integrated, and intensive), and the over 200 species of fish and shellfish involved (Austin, 1985; Arthur *et al.*, 2000; Costa-Pierce, 2003; 2010; Naylor and Burke, 2005; Asche *et al.*, 2008; 2010; Asche, 2009; Diana, 2009). Collection of information about antimicrobial use in aquaculture is further complicated by a wide range of proprietorship (family units, village ownership, small businesses, international conglomerates) (Austin, 1985; Costa-Pierce, 2003; 2010; Naylor and Burke, 2005; Asche *et al.*, 2008; Asche, 2009; Diana, 2009; Rodgers and Furones, 2009) as well as by differing national regulations which often do not encourage data collection for purposes of animal and public health and epidemiology (Asche *et al.*, 2008; Asche, 2009; Burrridge *et al.*, 2010; Millanao *et al.*, 2011).

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Despite these impediments, available information has revealed widespread geographical heterogeneity in the amounts and classes of antimicrobials used in aquaculture (Burridge *et al.*, 2010; Millanao *et al.*, 2011; Ndi and Barton, 2012). It has also indicated that intensive aquaculture in some countries is an especially important source for passage of antimicrobials into the aquatic environment with potential effects on the health of fish, terrestrial animals, human beings, and the environment in general (Burridge *et al.*, 2010; Millanao *et al.*, 2011; Miranda, 2012). One of us has previously succinctly reviewed antimicrobial use in aquaculture and the implications of this use for biodiversity and human health (Cabello, 2006). This present more comprehensive review examines recently emerging and past information about antimicrobial use in aquaculture and its impact on the molecular genetics and evolution of antimicrobial resistance in the environment. Some aspects of this review concentrate on salmon aquaculture because of the availability of relatively reliable information obtained by us about this industry's usage of antimicrobials (Millanao, 2002; Barrientos, 2006; Gómez, 2009; Millanao *et al.*, 2011) and because of the important potential impacts of this rapidly growing industry on aquatic biodiversity, antimicrobial resistance evolution, and piscine, terrestrial animal and human health.

Antimicrobial use in aquaculture

Classes and amounts

A large proportion, perhaps half, of the world's industrial production of antimicrobials is consumed in terrestrial animal agriculture; their use as prophylactics and as growth promoters far outweighs their use as therapeutics (Mellon *et al.*, 2001; Sarmah *et al.*, 2006; Davies, 2009; Davies and Davies, 2010; Levy and Marshall, 2010; Bush *et al.*, 2011; Marshall and Levy, 2011). Antimicrobials are used in aquaculture not to promote growth but rather to prevent and treat bacterial infections in fish and invertebrates. These arise as a consequence of lowered host defences associated with culture at high density with sub-optimal hygiene in enclosures in close proximity (Austin, 1985; Barton and Iwama, 1991; Grave *et al.*, 1999; Arthur

et al., 2000; Woo *et al.*, 2002; Beveridge, 2004; Armstrong *et al.*, 2005; Defoirdt *et al.*, 2007; Sapkota *et al.*, 2008; Grave and Hansen, 2009; Rodgers and Furones, 2009; Burridge *et al.*, 2010; Millanao *et al.*, 2011; Austin and Austin, 2012). These conditions, often associated with efforts to increase productivity, in turn favour development and epizootic dissemination of bacterial infections among aquaculture units in a geographical area (Barton and Iwama, 1991; Burka *et al.*, 1997; Grave *et al.*, 1999; Sørum, 2000; 2006; Woo *et al.*, 2002; Beveridge, 2004; Cabello, 2006; Cole *et al.*, 2009; Grave and Hansen, 2009; Asche *et al.*, 2010; Barton and Floydsand, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011). In salmon aquaculture, the need to grow different developmental stages in fresh and salt water and the manipulations to transport them between these two environments also increases stress and the opportunities for contact between different populations of fish, thus increasing opportunities for cross infection (Woo *et al.*, 2002; Beveridge, 2004; Ibieta *et al.*, 2011).

Aquacultural use of antimicrobials in developed countries has generally been restricted to avoid potential selection for human pathogens resistant to antimicrobials effective in clinical practice (Grave *et al.*, 1999; Collignon *et al.*, 2009; Grave and Hansen, 2009; Heuer *et al.*, 2009; Burridge *et al.*, 2010). Canada, Norway and the United States permit aquacultural use of oxytetracycline, Canada and Norway permit use of florfenicol, and Norway permits aquacultural use of quinolones (Table 1) (Grave *et al.*, 1999; Sapkota *et al.*, 2008; Rodgers and Furones, 2009; Burridge *et al.*, 2010). Information regarding classes of antimicrobials used in aquaculture is undoubtedly incomplete even in industrialized countries because regulatory agencies have failed to collect this information (Sapkota *et al.*, 2008; Burridge *et al.*, 2010; Marshall and Levy, 2011).

The situation is more problematic in countries where control is less stringent or lacking (Sapkota *et al.*, 2008; Burridge *et al.*, 2010; Marshall and Levy, 2011; Millanao *et al.*, 2011). In contrast to the United States, Norway and Canada, Chile, the second largest producer of cultured salmon after Norway, not only permits aquacultural use of oxytetracycline, florfenicol, and quinolones, but also

Table 1. Antimicrobials currently authorized for use in salmon aquaculture in various countries.^a

	Oxytetracycline	Florfenicol	Sulfa/trimethoprim derivatives	Quinolones	Others
Canada	+	+	+		
Chile	+	+		+	(Oxolinic acid, Flumequin, others)
Norway	+	+		+	(Oxolinic acid, Flumequin)
United States	+		+		Amoxicillin, Erythromycin, Furazolidin Chloramphenicol, Gentamycin

a. Burridge *et al.* (2010)

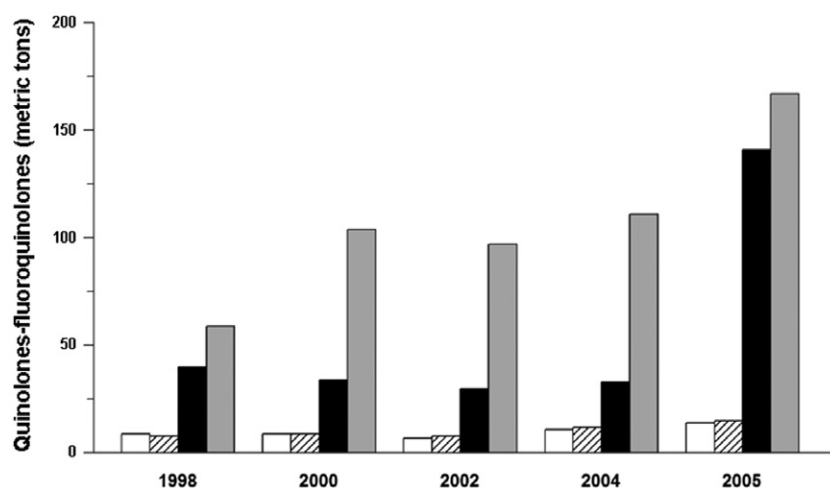


Fig. 1. Authorized and imported quantities (metric tons) of quinolones and fluoroquinolones for human and veterinary medical use in Chile, 1998–2005. Quinolones-fluoroquinolones authorized for import for human use by the Chilean Institute of Public Health (white bars) and actually imported (hatched bars). Quinolones-fluoroquinolones authorized for import for veterinary use by the Chilean Agricultural and Livestock Service (black bars) and actually imported (grey bars). Modified from (Millanao, 2002; Barrientos, 2006; Millanao *et al.*, 2011; A. Millanao and H. Dölz, unpublished).

allows use of amoxicillin, erythromycin and several other antimicrobials (Table 1) (Grave *et al.*, 1999; Sapkota *et al.*, 2008; Grave and Hansen, 2009; Rodgers and Furones, 2009; Burrige *et al.*, 2010). According our own investigations, agricultural regulators in Chile have consistently failed to successfully track and limit veterinary use of antimicrobials (Millanao, 2002; Barrientos, 2006; Millanao *et al.*, 2011). Between 1998 and 2004, 1.5 to 3.4 times more quinolones and fluoroquinolones were imported into Chile for veterinary medicine and used preferentially in aquaculture than were authorized by the national Livestock and Agricultural Service (Fig. 1). This ratio fell to 1.2 in 2005 (although use was still high) perhaps because permits were increased (Fig. 1), suggesting there was previously an unregulated market for veterinary use of these drugs (Millanao, 2002; Barrientos, 2006; Millanao *et al.*, 2011; A. Millanao and H. Dölz, unpublished). Quinolones such as oxolinic acid and flumequine comprise most of the quinolones imported to Chile and together with florfenicol are mostly used in aquaculture (Millanao, 2002; Barrientos, 2006; Millanao *et al.*, 2011). Studies of antimicrobial resistance in lower intensity fish and shrimp aquacultural settings also suggest that many classes of antimicrobials are employed in these activities as well (Holmström *et al.*, 2003; Le and Munkage, 2004; Le *et al.*, 2005; Hastein *et al.*, 2006). Detection of nitrofurans by the Food and Drug Administration in aquacultural products imported to the United States from China (Burrige *et al.*, 2010; Love *et al.*, 2011), and detection of chloramphenicol and metronidazole by the European Union regulatory authorities in seafood imported from China, Indonesia, Taiwan, Thailand and Vietnam provide additional evidence for lax control of antimicrobial use in other less industrialized countries (Rodgers and Furones, 2009; Love *et al.*, 2011).

There is a great variability in the amounts and classes of antimicrobials used in salmon, shrimp and other forms of aquaculture from country to country (Grave *et al.*, 1999; 2006; Holmström *et al.*, 2003; Le and Munkage, 2004; Grave and Hansen, 2009; Burrige *et al.*, 2010; Millanao *et al.*, 2011). For example, Japanese aquaculture used 179 metric tons of antimicrobials in 2001, slightly more than a third of the amount used in human medicine that year (Furushita and Shiba, 2007). The situation was quite different in intensive salmon aquaculture in Chile. Importation of antimicrobials to Chile in the period 2000–2007 for use in veterinary medicine increased in parallel with salmonid production (Figs 1 and 2). It was several times greater than importation of antimicrobials for human medicine which increased only slightly over this time (Millanao, 2002; Barrientos, 2006; Gómez, 2009; Millanao *et al.*, 2011). While Norway, the United Kingdom and Canada used approximately 0.0008 kg, 0.0117 kg, and 0.175 kg, respectively, of antimicrobials for each metric ton of salmon produced in 2007, Chile used at least 1.4 kg per metric ton (Fig. 2) (SalmonChile, 2008; Gómez, 2009; Burrige *et al.*, 2010; Millanao *et al.*, 2011; A. Millanao and H. Dölz, unpublished). Thus, considerably more antimicrobials were used in Chile than in Norway or Canada to produce one metric ton of salmon (approximately over 1500 and eight times more respectively) (Millanao, 2002; Barrientos, 2006; Gómez, 2009; Burrige *et al.*, 2010; Millanao *et al.*, 2011; A. Millanao and H. Dölz, unpublished). Approximately 471, 233, and 226 metric tons of tetracycline, florfenicol, and quinolones, respectively, were estimated to have been used in Chile in 2007 in veterinary medicine (Millanao, 2002; Barrientos, 2006; Millanao *et al.*, 2011). These 930 metric tons of antimicrobials were mostly used in salmon aquaculture (Millanao, 2002; Barrientos, 2006; Gómez, 2009; Millanao *et al.*, 2011).

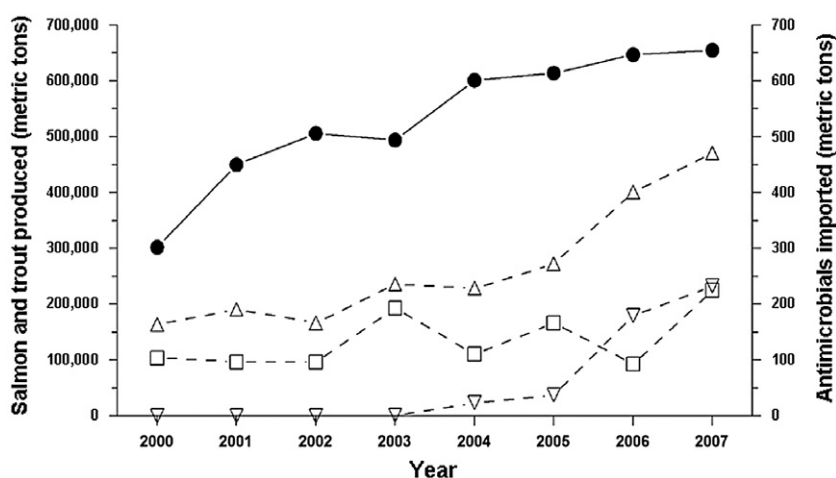


Fig. 2. Production of salmon and trout in Chile and net imports of selected antimicrobials for veterinary use (both in metric tons) to Chile, 2000–2007. Production of salmon and trout (●); imports of tetracyclines (Δ), quinolones/fluoroquinolones (□), and florfenicol (▽). Modified from (Millanao, 2002; Barrientos, 2006; SalmonChile, 2008; Gómez, 2009; Millanao *et al.*, 2011; A. Millanao and H. Dölz, unpublished).

Antimicrobials in water and sediments

Antimicrobials used in aquaculture are administered to fish mostly in food and only rarely by injection or bath (Capone *et al.*, 1996; Herwig *et al.*, 1997; Armstrong *et al.*, 2005; Sørsum, 2006; Rodgers and Furones, 2009). This method of administration leads to their affecting both diseased and healthy fish (metaphylaxis) in the population (Sørsum, 2006). Unconsumed medicated food (perhaps as much as 30% of that supplied if fish are diseased and anorexic) is deposited by gravity in sediments under and around aquaculture sites (Björklund *et al.*, 1990; Capone *et al.*, 1996; Herwig *et al.*, 1997; Armstrong *et al.*, 2005; Sarmah *et al.*, 2006; Sørsum, 2006; Sapkota *et al.*, 2008; Pelletier *et al.*, 2009; Rodgers and Furones, 2009). Of the ingested antimicrobials, approximately 80% pass into the environment in unabsorbed form in faeces or after absorption, in secreted forms in urine and other secretions (Björklund *et al.*, 1990; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Burka *et al.*, 1997; Le and Munekage, 2004; Armstrong *et al.*, 2005; Sørsum, 2006). These also accumulate in the sediments under and around the aquaculture pens (Björklund *et al.*, 1990; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Arthur *et al.*, 2000; Coyne *et al.*, 2001; Le and Munekage, 2004; Armstrong *et al.*, 2005) from where they can be carried by water currents to sediments at distant sites (Samuelsen *et al.*, 1992b; Capone *et al.*, 1996; Coyne *et al.*, 1997; 2001; Arthur *et al.*, 2000; Fortt *et al.*, 2007; Buschmann *et al.*, 2012). In places where hundreds of metric tons of antimicrobials are used per year in a limited geographical area, antimicrobials may remain in large amounts for far longer periods of time than was previously thought to occur (Asche *et al.*, 2010; Burrige *et al.*, 2010; Millanao *et al.*, 2011; Buschmann *et al.*, 2012). Antimicrobials leached from sediments as well as from ingestion of uneaten medicated feed can also potentially affect free-ranging

fish, shellfish and other animals in proximity to aquaculture sites (Björklund *et al.*, 1990; Samuelsen *et al.*, 1992b; Capone *et al.*, 1996; Coyne *et al.*, 1997; Fortt *et al.*, 2007).

The length of time untransformed and transformed antimicrobial activity remains in sediments is dependent on the initial concentrations of antimicrobials (i.e. proportional to the total amounts used at aquaculture sites), their chemical structures, and the half-life of these compounds (Björklund *et al.*, 1990; 1991; Husevåg *et al.*, 1991; Samuelsen *et al.*, 1994; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Kerry *et al.*, 1996; Arthur *et al.*, 2000; Chelossi *et al.*, 2003; Boxall *et al.*, 2004; Kummerer, 2009). Environmental chemical and physical variables such as sediment characteristics, water currents, temperature, light and pH also influence the length of time sediments retain antimicrobial activity (Capone *et al.*, 1996; Kummerer, 2009). Leaching into water and dispersion by currents appears to be the main mechanism mediating decreases in antimicrobial concentrations in sediments rather than degradation *per se*, but this has not been extensively studied (Björklund *et al.*, 1990; Samuelsen *et al.*, 1992a; 1994; Hektoen *et al.*, 1995; Kummerer, 2009). Field and laboratory investigations have indicated that detectable concentrations of biologically-active oxytetracycline remain in sediments for months to more than a year (Björklund *et al.*, 1990; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Coyne *et al.*, 2001; Koeyupdsu *et al.*, 2005). Studies on artificial marine sediments suggest that non-degradable quinolones such as oxolinic acid and flumequine may persist close to aquaculture sites months after their utilization (Hansen *et al.*, 1993; Samuelsen *et al.*, 1994; Hektoen *et al.*, 1995; Lai and Lin, 2009). Similar studies with sulfa drugs, trimethoprim and florfenicol also suggest that these remain active in sediments for several months (Samuelsen *et al.*, 1994; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Hoa

et al., 2008). Although florfenicol disappears in a few days, one of its derivatives, florfenicol amine, remains in sediments for months (Hektoen *et al.*, 1995). The half-life of antimicrobials within and under sediments is also prolonged; they continue to be able to exert selective pressure in this location for an extended period of time (Hektoen *et al.*, 1995; Capone *et al.*, 1996). Antimicrobials such as tetracycline can exert antimicrobial activity even if they adsorb to sediments and react with inhibitory cations such as Mg^{2+} and Ca^{2+} , especially in areas where large amounts are used and subinhibitory concentrations are maintained in the environment (Barnes *et al.*, 1995; Lunestad and Goksøyr, 2010). Some authors have claimed that antimicrobials such as tetracycline do not end up in sediments because only minimal amounts are detectable there (Smith, 1996; Miranda, 2012). The subinhibitory concentrations of antimicrobials in the sediment postulated by supporters of this hypothesis would still have sufficient biological activity to affect horizontal gene transfer (HGT) and mutagenesis in bacteria (Beaber *et al.*, 2004; Hastings *et al.*, 2004; Davies, 2009; Gullberg *et al.*, 2011). In fact, concentrations of antimicrobials detected in sediments in several studies are still many times greater than the minimal inhibitory concentrations for most bacteria (Samuelsen, 1989; Björklund *et al.*, 1991; Samuelsen *et al.*, 1992a; Capone *et al.*, 1996; Smith, 1996; Tello *et al.*, 2012).

Effects of antimicrobials in the aquacultural environment

Selection of antimicrobial-resistant bacteria

Significant concentrations of antimicrobials remaining for long periods of time in the aquatic environment are the principal selective pressure for antimicrobial resistance in bacteria in sediments and the overlying water column (Samuelsen *et al.*, 1994; Hektoen *et al.*, 1995; Capone *et al.*, 1996; Herwig *et al.*, 1997; Petersen *et al.*, 2002; Giraud *et al.*, 2006; Dang *et al.*, 2007; Baquero *et al.*, 2008; 2009; Ding and He, 2010; Marshall and Levy, 2011). The impact of this process leads to a major alteration of the biodiversity of the sediment and water by replacing susceptible communities of bacteria and other microorganisms with resistant ones. This impact has been extensively documented both in the laboratory and in the field (DePaola *et al.*, 1995; Capone *et al.*, 1996; Herwig and Gray, 1997; Herwig *et al.*, 1997; Holten Lützhøft *et al.*, 1999; Arthur *et al.*, 2000; Guardabassi *et al.*, 2000; Miranda and Zemelman, 2002a,b; Kim *et al.*, 2004; 2011; Le and Munksgaard, 2004; Alcaide *et al.*, 2005; Le *et al.*, 2005; Akinbowale *et al.*, 2006; 2007; Christensen *et al.*, 2006; Giraud *et al.*, 2006; Cordova-Kreylos and Scow, 2007; Dang *et al.*, 2007; 2011; Gonçalves Ferreira *et al.*, 2007; Gordon *et al.*,

2007; Miranda and Rojas, 2007; Heepngoen *et al.*, 2008; American Academy of Microbiology, 2009; Ding and He, 2010; Fernández-Alarcón *et al.*, 2010; Ishida *et al.*, 2010; Andersson and Hughes, 2011). Significant increases in the frequency of bacteria resistant to oxytetracycline, quinolones, sulfa/trimethoprim, florfenicol, and amoxicillin have been repeatedly found in proximity to aquaculture farms employing these antimicrobials, suggesting a causal relationship between these variables (DePaola *et al.*, 1995; Guardabassi *et al.*, 2000; Schmidt *et al.*, 2000; Dang *et al.*, 2007; Gordon *et al.*, 2007; Suzuki, 2010). Moreover, antimicrobial-resistant bacteria are found at aquaculture sites for a prolonged period of time after antimicrobial use, further suggesting the relevance of this selection over time (Husevåg *et al.*, 1991; Tamminen *et al.*, 2011b). Laboratory models using aquatic sediments have consistently demonstrated that introduction of antimicrobials is accompanied by increases in the frequency of antimicrobial-resistant bacteria (Hansen *et al.*, 1993; Herwig and Gray, 1997; Stepanauskas *et al.*, 2006) and, as expected from the modular clustering of antimicrobial resistant genetic elements, introduction of one antimicrobial can give rise to bacteria resistant to other antimicrobials that are not even in use in the area (Herwig and Gray, 1997; Le *et al.*, 2005; Alekshun and Levy, 2007; Stokes and Gillings, 2011). Whether these antimicrobials remain in the sediment or leach into the surrounding water, the end result is still selection of antimicrobial-resistant bacteria (Davies and Davies, 2010; Marshall and Levy, 2011; Buschmann *et al.*, 2012).

The fact that Chilean salmon aquaculture experienced epizootics and infestations resulting from unsanitary conditions strongly suggests that a large proportion of these antimicrobials were used for prophylaxis rather than for therapeutics (Godoy *et al.*, 2008; Kibenge *et al.*, 2009; Asche *et al.*, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011). In Chile at least, aquaculture rather than human and other veterinary medical activities would seem to be the most important source for passage of antimicrobials into the aquatic environment where they select for antimicrobial-resistant bacteria (Asche *et al.*, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011). In view of the continuing worldwide increase in aquaculture, the effects of antimicrobial use in this industry raise questions that deserve careful monitoring (FAO, 2010).

The emergence of antimicrobial-resistant bacteria may even be greater than that which has been detected since most studies have been limited to demonstrating this resistance in culturable bacteria, which constitute only a small proportion of the total bacteria present in the aquatic environment (Bissett *et al.*, 2006). There is a lack of information regarding microbial communities that change in numbers or even disappear in aquatic environments

because of their susceptibility to antimicrobials and the effect this phenomenon may have on metabolic activities of microbial communities and the health of the sediment (Bissett *et al.*, 2006; Edlund *et al.*, 2006; Ma *et al.*, 2006; Gonçalves Ferreira *et al.*, 2007). Deposition of food pellets and organic matter lacking antimicrobials onto sediments near aquaculture sites and in the laboratory have been shown to impact sediment microbial biodiversity and have been suggested to increase the fraction of antimicrobial-resistant bacteria present in them (Smith *et al.*, 1994; Kapetanaki *et al.*, 1995; Nogales *et al.*, 2011; Pitkanen *et al.*, 2011; Tamminen *et al.*, 2011a). However, these studies did not rule out the presence of other antimicrobial compounds in the food or in the sediments such as heavy metals (Zn, Cu, Hg), disinfectants, and organic antibacterial compounds, any or all of which might be responsible for these results (Smith *et al.*, 1994; Kapetanaki *et al.*, 1995; Akinbowale *et al.*, 2007; Tacon and Metian, 2008; Pitkanen *et al.*, 2011; Tamminen *et al.*, 2011a). Although the observed increase in antimicrobial-resistant bacteria could be explained by linkage of genes involved in metabolism of organic matter with antimicrobial resistance genes, the preponderance of evidence to date suggests that antimicrobial residues present in the environment where aquaculture takes place are the most relevant selective pressure to account for the increased fraction of antimicrobial-resistant bacteria there.

Mechanisms of bacterial selection

The genomes of aquatic bacteria are highly diverse and contain genetic elements and genes involved in the generation and dissemination of antimicrobial resistance genes similar to those previously characterized in terrestrial bacteria (Venter *et al.*, 2004; Baker-Austin *et al.*, 2009; Biers *et al.*, 2009; Sobecky and Hazen, 2009; Hazen *et al.*, 2010; McDaniel *et al.*, 2010; Wiedenbeck and Cohan, 2011). The total of all mobile genetic elements (MGE) of the genome of aquatic bacteria, the mobilome, include water current-transported naked DNA (Stewart and Sinigalliano, 1990; Sobecky and Hazen, 2009; Fondi and Fani, 2010; Taylor *et al.*, 2011; Domingues *et al.*, 2012), insertion sequences (Toleman and Walsh, 2011), insertion sequence elements with common regions (ISCR) (Toleman *et al.*, 2006; Toleman and Walsh, 2010; Xia *et al.*, 2010), integrons mobilized by plasmids, transposons and integrative and conjugative elements (ICE or SXT) (Rosser and Young, 1999; L'Abée-Lund and Sørum, 2001; Schmidt *et al.*, 2001b; Burrus *et al.*, 2006; Koenig *et al.*, 2008; Osorio *et al.*, 2008; Wozniak *et al.*, 2009; Cambray *et al.*, 2010; Daccord *et al.*, 2010; Rosewarne *et al.*, 2010; Wozniak and Waldor, 2010), genomic islands (Boyd *et al.*, 2002; 2008; Juhas *et al.*, 2009; Daccord *et al.*, 2010; Le Hello *et al.*, 2011), transposons and con-

jugative transposons (Rhodes *et al.*, 2000; Knapp *et al.*, 2008), conjugative and mobilizable plasmids (Baya *et al.*, 1986; Aoki *et al.*, 1987; Kim and Aoki, 1996b; Sobecky *et al.*, 1997; Schmidt *et al.*, 2001b; Furushita *et al.*, 2003; Kim *et al.*, 2004; Rhodes *et al.*, 2004; Gordon *et al.*, 2007; Cattoir *et al.*, 2008; Guglielmetti *et al.*, 2009; Sobecky and Hazen, 2009; Erauso *et al.*, 2011; Ma *et al.*, 2012), and bacteriophages, including phage-like elements designated gene transfer agents (GTA) (Suttle, 2007; Colomer-Lluch *et al.*, 2011; Lang *et al.*, 2012). GTA mediate HGT between heterologous bacteria and appear to have an important role in this process in marine bacterial communities (Lang *et al.*, 2012). It is not surprising that introduction of large amounts of antimicrobials into the aquatic environment is rapidly followed by emergence of significant numbers of multiple-resistant bacteria since antimicrobial resistance genes would enhance fitness for growth in sediments containing antimicrobials (Capone *et al.*, 1996; Kerry *et al.*, 1996; Sobecky *et al.*, 1997; Guardabassi *et al.*, 2000; Schmidt *et al.*, 2000; Furushita *et al.*, 2003; Groh *et al.*, 2007; Seyfried *et al.*, 2010). Moreover, contrary to well-documented reports showing that some antimicrobial resistance mechanisms have a fitness cost, the presence of the quinolone resistance gene *qnrA* in some aquatic bacteria and other antimicrobial resistance genes in *Shewanella* may enhance fitness in the absence of antimicrobials (Groh *et al.*, 2007; Michon *et al.*, 2011).

Conditions in aquatic environments that favour HGT include biofilms of aquatic bacteria on the epilithon (particulate organic matter coating benthic ecosystems), on clays and sand of sediments, and on aquacultural structures, coupled with the large concentrations of bacteriophages and GTAs in seawater, also favour HGT and dissemination of antimicrobial resistance (Hill *et al.*, 1992; Sobecky *et al.*, 1997; Bushman, 2002; Venter *et al.*, 2004; Furushita and Shiba, 2007; Suttle, 2007; McDaniel *et al.*, 2010; Marshall *et al.*, 2011; Sundell and Wiklund, 2011; Taylor *et al.*, 2011; Lang *et al.*, 2012; Lupo *et al.*, 2012; Toussaint and Chandler, 2012). Antimicrobials can potentially also stimulate HGT mediated by naked DNA generated by bacteriophage lysis, as well as that mediated by plasmids in the aquatic environment and in the intestines of fish and terrestrial animals (Stewart and Sinigalliano, 1990; Beaber *et al.*, 2004; Frost *et al.*, 2005; Aarestrup *et al.*, 2008; Allen *et al.*, 2011; Domingues *et al.*, 2012; Looft *et al.*, 2012). In addition, aquatic bacteriophages can contain antimicrobial resistance genes that may be expressed upon infection of bacteria (Colomer-Lluch *et al.*, 2011). Several aquatic bacteria such as *Vibrio* spp. are naturally competent for DNA uptake, thus also increasing the opportunities for transformation to occur in the aquatic environment (Stewart and Sinigalliano, 1990; Meibom *et al.*, 2005; Baharoglu *et al.*, 2012).

Bacteria from aquatic and terrestrial environments share similar antimicrobial genetic determinants (Table 2, Fig. 3) (Baquero *et al.*, 2008; Sobecky and Hazen, 2009; Marshall and Levy, 2011; Taylor *et al.*, 2011; Buschmann *et al.*, 2012), and HGT and recombination of these determinants between different bacterial species can be stimulated by residual and subinhibitory antimicrobial concentrations of tetracyclines and quinolones in sediments (Kruse and Sørum, 1994; Aarestrup *et al.*, 2000; Beaber *et al.*, 2004; Hastings *et al.*, 2004; Davies, 2009; Buschmann *et al.*, 2012). Bacteria in aquatic environments may in fact be the source of genetic elements of the mobilome such as SXT, ISCR, and integrons as well as previously unknown antimicrobial resistance determinants (Miranda *et al.*, 2003; Burrus *et al.*, 2006; Laroche *et al.*, 2009; Daccord *et al.*, 2010; Kristiansson *et al.*, 2011; Xu *et al.*, 2011a,b; Ma *et al.*, 2012). For example, *tetG* (Table 2), an independently evolved tetracycline resistance determinant, was first discovered in aquatic bacteria (Aoki *et al.*, 1987; Zhao and Aoki, 1992; Angulo, 1999). Several phenotypically tetracycline-resistant bacteria isolated from aquaculture sites also contained genetic determinants that could not be amplified by PCR with primers corresponding to the known tetracycline resistance determinants indicating that they carried unknown tetracycline resistance genes (Miranda *et al.*, 2003).

A number of antimicrobial resistance genes appear to have been first detected in aquatic bacteria before being detected and disseminating among human and animal pathogens. These include some of the emerging plasmid-mediated quinolone resistance (PMQR) genes found in aquatic *Vibrio*, *Shewanella* and *Aeromonas* (Table 2) (Poirel *et al.*, 2005; 2012; Cattoir *et al.*, 2007; 2008; Xia *et al.*, 2010); new β -lactamase genes from *Photobacterium damsela* (Table 2) (Morii, 2004) and *Oceanobacillus ihayensis* (Toth *et al.*, 2010); a novel fosfomycin resistance determinant isolated from the aquatic environment (Xu *et al.*, 2011b); the widely disseminated emerging *floR* gene of human pathogens (Kim and Aoki, 1996a; Angulo, 1999; Arcangioli *et al.*, 1999; 2000; Bolton *et al.*, 1999; Cloeckert *et al.*, 2000; 2001; Miranda and Rojas, 2007; Gordon *et al.*, 2008; Smith, 2008a,b; Cabello, 2009; Welch *et al.*, 2009; Fernández-Alarcón *et al.*, 2010; Hall, 2010); and the chloramphenicol resistance genes *catII*, *catB9* and *catB2* from aquatic *Photobacterium*, *Vibrio* and *Shewanella* respectively (Roberts and Schwarz, 2009; Roberts *et al.*, 2012). Moreover, antimicrobial resistance gene variants including those for β -lactams, aminoglycosides, tetracyclines, macrolides and heavy metals have been detected in the genome of the salmon pathogen *Renibacterium salmoninarum* and the aquatic opportunistic human pathogen *Stenotrophomonas maltophilia* suggesting that these aquatic bacteria may be repositories for

antimicrobial resistance genes (Crossman *et al.*, 2008; Wiens *et al.*, 2008).

Selection of antimicrobial-resistant bacteria in the aquatic environment can also occur by selection of spontaneous single mutants since water, sediments and piscine intestines all contain sufficiently large concentrations of bacteria to have detectable numbers of spontaneously arising antimicrobial-resistant mutants (Capone *et al.*, 1996; Levy and Marshall, 2004; Alekshun and Levy, 2007; Navarrete *et al.*, 2008; Navarro *et al.*, 2008; Nikaido, 2009). Moreover, the high density of fish and shellfish in aquacultural enclosures increases the opportunities for this selection to occur (Woo *et al.*, 2002; Beveridge, 2004; Austin and Austin, 2012). Mutants and bacteria tolerant to antimicrobials can clearly be selected by inhibitory and subinhibitory concentrations of antimicrobials (Miller *et al.*, 2004; Dorr *et al.*, 2009; Kohanski *et al.*, 2010). Though this mechanism may not be as effective for selection and dissemination of antimicrobial-resistant bacteria as selection of bacteria containing MGEs with multiple antimicrobial resistance genes (Akinbowale *et al.*, 2007; Davies, 2009; Davies and Davies, 2010), it may be relevant since persistent residual and subinhibitory concentrations of antimicrobials in sediments can trigger the SOS system (a bacterial reparative response to DNA damage). This system can increase the rate of mutagenesis by several mechanisms including generation of oxygen radicals (Kohanski *et al.*, 2007; 2010; Dorr *et al.*, 2009; Blazquez *et al.*, 2012). Subinhibitory concentrations of antimicrobials can also select resistant bacteria by non-SOS-mediated mechanisms such as DNA recombination, amplification, and selection for hypermutator strains (López *et al.*, 2007; Sun *et al.*, 2009; Blazquez *et al.*, 2012). These mechanisms may be especially relevant with quinolones located in sediments since these antibacterial agents are only slowly degraded and are well-known inducers of mutagenesis and antimicrobial tolerance (Dorr *et al.*, 2009; Lai and Lin, 2009; Kohanski *et al.*, 2010; Blazquez *et al.*, 2012).

Antimicrobial-resistant mutants selected in fish intestinal tracts and in the environment can also have their mutated genes captured by integrons, genetic elements with diverse antimicrobial resistance determinant cassettes that can be mobilized by transposons and plasmids to generate new permutations of resistance genes (Rowe-Magnus and Mazel, 1999; L'Abée-Lund and Sørum, 2001; Mazel, 2006; Boucher *et al.*, 2007; Jacobs and Chenia, 2007; Gillings *et al.*, 2008; Laroche *et al.*, 2009; Xia *et al.*, 2010; Stalder *et al.*, 2012). The frequent presence of integrons in aquatic bacteria, especially in bacteria from sediments impacted by anthropogenic activities such as aquaculture, may suggest an aquatic origin (Rosser and Young, 1999; Schmidt

Table 2. Some genetic elements and antimicrobial resistance genes shared between aquatic bacteria and human pathogens.

	β -proteobacteria	Shewanella	Vibrio	Photobacterium	Aeromonas	Moraxella	Acinetobacter	Y. ruckeri	Kluyvera	Reference
Elements										
ICE		+	+	+						a
Integron 1	+	+	+							b
ISCR		+	+	+	+					c
SXT/391		+	+	+						d
plncA/C			+	+	+			+		e
plncU			+	+	+					f
SGI1				+						g
Tn1721				+	+					h
Genes										
<i>floR</i>				+	+					i
β -lactamase				+						j
CTX- β -lactamase									+	k
<i>qnrA</i>		+								l
<i>qnrS</i>			+	+	+					m
<i>qnrVC</i>			+		+					n
<i>tetD</i> (E)			+		+					o
<i>telH</i>							+			p
<i>telC</i>						+				q
<i>telG</i>			+		+					r

- a. Burrus *et al.* (2006); Osorio *et al.* (2008); Toleman and Walsh (2011); Rodriguez-Blanco *et al.* (2012).
b. L'Abee-Lund and Serum (2001); Boucher *et al.* (2007); Gillings *et al.* (2008); Koenig *et al.* (2008); Laroche *et al.* (2009); Cambray *et al.* (2010).
c. Toleman *et al.* (2006); Toleman and Walsh (2011).
d. Wozniak *et al.* (2009); Wozniak and Waldor (2010).
e. McIntosh *et al.* (2008); Fricke *et al.* (2009); Welch *et al.* (2009).
f. Serum *et al.* (2003); Rhodes *et al.* (2004).
g. Briggs and Fratamico (1999); Boyd *et al.* (2008).
h. Rhodes *et al.* (2000).
i. Kim and Aoki (1996a); Arcangioli *et al.* (1999); Cloeckaert *et al.* (2000); Gordon *et al.* (2008); Smith (2008a,b); Cabello (2009); Welch *et al.* (2009).
j. Morii (2004).
k. Decousser *et al.* (2001); Rodriguez *et al.* (2004); Cantón and Coque (2006); Cantón *et al.* (2012).
l. Poirel *et al.* (2005).
m. Saga *et al.* (2005); Cattoir *et al.* (2007); Cattoir *et al.* (2008); Poirel *et al.* (2012).
n. Xia *et al.* (2010).
o. Furushita *et al.* (2003); Roberts and Schwarz (2009).
p. Miranda *et al.* (2003).
q. Furushita *et al.* (2003); Sandoz and Rockey (2010).
r. Zhao and Aoki (1992).

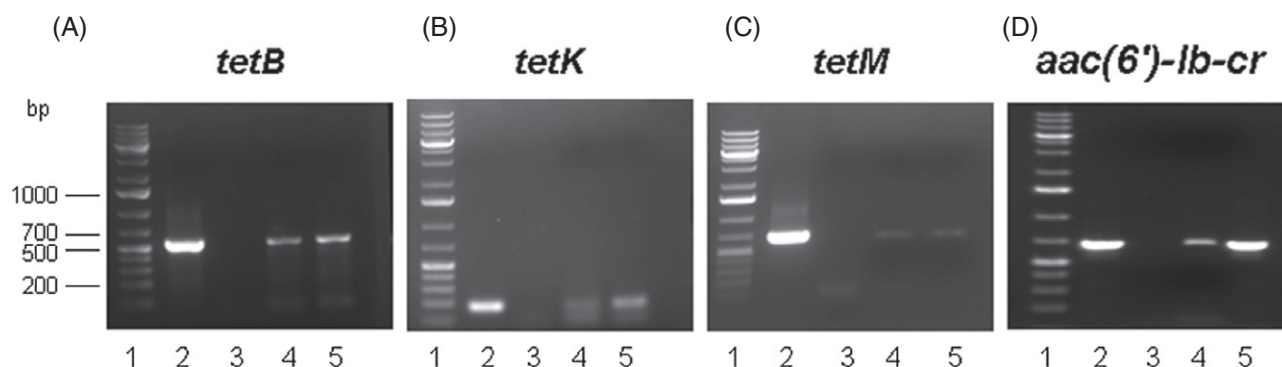


Fig. 3. Sharing of tetracycline and plasmid mediated quinolone resistance determinant *aac(6')-Ib-cr* between marine bacteria and four *Escherichia coli* clinical isolates (isolates 1–4) from human urinary tract infections in Region X, Chile. Amplicons of *tetB*, *tetK*, *tetM* and *aac(6')-Ib-cr* were detected by PCR as described (Ng *et al.*, 2001; Miranda *et al.*, 2003; Robicsek *et al.*, 2006). For all panels, lane 1, molecular weight standards; lane 2, positive control; lane 3, negative control (*E. coli* DH5 α); lane 4, marine bacterial isolate; lane 5, clinical isolate. A. *tetB*. Lane 2, *E. coli* DH5 α pET*tetB*1; lane 4, *Pseudoalteromonas* sp.; lane 5, *E. coli* isolate 1. B. *tetK*. Lane 2, *S. aureus* pT181; lane 4, *Pseudoalteromonas* sp.; lane 5, *E. coli* isolate 2. C. *tetM*. Lane 2, *E. coli* DH10B pJFP76; lane 4, *Shewanella* sp.; lane 5, *E. coli* isolate 3. D. *aac(6')-Ib-cr*. Lane 2, *E. coli* J53 pMG298; lane 4, *Rhodococcus* sp.; lane 5, *E. coli* isolate 4.

et al., 2001a; Rosewarne *et al.*, 2010; Gaze *et al.*, 2011). Stimulation of the SOS stress regulon by antimicrobials such as quinolones and β -lactamases can not only stimulate HGT by transformation and conjugation but can also affect integron recombination and plasticity. This latter is a result of the triggering of integrase activity with its resultant antimicrobial resistance cassette uptake and expression (Baharoglu *et al.*, 2010; 2012; Cambray *et al.*, 2011). Subinhibitory concentrations of antimicrobials from aquacultural activities could thus, besides selecting and inducing antimicrobial resistance in sediments and water, also mediate antimicrobial resistance genetic plasticity *in vivo* in the intestine of aquaculture species (Guerin *et al.*, 2009; Baharoglu *et al.*, 2010; Baharoglu and Mazel, 2011; Blazquez *et al.*, 2012; Hocquet *et al.*, 2012).

It has been suggested that antimicrobial resistance genes and antimicrobial-resistant bacteria arrive there in fish food and in exogenous contaminating effluents rather than being generated from local sources in the water and sediments at aquaculture sites (Smith *et al.*, 1994; Kapetanaki *et al.*, 1995; Kerry *et al.*, 1995; Smith, 2008b; Martinez, 2009b; 2012; Pitkanen *et al.*, 2011). This and the fact that fish food may increase antimicrobial resistance (Smith *et al.*, 1994; Kapetanaki *et al.*, 1995; Kerry *et al.*, 1995; Martinez, 2009b; Pitkanen *et al.*, 2011) are plausible hypotheses that deserve investigation. However, persistence and increase of these genes and these bacteria in aquatic environments will be sustained by the presence of antimicrobials no matter how they arrive (Akinbowale *et al.*, 2007; Davies, 2009; Nikaido, 2009; Davies and Davies, 2010). The modular

nature of the MGE involved in antimicrobial resistance in aquatic bacteria also facilitates selection of multiple antimicrobial resistances by a single antimicrobial compound and by other antimicrobial compounds used in aquaculture such as heavy metals and disinfectants (Herwig and Gray, 1997; Lawrence, 2000; Stepanauskas *et al.*, 2006; Akinbowale *et al.*, 2007; Alekshun and Levy, 2007; Davies, 2009; Seiler and Berendonk, 2012).

Antimicrobial resistance genes have been demonstrated in ancient bacterial DNA extracted from terrestrial permafrost and in collections of bacteria preceding introduction of antimicrobials (Datta and Hughes, 1983; Hughes and Datta, 1983; D'Costa *et al.*, 2011). While the effects of aquacultural antimicrobial use on aquatic sediments are most likely restricted to selecting those bacteria able to survive in their presence, the increase of antimicrobial-resistant bacteria that this produces and the particularities of the aquatic environment at aquaculture sites may provide new avenues for the generation and emergence of previously unknown and undescribed mechanisms for this selection as well as of new permutations of antimicrobial resistance genes as those generated by integron plasticity (Levesque *et al.*, 1995; Sobecky *et al.*, 1997; Sørum, 2006; Baquero *et al.*, 2008; Sobecky and Hazen, 2009; Baharoglu *et al.*, 2010; Cambray *et al.*, 2010; Taylor *et al.*, 2011; Hocquet *et al.*, 2012). In aquaculture and the aquatic environment, antimicrobials clearly appear to display their hormetic properties: higher concentrations of antimicrobials select for resistant bacteria, while subinhibitory concentrations of their residues might stimulate HGT and mutagenesis (Linares *et al.*, 2006).

Table 3. Some fish-associated bacterial zoonoses.^{a,b,c,d,e,f}

Mechanism of transmission	Disease
Contact-borne <i>Mycobacterium marinum</i> , <i>M. fortuitum</i> , <i>M. ulcerans</i> <i>Streptococcus iniae</i> <i>Aeromonas hydrophila</i> , <i>A. sobria</i> , <i>A. caviae</i> <i>Vibrio damsela</i> , <i>V. vulnificus</i> , <i>V. mimicus</i> , <i>V. fluvialis</i> , <i>V. alginolyticus</i> <i>Edwardsiella tarda</i> <i>Erysipelothrix rhusopathiae</i> <i>Stenotrophomonas maltophilia</i> (?) <i>Kluyvera</i> (?)	Fish handler disease, tank granuloma Cellulitis, systemic infections Skin wound infections, systemic infections Skin and wound infections, systemic infections Cellulitis, gastroenteritis, bacteraemia Skin infections, systemic infections Pneumonia, systemic infections Gastroenteritis, bacteraemia
Food-borne <i>Vibrio parahaemolyticus</i> , <i>V. cholerae</i> <i>Aeromonas hydrophila</i> <i>Salmonella</i> <i>Listeria monocytogenes</i> <i>Clostridium botulinicum</i> , <i>C. perfringens</i> <i>Plesiomonas shigelloides</i>	Diarrhoea Diarrhoea, systemic infections Diarrhoea, systemic infections Diarrhoea, systemic infections Botulism, diarrhoea Diarrhoea

- a. Sarria *et al.* (2001).
b. Looney *et al.* (2009).
c. Lowry and Smith (2007).
d. Iwamoto *et al.* (2010).
e. Boylan (2011).
f. Austin and Austin (2012).

Effects of aquacultural use of antimicrobials on animal and human health

Animal health

The most obvious detrimental effect of extensive use of antimicrobials in aquaculture is selection of fish and shellfish pathogens resistant to multiple antimicrobials which in turn produce difficult or impossible to treat epizootics (L'Abée-Lund and Sørsum, 2002; Murray and Peeler, 2005; Toranzo *et al.*, 2005; Asche *et al.*, 2010; Barton and Floydsand, 2010; Pulkkinen *et al.*, 2010; Ibieta *et al.*, 2011). The clinical problems generated in veterinary and human medicine by antimicrobial-resistant bacteria are well reviewed (Aarestrup *et al.*, 2000; 2008; Anderson *et al.*, 2003; Angulo *et al.*, 2004; Molbak, 2006; Sapkota *et al.*, 2008; Le Hello *et al.*, 2011; Marshall and Levy, 2011), and fish and shellfish pathogens resistant to multiple antimicrobials used in aquaculture have been described (Austin, 1985; Arthur *et al.*, 2000; Sørsum, 2000; 2006; Armstrong *et al.*, 2005; Toranzo *et al.*, 2005). These include *Aeromonas salmonicida*, *A. hydrophila*, *A. caviae*, *A. sobria*, *E. ictaluri*, *E. tarda*, *P. damsela*, *P. piscicida*, *Vibrio anguillarum*, *V. salmonicida*, *V. ordalii*, *Flavobacterium psychrophilum*, *Pseudomonas fluorescens*, *Streptococcus iniae*, *Renibacterium salmonicarium*, *Yersinia ruckeri* and *Piscirickettsia salmonis* (Table 2). In most of them, antimicrobial resistance is mediated by plasmids and MGE, often conjugative, and with potential for HGT (Austin, 1985; Arthur *et al.*, 2000; Rhodes *et al.*, 2000; Sørsum, 2000; 2008; Schmidt *et al.*, 2001a; Armstrong *et al.*, 2005; Casas *et al.*, 2005; Toranzo *et al.*, 2005; Erauso *et al.*, 2011). Some of these pathogens, e.g. *Edwardsiella*,

Aeromonas, and *Streptococcus*, can infect humans and generate antimicrobial-resistant zoonotic infections (Table 3) (Novotny *et al.*, 2004; Toranzo *et al.*, 2005; Lowry and Smith, 2007; Iwamoto *et al.*, 2010; Boylan, 2011; Naviner *et al.*, 2011; Austin and Austin, 2012; Leung *et al.*, 2012). *Kluyvera* spp. and *S. maltophilia* are additional aquatic bacteria related to fish that are emerging as opportunistic human pathogens (Sarria *et al.*, 2001; Looney *et al.*, 2009).

The worldwide occurrence of IncU, pRAS3, pRAS1 and pAr-32 plasmids in *Aeromonas* illustrates the relevance of the widespread dissemination of antimicrobial resistance genes coded by MGE in fish and shellfish pathogens (L'Abée-Lund and Sørsum, 2002; Sørsum *et al.*, 2003). Similarly, PMQR genes selected in aquatic bacteria as a result of aquacultural antimicrobial use could hypothetically pass by HGT to fish pathogens such as *F. psychrophilum*, *A. salmonicida* and *Y. ruckeri* expressing a mutated GyrA (Oppegaard and Sørsum, 1994; Izumi and Aranishi, 2004; Shah *et al.*, 2012a,b). Such an occurrence could increase quinolone resistance and increase quinolone concentrations required to prevent chromosomal mutations to these antimicrobials, and as a result complicate treatment of infections caused by these pathogens (Oppegaard and Sørsum, 1994; Izumi and Aranishi, 2004; Strahilevitz *et al.*, 2009; Shah *et al.*, 2012a,b).

While selection of antimicrobial-resistant bacteria in normal intestinal and other flora of fish as a result of aquacultural use of antimicrobials has not been extensively investigated, it is reasonable to suppose that antimicrobial resistance determinants present in normal piscine flora could be the source of resistance

determinants in piscine pathogens analogous to what has been shown to occur in terrestrial animals and human beings (Salyers and Shoemaker, 2006; Navarrete *et al.*, 2008; Marshall *et al.*, 2009; Martinez *et al.*, 2009; Nayak, 2010; Looft *et al.*, 2012). Antimicrobial resistance determinants in piscine pathogens could also be acquired from environmental antimicrobial-resistant bacteria that have been selected by residual antimicrobials in water and sediments (Kruse and Sørum, 1994; Davison, 1999; Alonso *et al.*, 2001; Fricke *et al.*, 2008; Cantón, 2009; Martinez, 2009a; 2012; Allen *et al.*, 2010; Fondi and Fani, 2010; Colomer-Lluch *et al.*, 2011; Stokes and Gillings, 2011; Dantas and Sommer, 2012). Both of these processes can be stimulated by the presence of antimicrobials in fish tissues and in the environment since (as previously mentioned) many of these antimicrobials are able to fuel HGT and mutagenesis (Aarestrup *et al.*, 2000; Beaber *et al.*, 2004; Couce and Blazquez, 2009; Kohanski *et al.*, 2010; Allen *et al.*, 2011; White and McDermott, 2011). Moreover, alterations produced by antimicrobials in the sediments and in the normal flora in the piscine intestinal tract may favour infection by pathogens (Navarrete *et al.*, 2008; Nayak, 2010). Excessive and prophylactic use of antimicrobials in aquacultural settings can thus be counterproductive by selecting and favouring untreatable infections with piscine and shellfish pathogens resistant to multiple antimicrobials, and result in the collapse of these activities (Lin, 1989; Holmström *et al.*, 2003; León-Muñoz *et al.*, 2007; Asche *et al.*, 2010; Barton and Floydsand, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011).

Salmon aquaculture requires growth of this anadromous species in both fresh and salt water. This results in differences in normal flora and pathogens in fish and the environment in these two locations, differences which in turn affect the outcome of the selective effects of antimicrobials (Woo *et al.*, 2002; Beveridge, 2004; Austin and Austin, 2012). For example, antimicrobials used in freshwater will select for antimicrobial resistance among the freshwater pathogens *F. psychrophilum* and *A. salmonicida* while those used in the marine stages will select among marine pathogens such as *Vibrio* spp. and *P. salmonis* (Woo *et al.*, 2002; Beveridge, 2004; Austin and Austin, 2012).

In Chile, the augmented use of antimicrobials that accompanied increases in salmon production coincided with surges in fish mortalities and emergence of new and resistant bacterial pathogens (Asche *et al.*, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011). It was in this period that *S. phocae*, *Rhodococcus qingshengi*, *F. chilensis* and *F. araucanum* emerged as potential new salmon pathogens (Valdés *et al.*, 2009; Avendaño-Herrera *et al.*, 2011; Kämpfer *et al.*, 2012). Moreover, approximately 90% of isolates of *F. psychrophilum*, the cause of cold water disease in salmon and trout, were resistant to the three

most commonly used antimicrobials (tetracyclines, florfenicol, oxolinic acid) making this disease practically untreatable with them (Henríquez-Núñez *et al.*, 2012). In other regions of the world, pathogens of aquacultured fish such as *Edwardsiella ictaluri* and *E. tarda* also display high levels of antimicrobial resistance (Dung *et al.*, 2008; Nadirah *et al.*, 2012). In Taiwan, the collapse of shrimp aquaculture during the late 1980s resulted from the emergence of multiple-resistant pathogens selected by the injudicious use of antimicrobials (Lin, 1989; Kautsky *et al.*, 2000). Preliminary observations suggest that the frequency of detection of antimicrobial resistance genes in aquaculturally-related aquatic bacteria can be correlated with the amounts of antimicrobials used in this activity in Norway and Chile (Shah, 2012).

Human health

There are increasing signs that antimicrobial use in aquaculture may have a long-term and permanent potential to select for antimicrobial-resistant bacteria in the aquatic environment at multiple levels (DePaola *et al.*, 1995; Capone *et al.*, 1996; Schmidt *et al.*, 2001a; Holmström *et al.*, 2003; Miranda *et al.*, 2003; Sørum, 2006; Miranda and Rojas, 2007; Seyfried *et al.*, 2010). This may be particularly relevant to human health in those countries where aquacultural use is heavy, prophylactic and uncontrolled, since bacteria and archaea in the aquatic environment share a large assortment of MGE and antimicrobial resistance genes with a wide range of terrestrial bacteria (Furushita *et al.*, 2003; Hastings *et al.*, 2004; Furushita and Shiba, 2007; Sobecky and Hazen, 2009; McDaniel *et al.*, 2010; Erauso *et al.*, 2011; Millanao *et al.*, 2011; Taylor *et al.*, 2011; Buschmann *et al.*, 2012). Indeed, there is strong laboratory and field evidence for readily detectable frequencies of HGT between bacteria in the aquatic environment and human pathogens, as would be expected of genetic exchange communities linked by HGT in spite of the oligotrophy of the aquatic environments (Sandaa *et al.*, 1992; Goodman *et al.*, 1993; L'Abée-Lund and Sørum, 2002; Furushita and Shiba, 2007; Baquero *et al.*, 2008; Guglielmetti *et al.*, 2009; Taylor *et al.*, 2011; Lupo *et al.*, 2012). As a result of HGT, these new genetic entities may be incorporated into the pangenome of terrestrial bacteria including human pathogens, linking the aquatic and terrestrial resistomes and complicating the treatment of human infections (Sandaa *et al.*, 1992; Furushita *et al.*, 2003; Medini *et al.*, 2005; Sørum, 2006; Sobecky and Hazen, 2009; Martinez, 2009a; Fondi and Fani, 2010; Erauso *et al.*, 2011; Forsberg *et al.*, 2012). The power of HGT to generate genetic diversity from aquatic bacteria is demonstrated by the ability of human intestinal *Bacteroides* to acquire genes needed for degradation of algal polysaccharides

from aquatic bacteria (Hehemann *et al.*, 2010; 2012). This gene flow may not be directly from aquatic bacteria to human pathogens but may involve intermediaries such as other environmental bacteria or commensal flora of animals and humans (Roberts, 2009; Skippington and Ragan, 2011). This complex ecological web makes tracking the flow of antimicrobial resistant genes and their history difficult but not impossible (Roberts, 2009; Skippington and Ragan, 2011). While genetic flow between aquatic and terrestrial bacteria might well be restricted by molecular mechanisms against DNA transfer (Thomas and Nielsen, 2005; Martinez *et al.*, 2009; Skippington and Ragan, 2011; Wiedenbeck and Cohan, 2011), it still might frequently occur because the strong selective pressure in aquatic sediments contaminated with antimicrobials could overcome these restrictive mechanisms (Hastings *et al.*, 2004; Thomas and Nielsen, 2005; Aminov and Mackie, 2007). The potential bidirectional flow of antimicrobial resistance genes between aquatic bacteria and human pathogens increases the danger to human health if this flow results in high risk clones that can disseminate widely among human populations (Woodford *et al.*, 2011).

An example of such genetic flow is the occurrence of similar IncU incompatibility group plasmids containing Tn1721 TetA determinants and integron1 in piscine and human pathogenic *Aeromonas* and in *Escherichia coli* isolated in hospitalized patients (Rhodes *et al.*, 2000; 2004; Sørsum *et al.*, 2003). Sharing of the quinolone resistance genes *qnrA*, *qnrS* and *qnrVC* by aquatic *Shewanella*, *Photobacterium*, *Aeromonas* and *Vibrio* (Table 2) with a large array of Gram-negative human pathogens (e.g. *E. coli* and *Klebsiella*) is another example of such gene flows (Poirel *et al.*, 2005; 2012; Saga *et al.*, 2005; Cattoir *et al.*, 2007; 2008; Martínez-Martínez *et al.*, 2008; Strahilevitz *et al.*, 2009; Xia *et al.*, 2010; Hernández *et al.*, 2011). We ourselves have found the PMQR gene *aac(6')-Ib-cr*, commonly found in clinical isolates, in marine bacteria such as *Rhodococcus* spp. (Fig. 3) (Robicsek *et al.*, 2006; Buschmann *et al.*, 2012; Poirel *et al.*, 2012). The current dissemination of CTX-M-type extended-spectrum β -lactamases among enteric pathogens may be a third example of human pathogens probably acquiring antimicrobial resistance genes from aquatic bacteria (Rodriguez *et al.*, 2004; Cantón and Coque, 2006; Cantón *et al.*, 2012). In this case, it has been postulated that the CTX-M gene was acquired from *Kluyvera*, a genus encountered in the aquatic environment in fish intestines and an opportunistic human pathogen (Tables 2 and 3) (Decousser *et al.*, 2001; Sarria *et al.*, 2001; Humeniuk *et al.*, 2002; Rodriguez *et al.*, 2004; Cantón and Coque, 2006; Navarrete *et al.*, 2008; Rossolini *et al.*, 2008; Cantón *et al.*, 2012). Plasmids of the IncA/C incompatibility group harbouring a variety of

antimicrobial resistance genetic elements and metal resistance genes have been recently found to be shared by fish pathogens such as *Y. ruckeri*, *Aeromonas*, *Edwardsiella* (Table 2) and human pathogens such as *Y. pestis*, *Salmonella* and *V. cholerae* (Welch *et al.*, 2007; 2009; McIntosh *et al.*, 2008; Pan *et al.*, 2008; Fricke *et al.*, 2009; Call *et al.*, 2010; Douard *et al.*, 2010; Toleman and Walsh, 2010). It has also been postulated that bacteria such as *Aeromonas* exposed to antimicrobials in an aquatic environment may have facilitated the transfer of the IncA/C plasmids between bacteria of different environments to human pathogens (McIntosh *et al.*, 2008; Fricke *et al.*, 2009; Parker and Shaw, 2011). A similar role could be played by *Edwardsiella* and *Vibrio* (Pan *et al.*, 2008; Welch *et al.*, 2009; Leung *et al.*, 2012).

The unrestricted transmission of STX/R391 (an ICE able to harbour a multiple antimicrobial resistance integron and to mobilize genomic islands) between aquatic *V. cholerae*, the opportunistic human pathogens *Providencia* and *Proteus*, the fish pathogen, *P. damsela*, and the environmental aquatic, *Shewanella*, underscores the potential for HGT between bacteria from the aquatic environment and human pathogens (Burrus *et al.*, 2006; Osorio *et al.*, 2008; Wozniak *et al.*, 2009; Daccord *et al.*, 2010; Wozniak and Waldor, 2010; Toleman and Walsh, 2011; Rodriguez-Blanco *et al.*, 2012). ICE elements are genetically related to the IncA/C plasmids with the potential of genetic recombination and interactions between them that facilitate their host range and dissemination (Burrus *et al.*, 2006; Osorio *et al.*, 2008; Wozniak *et al.*, 2009; Daccord *et al.*, 2010; Wozniak and Waldor, 2010; Guglielmini *et al.*, 2011; Toleman and Walsh, 2011). Ready distribution and transfer of antimicrobial resistance genes between bacteria in the aquatic environment and terrestrial bacteria and human pathogens is further demonstrated by the sharing of *tetG* and *floR* resistance determinants of an antimicrobial-resistance *Salmonella* genomic island 1 (SGI1) between *P. damsela piscicida* and epidemic *S. enterica* serovar Typhimurium DT104, fish-transmitted serovar Paratyphi B, serovar Agona and serovar Albany (Zhao and Aoki, 1992; Kim and Aoki, 1996a; Angulo, 1999; Arcangioli *et al.*, 1999; 2000; Bolton *et al.*, 1999; Briggs and Fratamico, 1999; Cloeckert *et al.*, 2000; 2001; Boyd *et al.*, 2002; 2008; Meunier *et al.*, 2002; Doublet *et al.*, 2003; Smith, 2008a,b). It is also demonstrated by the suspected potential passage of *tetC* tetracycline island mediated by insertion element IS_{CS605} (an insertion element also present in *Helicobacter pylori*) (Lau *et al.*, 2008; Roberts, 2009; Roberts and Schwarz, 2009; Sandoz and Rockey, 2010) from aquatic *A. salmonicida* or the opportunistic piscine-originated *Laribacter hongkongensis* to *Chlamydia suis*, a pig pathogen (Lau *et al.*, 2008; Roberts, 2009; Roberts and Schwarz, 2009; Sandoz and Rockey, 2010; Roberts *et al.*, 2012), and by

the assumed origin in aquaculture of SGI1 variant K in internationally disseminated *S. enterica* serovar Kentucky ST198 resistant to ciprofloxacin (Le Hello *et al.*, 2011).

The wide spectrum of potential interactions between these antimicrobial resistance MGEs of aquatic and terrestrial bacteria is further illustrated by a recent report that SGI1 can be mobilized between different bacteria by antimicrobial resistance plasmids of incompatibility group IncA/C found in piscine (*Aeromonas*, *Photobacterium*) and human pathogens (*Salmonella*, *Proteus*, *Vibrio*) (Douard *et al.*, 2010). Undoubtedly, the possibilities of HGT between bacteria of the aquatic environment and human pathogens are increased in settings where injudicious use of antimicrobials in aquaculture facilitates passage of large amounts of antimicrobials into the aquatic environment (Cabello, 2006; Asche *et al.*, 2010; BurrIDGE *et al.*, 2010; Millanao *et al.*, 2011; Buschmann *et al.*, 2012). There the antimicrobials can select for antimicrobial-resistant bacteria increasing their numbers, stimulate mutagenesis and HGT, and facilitate dissemination of antimicrobial resistance genes from the aquatic resistome to the terrestrial one (Baya *et al.*, 1986; Baquero *et al.*, 2008; Cantón, 2009; Couce and Blazquez, 2009; Martinez, 2009a; Forsberg *et al.*, 2012; Lupo *et al.*, 2012; Tello *et al.*, 2012). The use of antimicrobials in aquaculture may also negatively influence human health in areas where the marine aquatic environment is the source of epidemics of shellfish-borne *V. parahaemolyticus* (Cabello *et al.*, 2007; García *et al.*, 2013). Selecting for antimicrobial-resistant Vibrios in the environment and facilitating the transfer and mutagenesis of its chromosomal *qnr*-like loci and alternative antimicrobial resistance genes to other pathogens (Saga *et al.*, 2005; Cabello *et al.*, 2007). Contamination of the aquatic environment with human pathogens, as is common in lakes, rivers and the marine littoral in developing countries, will further facilitate genetic flow between aquatic bacteria and these pathogens (Silva *et al.*, 1987; Miranda and Zemelman, 2001; Baquero *et al.*, 2008; Luna *et al.*, 2010; Ribeiro *et al.*, 2010; Tello *et al.*, 2012). The aquatic environment at aquacultural sites, as suggested by Sørum and Baquero, may constitute a bona fide reactor for facilitating and accelerating evolution towards antimicrobial resistance of a wide range of aquatic and terrestrial bacteria including human pathogens (Sørum, 2006; Baquero *et al.*, 2008). In the case of salmonid aquaculture, transport of juvenile fish from hatcheries and lakes to the marine environment will also play a role in disseminating antimicrobial resistant bacteria and genes between these two aquatic environments (Sørum, 2006; Miranda, 2012).

In addition to selection and dissemination of antimicrobial-resistant bacteria, excessive use of antimicrobials in aquaculture can potentially have other detrimental impacts on human health (Austin, 1985; Burka

et al., 1997; Arthur *et al.*, 2000; Haya *et al.*, 2001; Cabello, 2006; Hastein *et al.*, 2006; Sarmah *et al.*, 2006; Sapkota *et al.*, 2008; Heuer *et al.*, 2009; Rodgers and Furones, 2009; BurrIDGE *et al.*, 2010; Abraham, 2011; Naviner *et al.*, 2011). Fish products for human consumption can become contaminated with antimicrobial residues at doses higher than Maximum Residue Limits (Silva *et al.*, 1987; Cabello, 2006; Silley, 2007; Silbergeld *et al.*, 2008; Nogales *et al.*, 2011). When such products are eaten, they can potentially alter the human normal intestinal flora, select for antimicrobial-resistant bacteria, and aid infection with human pathogens while further facilitating HGT of antimicrobial resistance (Cabello, 2006; Salyers and Shoemaker, 2006; Silley, 2007; Nisha, 2008; Sapkota *et al.*, 2008; Silbergeld *et al.*, 2008). Passage of antimicrobials to humans in fish meat may be more common than supposed since regulatory agencies frequently detect antimicrobial residues in fish for human consumption despite the low proportion of aquacultured fish tested for the presence of these drugs (Arthur *et al.*, 2000; Sapkota *et al.*, 2008; Silbergeld *et al.*, 2008; Tacon and Metian, 2008; Rodgers and Furones, 2009; BurrIDGE *et al.*, 2010; Love *et al.*, 2011). Ingestion of free-ranging (wild) fish, shellfish and crustaceans from areas surrounding aquaculture sites can also result in passage of antimicrobials used in aquaculture to the human intestine since antimicrobials can reach other animals near these sites and remain in their tissues for some period of time (Björklund *et al.*, 1990; Sarmah *et al.*, 2006; Fortt *et al.*, 2007; Sapkota *et al.*, 2008). Similarly, antimicrobial-resistant bacteria selected in aquaculture sites can contaminate marketed aquacultural produce (Sarmah *et al.*, 2006; Naviner *et al.*, 2011; Castillo Neyra *et al.*, 2012; Nawaz *et al.*, 2012). In addition, there is the reasonable possibility that workers in food mills and aquaculture sites will become exposed to antimicrobials and antimicrobial resistant bacteria by aerosols and by direct contact with medicated food in aquacultural areas where annual usages of antimicrobials run into the metric tons, again shifting the normal flora of skin, intestine and mucosa of these workers towards antimicrobial-resistant bacteria (BurrIDGE *et al.*, 2010; Millanao *et al.*, 2011; Castillo Neyra *et al.*, 2012).

Concluding remarks

Although the available information is partial and fragmented it does not support the hypothesis that the aquatic environment and its bacteria are unique. On the contrary, it strongly suggests that aquaculture, like terrestrial animal farming, is an important source for passage of large amounts of a variety of antimicrobials into the environment. Better information is needed to provide more accurate assessment of the classes and amounts of

antimicrobials used in aquaculture in order to determine their potential impact on the general environment and on animal and public health (Aarestrup *et al.*, 2000; 2008; Collignon *et al.*, 2009; Heuer *et al.*, 2009; Love *et al.*, 2011). Despite the lack of accurate information, it is clear that excessive amounts of antimicrobials are used in aquaculture in some countries for both therapeutic and prophylactic purposes (Arthur *et al.*, 2000; Armstrong *et al.*, 2005; Sapkota *et al.*, 2008; Rodgers and Furones, 2009; Asche *et al.*, 2010; Barton and Floydsand, 2010; Burrridge *et al.*, 2010; Ndi and Barton, 2012). This veterinary use includes antimicrobials also used clinically in human medicine (Millanao, 2002; Collignon *et al.*, 2009; Heuer *et al.*, 2009; Millanao *et al.*, 2011). Previous experience regarding use of antimicrobials in terrestrial animal husbandry and an analysis of extant information regarding genetic aspects of antimicrobial resistance in aquatic bacteria strongly suggests that antimicrobial use in aquaculture is also likely to select antimicrobial-resistant bacteria (including piscine pathogens) in aquacultural environments (Aarestrup *et al.*, 2000; Cabello, 2006; Nikaido, 2009; Levy and Marshall, 2010; Davis *et al.*, 2011; Marshall and Levy, 2011; Buschmann *et al.*, 2012). Evidence also exists suggesting that the resistome of aquatic bacteria contains novel antimicrobial genetic determinants (Miranda *et al.*, 2003; Cattoir *et al.*, 2007; 2008). Passage of such antimicrobial resistance determinants from aquatic to terrestrial bacteria will be facilitated by excessive antimicrobial use and the common mobilome of aquatic and terrestrial bacteria (Sørum, 2006; Sobecky and Hazen, 2009; Millanao *et al.*, 2011). These novel antimicrobial resistance elements may ultimately reach human pathogens and complicate therapy of infections caused by them (Aarestrup *et al.*, 2000; 2008; Miranda *et al.*, 2003; Cattoir *et al.*, 2007; 2008; Roberts, 2009). The presence of residual antimicrobials in the meat of target and free-ranging species surrounding aquaculture sites and the exposure to antimicrobials of workers that manipulate medicated food is yet another way in which excessive use of antimicrobials in aquaculture may impact human health (Samuelsen *et al.*, 1992b; Fortt *et al.*, 2007; Sapkota *et al.*, 2008). These considerations suggest that excessive aquacultural use of antimicrobials may potentially have major effects on animal and human health as well as on the environment.

The global reach of the problem of antimicrobial resistance indicates that the potential complications of antimicrobial use in aquaculture need to be addressed globally (Angulo, 1999; Anderson *et al.*, 2003; Davies, 2009; Martinez *et al.*, 2009; Levy and Marshall, 2010). This assessment must include an evaluation of governmental regulations as well as determination of the classes and amounts of antimicrobials used in aquaculture in different countries throughout the world (Davies, 2009; Martinez

et al., 2009; Burrridge *et al.*, 2010), and investigation of the reasons aquacultural conglomerates show drastic differences in antimicrobial use in different countries (Grave *et al.*, 1999; 2006; Millanao, 2002; Grave and Hansen, 2009; Burrridge *et al.*, 2010; Millanao *et al.*, 2011). Such information is a prerequisite to regulating aquacultural use, especially for those antimicrobials important to human therapeutics. It is also crucial to anticipating potential problems of antimicrobial resistance related to piscine and human health stemming from this use which still goes undetected in a number of countries (Grave *et al.*, 1999; 2006; Grave and Hansen, 2009; Asche *et al.*, 2010; Burrridge *et al.*, 2010; Ibieta *et al.*, 2011; Millanao *et al.*, 2011; Ndi and Barton, 2012). In parallel with this increased assessment of antimicrobial use, there is a need for increased awareness and research focused on the aquatic resistome and the potential passage of genetic elements and antimicrobial resistant determinants from this resistome to the resistomes of fish and human pathogens (Wright *et al.*, 2008; Cantón, 2009; Wright, 2010). In this regard, the use of metagenomics with cloning, next generation DNA sequencing and molecular epidemiological tools are already helping to improve definition of the resistome of environmental, animal and human bacteria sharing of antimicrobial resistance genes (Sørum, 2006; Fondi and Fani, 2010; Sommer *et al.*, 2010; Kristiansson *et al.*, 2011; Sommer and Dantas, 2011; Forsberg *et al.*, 2012).

Regulation of antimicrobial use in farmed animals in Europe and in salmon farms in Norway has demonstrated that reducing the use of antimicrobials is not incompatible with economically feasible animal farming (Markestad and Grave, 1997; Aarestrup *et al.*, 2000; 2008; Wegener, 2003; Sørum, 2006; Midtlyng *et al.*, 2011; White and McDermott, 2011). There is thus a critical need to educate all stakeholders (including aquacultural corporations) to understand that sacrificing fish hygiene and well-being for short-term economic gains is not a winning strategy, and that appropriate use of prebiotics, probiotics and vaccines can replace excessive use of antimicrobials (Markestad and Grave, 1997; Bravo and Midtlyng, 2007; Defoirdt *et al.*, 2007). The continuous growth of aquaculture and the potential increase of fish diseases generated by global warming and globalization increases the urgency of coupling these approaches so that all can reap maximal benefits from antimicrobial use while avoiding the negative effects of their excessive use on the environment and on animal and human health (Sapkota *et al.*, 2008; Sørum, 2008; Asche, 2009; Asche *et al.*, 2010).

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