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; Burridge 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; 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; Burridge et al., 2010; Millanao et al., 2011).
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 Floysand, 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>
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<tr>
<th>Antimicrobials</th>
<th>Oxytetracycline</th>
<th>Florfenicol</th>
<th>Sulfamethoxazole/trimethoprim</th>
<th>Quinolones</th>
<th>Others</th>
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<td>Canada</td>
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<td>+ (Oxolinic acid, Flumequin, others)</td>
<td>Amoxicillin, Erythromycin, Furazolidin Chloramphenicol, Gentamycin</td>
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<td>Norway</td>
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<td>+ (Oxolinic acid, Flumequin)</td>
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<td>United States</td>
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a. Burridge et al. (2010)
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; Burridge 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 (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 (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 (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 (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 (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 (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 (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 (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 (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 (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 (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 (Millanao, 2002; Barrientos, 2006; Millanao et al., 2011).
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; Sørum, 2006; Rodgers and Furones, 2009). This method of administration leads to their affecting both diseased and healthy fish (metaphylaxis) in the population (Sørum, 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; Sørøm, 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., 1996; Capone et al., 1996; Burka et al., 1997; Le and Munekage, 2004; Armstrong et al., 2005; Sørøm, 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; Burridge 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; Koepypudsa 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., 1996;
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 Lützhoft et al., 1999; Arthur et al., 2000; Guardabassi et al., 2000; Miranda and Zemelman, 2002a,b; Kim et al., 2004; 2011; Le and Munekage, 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; Heepngo et al., 2008; American Academy of Microbiology, 2009; Ding and He, 2010; Fernández-Alarcon 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., 2006; Edlund et al., 2006; 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; Loof 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 mobiliome 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 damselae (Table 2) (Mori, 2004) and Oceanobacillus iheyensis (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; Arcangiol et al., 1999; 2000; Bolton et al., 1999; Cloeckaert 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 catI, 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; Nikaio, 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’Abee-Lund and Serum, 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.

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<thead>
<tr>
<th>Elements</th>
<th>β-proteobacteria</th>
<th>Shewanella</th>
<th>Vibrio</th>
<th>Photobacterium</th>
<th>Aeromonas</th>
<th>Moraxella</th>
<th>Acinetobacter</th>
<th>Y. ruckeri</th>
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a. Burres et al. (2006); Osorio et al. (2008); Toleman and Walsh (2011); Rodriguez-Blanco et al. (2012).
b. L’Abee-Lund and Sørum (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); Friske et al. (2009); Welch et al. (2009).
f. Sørum 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).
k. Decousser et al. (2001); Rodríguez 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).
q. Furushita et al. (2003); Sandoz and Rockey (2010).
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; Serum, 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.\textsuperscript{a,b,c,d,e,f}

<table>
<thead>
<tr>
<th>Mechanism of transmission</th>
<th>Disease</th>
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<tr>
<td>Contact-borne</td>
<td>Fish handler disease, tank granuloma</td>
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<tr>
<td>Mycobacterium marinum, M. fortuitum, M. ulcerans</td>
<td>Cellulitis, systemic infections</td>
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<tr>
<td>Streptococcus iniae</td>
<td>Skin wound infections, systemic infections</td>
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<tr>
<td>Aeromonas hydrophila, A. sobria, A. caviae</td>
<td>Cellulitis, gastroenteritis, bacilaeria</td>
</tr>
<tr>
<td>Vibrio damselae, V. vulnificus, V. mimicus, V. fluvialis, V. algindoiycicus</td>
<td>Skin infections, systemic infections</td>
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<tr>
<td>Edwardsiella tarda</td>
<td>Pneumonia, systemic infections</td>
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<tr>
<td>Enyipelothrix thysanotaenia</td>
<td>Gastroenteritis, bacilaeria</td>
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<td>Stenotrophomonas maltophilia (?)</td>
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<td>Klyuvera (?)</td>
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<tr>
<td>Food-borne</td>
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<tr>
<td>Vibrio parahaemolyticus, V. cholerae</td>
<td>Diarrhoea</td>
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<tr>
<td>Aeromonas hydrophila</td>
<td>Diarrhoea, systemic infections</td>
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<td>Salmonella</td>
<td>Diarrhoea, systemic infections</td>
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<td>Listeria monocytogenes</td>
<td>Diarrhoea, systemic infections</td>
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<tr>
<td>Clostridium botulinicum, C. perfringens</td>
<td>Botulism, diarrhoea</td>
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<tr>
<td>Plesiomonas shigellodes</td>
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b. Looney et al. (2009).
c. Lowry and Smith (2007).
d. Iwamoto et al. (2010).

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’Abee-Lund and Serum, 2002; Murray and Peeler, 2005; Toranzo et al., 2005; Asche et al., 2010; Barton and Flóysand, 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 and Leung, 2006; Ibieta et al., 2011). While selection of antimicrobial-resistant bacteria in aquaculture 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.

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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 Floysand, 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 qingshengyi*, *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 resistance 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; Serum, 2006; Miranda and Rojas, 2007; Seyfried et al., 2010). This may be particularly relevant to human health in those countries where aquaculture 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 (Furusu 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’Abee-Lund and Serum, 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; Serum, 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; Serum 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; Martinez-Martínez et al., 2008; Strahlievitz 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; Rodríguez 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. damselae, 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. damselae 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; Cloeckaert et al., 2000; 2001; Boyd et al., 2002; 2008; Meunier et al., 2002; Doublet et al., 2003; Smith, 2005, 2008a,b). It is also demonstrated by the suspected potential passage of tetC tetracycline island mediated by insertion element IScs605 (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; Burrudge 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 assumed origin in aquaculture of SGI1 variant K in internationally disseminated *S. enterica* serovar Kentucky ST198 resistant to ciprofloxacin (Le Hello et al., 2011).

Aquacultural antimicrobial use and antimicrobial resistance

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 conditions...
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 Floysand, 2010; Burridge 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 aquatic 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 (Serum, 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; Burridge 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; Burridge 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; Burridge 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; Serum, 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; Serum, 2008; Asche, 2009; Asche et al., 2010).

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