ABSTRACT

Important antibiotics in human medicine have been used for many decades in animal agriculture for growth promotion and disease treatment. Several publications have linked antibiotic resistance development and spread with animal production. Aquaculture, the newest and fastest growing food production sector, may promote similar or new resistance mechanisms. This review of 650+ papers from diverse sources examines parallels and differences between land-based agriculture of swine, beef, and poultry and aquaculture. Among three key findings was, first, that of 51 antibiotics commonly used in aquaculture and agriculture, 39 (or 76%) are also of importance in human medicine; furthermore, six classes of antibiotics commonly used in both agriculture and aquaculture are also included on the World Health Organization's (WHO) list of critically important/highly important/important antimicrobials. Second, various zoonotic pathogens isolated from meat and seafood were observed to feature resistance to multiple antibiotics on the WHO list, irrespective of their origin in either agriculture or aquaculture. Third, the data show that resistant bacteria isolated from both aquaculture and agriculture share the same resistance mechanisms, indicating that aquaculture is contributing to the same resistance issues established by terrestrial agriculture. More transparency in data collection and reporting is needed so the risks and benefits of antibiotic usage can be adequately assessed.

Keywords: Agriculture, Aquaculture, Antibiotic Resistance, Resistance Mechanisms

INTRODUCTION

Antibiotics are arguably the most successful and important family of drugs developed for the protection of human health. Since the discovery of penicillin in 1928, over 100 antibiotics have been discovered and used, with the majority of these being introduced before 1970 (1). With the unveiling of each new antibiotic class, resistant bacterial strains were soon identified thereafter, and treatment of some are now a major medical challenge. Today, approximately 70% of characterized nosocomial infections are resistant to at least one clinically relevant antibiotic (2). Moreover, many strains have been discovered that exhibit multi-drug resistance (MDR) to nearly all commonly available classes of antibiotics (3). Coded by antibiotic resistance genes (ARGs), resistance mechanisms such as efflux pumps have made many zoonotic pathogens extremely difficult to treat, forcing doctors to use antibiotics of last resort, example, the fluoroquinolone ciprofloxacin, to treat pathogenic *Escherichia coli* strains (4).

Usage of antibiotics in the production of food animals to sustain and nurture the world's continually increasing human population has contributed to the development of antibiotic resistance (5). In agriculture – referred to in this review as the farming of swine, poultry, and cattle – uses of antibiotics include disease prevention, treatment, control, and application as growth-promoting antibiotics (GPA) in order to improve feed utilization and production (5). The jurisdictions for specific antibiotics allowed and their usage in agriculture vary depending on the location; for example in the European Union (EU), use of antibiotics for growth promotion is not allowed (6). In aquaculture – referred to in this review as the production of aquatic seafood in captivity but excluding plants – application of antibiotics is regulated sparingly, differing greatly from country to country with little to no enforcement in many of the countries that produce the majority of the world's aquaculture products (7). Usage purposes are the same as those in agriculture, with the exception that in aquaculture, prophylactic treatment is more common (8). Previous research has linked agricultural antibiotic usage practices with antibiotic resistance development, resulting in calls for more judicious usage of antibiotics (5, 9). Many studies have found drug resistant bacterial strains in agricultural facilities, whether originating in the meat itself (10-12) or in the surrounding environment (13-15). The same has been shown for aquaculture (16-18), triggering repeated calls for improved regulation and enforcement (7). Efforts to document resistance have increased in recent years, a notable one being the National Antimicrobial Resistance Monitoring System (NARMS) that was established in 1996 as a collaboration between the US Food and Drug Administration (FDA) Center for Veterinary Medicine (19), the US Department of Agriculture (USDA), and the Centers for Disease Control and Prevention (CDC). However, the role of antibiotic usage in agriculture and aquaculture in the development of resistance and dissemination of ARGs is still poorly understood.

Acknowledging the recent growth of aquaculture as a major agricultural sector, this review explors similarities and differences between antibiotic resistance risks associated with agriculture and aquaculture. Specifically, we address whether the recent rise of aquaculture is creating new resistance issues or whether it is simply exacerbating the same ones already established for agriculture. To answer this question, we first discuss how antibiotics have been traditionally used in these industries around the world. We then focus on peer-reviewed academic literature contributions containing data on resistance development in foodborne pathogens. And finally, we use the United States as a case study to discuss in more detail specific issues identified in the global analysis.

METHODOLOGY

A systematic review was conducted of over 650 reports extracted from the peer-reviewed academic literature, non-government organizations (NGOs), industry, and government (see Supplemental Information for full list of documents reviewed). Initial searches started with Web of Science and Google Scholar using key terms “antibiotics”, “livestock”, “agriculture”, “aquaculture”, and “food production”. Additional articles were identified using each article’s reference section and further searches were conducted depending on the topic section. Information was also obtained through conversations with food production experts. When possible, the most recent peer-reviewed academic literature was used as the cited reference. A total of 98 key sources are cited in-text to illustrate key issues, show novel data or ways of analysis, and highlight key research gaps still awaiting attention in future studies. A full list of references is available as supplemental information.

Animal Farming and Antibiotic Usage. In addition to the search terms above, various country/region names were searched alongside (European Union, Brazil, China, etc.). Each jurisdiction’s official government website was further surveyed to collect relevant data. Non-government documents such as ones from the Food and Agricultural Organization (FAO) were also extensively reviewed in this section.

Foodborne Pathogens and Antibiotic Resistance Mechanisms. A separate search was conducted to analyze the link between antibiotic resistance and animal production. The initial search of literature on Web of Science started with the search terms “antibiotics, resistance, and agriculture" and "antibiotics, resistance, and aquaculture/seafood" (see supplemental information). These results were then filtered based on title to exclude topics that are not covered in this review (see exclusion criteria in supplemental information). Further literature searches were conducted as needed using terms such as "drug resistance, seafood, and antimicrobials" in order to find articles not captured in the primary search.

United States Agriculture and Aquaculture. Much of these data were collected from governmental websites and through personal communications with personnel from various organizations such as the National Oceanic and Atmospheric Administration (NOAA) and the National Resources Defense Council (NRDC).

The cutoff date for the literature search was September 1, 2014. Information from the 2007 US Agriculture Census, kindly provided by the Food and Water Watch in raw and processed data formats, served to create the composite Geographic Information Systems (GIS) illustrations in Fig. 5. Whenever possible, an update to currently reported data is provided.

The use of terminology in the field of drug resistance is not always consistent. In this paper, we define prophylaxis as the precautionary administration of antibiotics at levels predetermined to be therapeutic in the absence of disease (sometimes also termed "disease prevention"). "Sub/non-therapeutic" usage of antibiotics refers to the usage of these compounds for growth promotion at concentrations lower than the dosages required to effectively inhibit the growth of harmful bacteria.

AGRICULTURE VS. AQUACULTURE

Animal Farming and Antibiotic Usage

Over the last sixty years, worldwide production of swine, poultry, and cattle has grown continuously, with poultry outpacing the others (Figure 1A). World aquaculture production only became a major animal production industry around 1985 (Figure 1B). Before then, it was a largely non-commercial affair, representing a traditional way of life for centuries and often providing the sole reliable source of nourishment for its producers (20). Reasons for the recent growth of aquaculture include an increased demand for what is now recognized as a healthy protein choice, advances in seafood feed production, depleted wild fish stocks, and improvements in farming facilities enabling high-density farming (16, 20). Total seafood production is now almost evenly split between wild-caught and farmed with the former steadily becoming stagnant in volume for the past two decades.

Figure 1 panels C-E show the top countries that produce cattle, swine, and aquacultured seafood. Perhaps the most important detail here is that the majority (>90%) of aquaculture occurs in Asia whereas agriculture's concentrated animal feeding operations (CAFOs) that confine large populations of animals in buildings or feedlots (9) can be found distributed across several regions. Aquaculture facilities vary in design, with some keeping animals contained in ocean nets and others in secluded ponds or reservoirs. In Asia, aquaculture often links to the natural water environment (21). Many of these freshwater farms irrigate or flow through ponds that often tie with water reservoirs, lakes, and rivers (22). Brackish water aquaculture has more than doubled over the past decade and is primarily producing shrimp in coastal ponds and tanks (22).

Data regarding the classes and amounts of antibiotics used for agriculture and aquaculture depends on the region. For example, in 2003, salmon aquaculture in Chile used about 0.5 kg of antibiotic for each kg of salmon produced, whereas the amount in Norway was 0.002 kg (23). Figure 2 shows the most recent data available regarding antibiotic sales in the US and the EU (25 countries). It is important to keep in mind that antibiotic sales do not equate to antibiotic usage, and usage information is not readily available or even reported in most cases. In both regions, the tetracycline class is the largest class of antibiotics sold, comprising about 40% of total sales. Similar reliable data from other regions of the world proved to be unavailable. Antibiotic sales and usage in India are not regulated (24, 25). In China, two different reports of antibiotic usage were found, one stating the annual usage in animal feeds as 6000 tons (26) and the other stating over 8000 tons were used annually in animal husbandry (27). In Brazil, it has been reported that the most commonly used antibiotic classes are fluoroquinolones (34% of total antibiotics), ionophores (20%), and macrolides (10%) (28). Overall, worldwide usage of antibiotics in both animal production and human medicine has increased in recent decades; agriculture accounts for the majority of drugs used, and the mass of antibiotics used for the production of terrestrial food animals is estimated to exceed the amount of drugs used in aquaculture (29).

How the antibiotics are used depends on the location and is not typically reported. Global trends in agriculture, aquaculture, and human medicine point to a steady increase in the usage of antibiotics. The most important delineation in usage is whether antibiotics are used for growth promotion. Among the top five cattle- and swine-producing countries (see Figure 1C-D), only the EU has a confirmed ban on use of GPAs (6). In the US, ionophores are used only in animals for growth promotion; a usage which is probably true in Brazil as well where ionophores are also reported to be commonly used (28). It should be noted that ionophores are typically reserved for animal usage and not for human usage, unlike the other antibiotic classes (30). These drugs can alter the stomach microorganisms in livestock to increase feed efficiency and energy extraction in the conversion of feeds (31). As Figure 2 shows, ionophores are absent from EU antibiotic sales because of the 2006 ban on usage of GPAs in food animals (6, 32). Although there is no law against GPA usage in the US, the FDA has recently issued formal guidance to industry strongly urging drug companies to withdraw their GPAs and/or convert their usage guidelines to “therapeutic only” (33). In China and Russia, antibiotic usage in animals is restricted to using only non-human medicine drugs (34) and since 2003, several reforms have been attempted in China to improve food safety (35). However, reports of medically-important antibiotics such as tetracyclines being used (36) and detections of illegal veterinary antibiotics like chloramphenicol in Chinese waters suggest that enforcement of the regulation is lax (27, 37). Today, unlike in the EU (32), no veterinary prescriptions are required in China for use of antibiotics in animals (32). One of the first steps that can be taken to ensure better monitoring of antibiotic usage is to require veterinary prescriptions when antibiotics are used in animals (Mathew 2007, Cabello 2006, Maron 2013). This approach is being favored in India, as reported in 2011 in a national policy document outlining details to contain antibiotic resistance (25). Whereas data on actual implementation of such measures are scarce, the current trend in published papers indicates that many countries are taking steps to better regulated and report antibiotic usage.

The data presented above is for all antibiotics used in animal production, which includes aquaculture. Specific data for antibiotic usage patterns in aquaculture is available mostly in non-academic literature from the FAO and reports based on surveys as to what antibiotics are commonly used. In 2008, a review article identified three antibiotics to be in common use in aquaculture: oxytetracycline, oxolinic acid, and chloramphenicol (16). A more recent survey conducted by the FAO of 21 countries engaging in aquaculture confirmed continued use of oxytetracycline as the top antibiotic applied in the treatment of disease in all major seafood species (38). Florfenicol and trimethoprim/sulfadiazine were next in line with respect to usage frequency. Oxytetracycline was also reported as the most widely used antibiotic for prophylactic treatment. A total of 24 countries were surveyed, including 11 of the top 15 aquaculture producers; the four countries missing from the survey were Egypt, Japan, South Korea, and Myanmar.

To assess the similarities and differences in antibiotics used for agriculture, aquaculture, and human health, the 2011 World Health Organization (WHO) list of important antimicrobials was compared to the above data (39). The WHO list is a categorization system of 260 antimicrobials created in an effort to contain antimicrobial resistance development and spread and to reserve key drugs for human medicine (40). This list was intended for public health and animal health authorities as a reference for prioritizing risk assessment with respect to antibiotic resistance development. Two criteria are considered for inclusion on this list: first, the antibiotic must be the sole or one of a few limited available therapies to treat serious human diseases; and second, it must be used to treat diseases caused either by a) organisms that may be transmitted to humans from non-human sources or b) human diseases caused by organisms that may acquire resistance genes from non-human sources. “Critically important” antimicrobials (*n*=162) meet both criteria. “Highly important” antimicrobials (*n*=88) meet one of the two criteria, and “important” antimicrobials (*n*=10) meet neither criterion but are still recognized as drugs of importance in human medicine. In this paper, antibiotics from all three classes were screened for usage similarity with results shown in Figure 3 (excluding antibiotics listed for veterinary use only). Six common classes of antibiotics (aminoglycosides, macrolides, penicillins, quinolones, sulfonamides, tetracyclines) on the WHO list are regularly used in agriculture and aquaculture. Of the 51 antibiotics reported to be used by the top agriculture and aquaculture countries, 39 are on the WHO list. Of these 39 antibiotics, only 2 are listed as “important”; the other 37 are either “critically important” or “highly important”. These numbers indicate that there is extreme crossover of antibiotic usage in human medicine and animal food production. It is important to note that data provided in Figure 3 most likely underestimate the antibiotics actually used as this information is not reported and recorded systematically. The most important message from these data is that several of the same classes of antibiotics are used for both human medicine and animal production. This parallel antibiotic usage may be promoting similar resistance issues in both aquaculture and agriculture.

Foodborne Pathogens and Antibiotic Resistance Mechanisms

As shown in the previous section, the antibiotics used in agriculture and aquaculture span many of the same antibiotic classes. Thus, as agriculture has been using antibiotics for much longer than aquaculture has, we ask whether the same resistance mechanisms exist in both or if the latter is promoting the development of new ones. In this section, we identified reported bacterial pathogens from meat and seafood, characterized how resistance may develop, and looked for resistance development pathways in agriculture and aquaculture. To relate the isolated strains to human health risks, we focused our identified strains on zoonotic foodborne pathogens.

The most prevalent and serious emerging pathogens in agricultural meat products are *Campylobacter jejuni*, *Salmonella enterica* serovar Typhimurium DT104, and *E. coli* O157:H7 (41). Often, these products are contaminated during handling and processing in the CAFOs where the animals are slaughtered. Pathogens present in feces and/or animal hides often are transferred to edible fractions, or spread as aerosols produced during dehiding, evisceration, and carcass splitting (41). In aquaculture, foodborne diseases are not as well documented, but the literature shows that *Salmonella* and *Vibrio* spp. are likely to be the most common pathogens detected in seafood, with *Listeria monocytogenes*, *Aeromonas*, and *Clostridium* spp. becoming emerging threats (42-44). Cases of human infections from seafood most often arise from handling, such as contact with the wash water or through processing in the food industry, and by oral consumption of infected fish or related products (45).

Aside from the potential to cause infections in the people that are exposed, these bacteria, along with others that are less often found, are capable of developing and spreading antibiotic resistance. In both agriculture and aquaculture, development/persistence of resistance can occur when these bacteria are exposed to sub-therapeutic concentrations of antibiotics (46). In terrestrial agriculture, this exposure occurs when antibiotics used for growth promotion are added as a CAFO feed additive over a period of time for fattening and for increased feed efficiency (47). In the US, about 55% of all antibiotic usage in cattle is during the feedlot stage of cattle production (48). The feedlot stage is when the animals weigh in between 700 and 1200 lbs, with average antibiotic dosages estimated at 80 mg/animal/day for about 120 days (48). This means that these cattle are subject to sub-therapeutic antibiotic concentrations for almost one third of a year.

The commonly cited rationale behind using GPAs is an economic benefit, with average increases in animal mass reported in the range of four to eight percent (49). Other advantages reported in the literature include an improvement of animal health, decreases of bacterial contamination in animal products, a reduction of adverse environmental impacts such as greenhouse gas emissions, and prevention of water eutrophication (50). However, an economic analysis of using antibiotics in commercial broiler chickens for growth promotion showed that the net economic effect of using GPAs is negative, with an estimated lost value of $0.0093 per chicken or about 0.45% of the total cost; the positive production changes associated with antibiotic use reportedly were insufficient to offset the cost of more expensive feed (51). The latter study did not consider the potential benefits of GPA removal in terms of preventing external costs from medical and public health burdens resulting from antibiotic-resistance infections. Considering such would further increase the cost incurred by the use of antibiotics. No other such analysis is available in the literature, and more are needed to assess the economic impact of using GPAs.

In aquaculture, sub-therapeutic exposure concentrations are mostly encountered after the prophylactic use of antibiotics. Unconsumed fish feed and feces can contain residues that persist in the surrounding environment (52), allowing for bacteria to be exposed to low concentrations that can select for resistance. The exposed bacteria then can spread ARGs to the natural microbiota in nearby ecosystems, which may pose a greater threat than low levels of residues, as resistance genes may persist for decades due to the marginal impact of gene maintenance on fitness (7). As previous studies suggest that the environment already harbors ARGs (53), the mixing of residues that is made easier via the water pathway make aquaculture more likely to spread contaminants compared to agriculture. In many cases, these compounds are only slightly transformed, or even unchanged and conjugated to polar molecules, allowing for easier dispersion in water (54). The added potential impacts on the environment include direct antibiotic toxicity in natural microbiota, flora, and fauna, have been voiced in literature (21, 55). However, not all detected antibiotic concentrations are environmentally relevant enough to negatively impact invertebrates and fish (56, 57). These reports in literature indicate that the risks associated with antibiotic residues in aquaculture may vary depending on the situation and that there is a gap in knowledge regarding residues and their effects on resistance development. It must be noted that the usage of antibiotics in animal production has provided many benefits as well. Antibiotics have allowed for animal health to be improved, increasing economic gain for the farmers, as pathogens are significantly reduced when antibiotics are utilized (47, 50). However, despite these benefits, we cannot ignore the risks and potential negative human health and environmental impacts.

To compare the potential for agriculture and aquaculture to be developing the same mechanisms of antibiotic resistance, we reviewed reports in literature of bacterial isolates resistant to commonly used antibiotics in these food production industries. In agriculture, four common resistance mechanisms have been identified (Figure 4). These categories are presented very broadly to be more inclusive; “altered intracellular target” can mean any mutation that allows for ribosomal active site changes or an RNA polymerase mutation that leads to reduced binding of the antibiotic (58). Antibiotics in many classes can be ineffective against these mechanisms; both macrolides and penicillins can be pumped out of the bacterial cell by efflux pumps, for example. In other words, co-resistance can occur with any of these mechanisms. The zoonotic pathogens of concern listed in Figure 4 are typical examples of bacteria exhibiting the common resistance mechanisms. For example, *P. aeruginosa* is well known for expressing MDR efflux pumps (59). Examples of these pathogens isolated from agriculture that have been molecularly shown to harbor each resistance mechanism’s ARGs are also shown in Figure 4. Many are resistant to several antibiotics, but ones commonly used in agriculture are noted.

The same four mechanisms were also found to be associated with aquaculture. Zoonotic pathogens resistant to aquaculture antibiotics have been isolated from seafood containing all of the four resistance mechanisms (18, 60-62). Some of these microbes are relevant pathogens in agricultural products as well (i.e. *Salmonella*). Tetracycline resistance is the most commonly seen resistance among bacterial isolates from aquaculture; a recent study showed that as the number of resistance reports increased, so did the incidence of tetracycline resistance (63). Among 23 publications on drug resistant bacteria isolated from seafood for human consumption, 21 reported resistance to at least one antibiotic belonging to the class of tetracyclines. This previous study only reported publications from 2003-2013 and limited the search to bacterial strains from seafood products only (excluding aquaculture facilities, the surrounding water, etc.). If the exclusions were not applied, the number of resistant strains isolated would most likely increase. The major issue with detections of specific resistance determinants such as efflux pumps is the ability of these genes to be spread via horizontal gene transfer, possibly to bacteria that are even more pathogenic to humans. In both aquaculture and agriculture, native environmental bacteria are mixed with zoonotic bacteria, providing a situation where resistance can develop, spread, and linger amongst them. The biggest human health risk is coming into contact with pathogenic bacteria that are also resistant to multiple antibiotics, especially ones from different classes. As noted above, several such cross-resistant isolates have already been found in terrestrial agriculture and aquaculture. These data suggest that identical resistance mechanisms are being promoted and developed in both agriculture and aquaculture. Alarmingly, some of the same pathogens have been isolated from both seafood and meat. Different strains of MDR *Salmonella* were isolated containing the same resistance genes from both shellfish and pork (64). Similarly, *E. coli* strains isolated from pork, beef, poultry, and fish were resistant to several tetracyclines (65). This review only focuses on human health risks posed by edible animal products themselves, but it should be noted that additional risks result from the processing and handling of all materials involved, such as the disposal of animal feces containing resistant bacteria (66). The studies available and examined for this work show that the same resistance mechanisms are being promoted in agricultural and aquacultural environments (including processing and handling), thereby allowing for resistance to develop and spread via food and the environment, resulting in significant human health threats.

CASE STUDY: UNITED STATES AGRICULTURE AND AQUACULTURE

Animal Farming and Antibiotic Usage

The US is one of the largest producers of agriculture in the world, ranking (counting beginning year stock numbers) 4th in 2013 cattle production at approximately 89 million head and 3rd in swine production at approximately 66 million head (67). As seen in Figure 5, the cattle and swine industries dominate over the poultry industry, with much higher densities reported for many of the US counties and states shown. These data (Figure 5A-D) are from the 2007 USDA Agricultural Census, which conducts a new survey every five years (the 2012 report is expected to be released within the next year). Shown at the county level, the majority of the US cattle, swine, and poultry farming is done in the Great Plains states and along the west border of the Mississippi river. These geographic locations differ, as one would expect, from the locales of aquaculture, which are largely situated near the ocean and along the Gulf of Mexico (Figure 5E).

Aquaculture can be divided into freshwater and saltwater culture (Figure 5E). By value of production, saltwater and freshwater aquaculture in the US contributed approximately $800 and $550 million dollars, respectively, in 2011 (68). About two-third by value of saltwater (or marine) aquaculture consists of mollusks such as oysters, clams, and mussels (69). This type of aquaculture takes place in cages that are located on the ocean floor or suspended in water column (70). The majority of this farming is done in the northwest region of the US (see Figure 5E for blue pie chart inserts) and in Washington and Oregon. Freshwater aquaculture is predominated by trout, catfish, and tilapia (68). Figure 5 only shows the density of aquaculture farms contained in each state based on the 2005 Agricultural Census, but these numbers don’t necessarily reflect the amount of production. The top 5 aquaculture states by value in 2005 were as follows: Mississippi, Arkansas, Alabama, Louisiana, and Washington, together producing about a half a billion dollars worth of products, which is about half of the total US value produced (71).

As production of cattle, poultry, and swine expanded to large-scale productions over the last half-century, the usage of antibiotics in agriculture has also become the norm and has greatly increased. Based off of FDA reports, we calculated that in 2011, 80% of the antibiotics sold by weight were designated for animal usage (72, 73). This percentage was calculated from the annual FDA released summary report on antimicrobials sold/distributed for food-producing animals (13.5 million kg) and from the FDA drug use review, where sales numbers for human medicine usage (3.29 million kg) were obtained (73). Similar numbers have previously been reported by several other NGOs, including the Natural Resources Defense Council (74, 75), the UCS, and the Center for Science in the Public Interest, among others (Table 1). These organizations primarily based their estimates on annual FDA summary reports for antimicrobials. However, the numbers reported by the Animal Health Institute (AHI) are much different, resembling those reported by the US Farmers and Ranchers Alliance, another entity representing the industry. The AHI estimates that only about 35% of antibiotics in the US is used in animals for food production (48).

A second data discrepancy requiring more transparency is what antibiotics are annually used in animal production as well as their frequency of usage. This reporting is difficult in part because animal producers are not required to report this information, but also because "non-therapeutic" or "sub-therapeutic" usage of antibiotics can mean different things. As the FDA allows antibiotics to be used for growth promotion, feed efficiency, disease and metaphylaxis, it is hard to specifically enumerate the amount of antibiotics used in each of these categories (76). Thus, it must be noted that the numbers reported in Table 1 column "Reported Sub-Therapeutic Usage" are only estimates by a few organizations and that these numbers may not reflect the situation accurately. As the FDA is now required to report antimicrobial usage numbers, the next step would be to report what the antibiotics are used for. Recent FDA/CVM guidance now provides recommendations for industry to voluntarily align their products with FDA #209 (77). This guidance includes two principles: 1) limiting medically important antimicrobials to uses in food-producing animals that are considered necessary for assuring animal health and 2) limiting these usages to only those with veterinary oversight or consultation (77). These guidelines encourage better documentation and usage practices.

With regards to aquaculture production, the US produces a relatively low amount compared to other countries. This is partly due to the fact that China provides close to 70% of total aquaculture products, as well as the fact that the US imports about 90% of its seafood. There is a major effort in place to expand the aquaculture industry in the US, so that the reliance on imported fish is reduced. The US is a leading global consumer of fish and fishery products, and yet only about 5-7% of the national supply comes from its aquaculture industry (70). It has been estimated that up to 433,000 lbs (approximately 196,000 kg) of antibiotics were used in 2002 in US aquaculture (78). These data indicates that the vast majority (approximately 80%) of animal antibiotics used in the US are used in agricultural animal production (see table 1). Antibiotics do not improve growth or feed efficiency in fish like they have been reported to do in certain livestock (79). The usage of vaccines has also greatly limited antibiotic usage in the US, and at present, only three antibiotics are registered and sold for disease control in fish: oxytetracycline, florfenicol, and sulfadimethoxine/ormetoprim (80). Thus, it can be assumed that the majority of the antibiotics used for food-producing animals in the US is in livestock, which is most likely the case in other countries as well (29).

Foodborne Pathogens and Detected Resistance

In the US, foodborne pathogens of concern in agricultural meats are *E. coli, Salmonella,* and *Campylobacter*. The NARMS Retail Meat Annual Report of 2011 identifies *E. coli* as the most commonly detected bacterium in all retail meat products (19). Out of 1,920 retail meats tested in 2011, 55.7% were found to culture positive for *E. coli.* Although no isolates were resistant to ciprofloxacin, some isolates were shown to be resistant to third-generation cephalosporins, and co-resistances to other *β*-lactam compounds were reported. For *Salmonella*, the three serotypes most commonly detected were Typhimurium, Kentucky, and Heidelberg. Resistance to ampicillin rose from 17% of isolates in 2002 to 41% in 2011. A similar trend was seen for third-generation cephalosporins (from 10% to 34%). Most concerning is the fact that 45% of retail chicken harbored isolates featuring resistance to three or more classes of antimicrobials. Approximately 27% showed resistance to at least 5 classes. With regards to *Campylobacter*, the species *jejuni* and *coli* were most commonly detected. The majority of the isolates (90%) were from retail chicken. Although macrolide resistance has remained low, tetracycline resistance increased by about 10% of isolates for both species from 2010 to 2011. MDR was low in *Campylobacter,* as only 9 out of 634 isolates were resistant to at least three antimicrobial classes. *Enterococcus* (*faecalis* and *faecium*) is used as a sentinel for antibiotic selection pressures by anti-gram-positive antibiotics. Vancomycin resistance was not detected, and streptogramin resistance has significantly decreased in retail chicken from 56% of isolates in 2002 to 27% in 2011. Overall, it seems that most of the risk is from gram-negative bacteria and gram-positive bacteria pose a lesser risk to humans in retail meats. In reference to Figure 4's resistance pipelines, these data support the notion that feeding food production animals with antibiotics like ampicillin and tetracycline may contribute to the increased drug resistances observed in the US as shown in NARMS data (19).

In US aquaculture, as most of the seafood is imported, foodborne pathogens of concern are often ones that are considered food safety risks overseas as well. In 2004, it was reported that eating contaminated seafood resulted in about 15% of the reported foodborne outbreaks in the US. This is a greater percentage than was found for either meat or poultry, which are consumed at volumes eight and six times higher than those of seafood (81). Our literature search shows that *Vibrio* spp. and *Salmonella* are the most commonly isolated zoonotic pathogens from seafood. Specifically, *V. vulnificus,* followed by *parahaemolyticus,* are the two most important *Vibrio* spp. noted, causing gastroenteritis that may lead to septicemia (82). *Vibrio* spp. are a natural inhabitant of many aquatic organisms and are the leading cause of seafood-related deaths in the US (83). Mostly a concern in oysters, *Vibrio* spp. have been isolated and characterized in several studies (84-86). Antibiotic residue in bivalves is not a significant concern because they are not fed feed as they are filter feeders that survive on particles in the water (79). *Salmonella* are an issue in almost all types of seafood, and species distribution is broad, with frequently reported serotypes including Weltevreden, Senftenberg, Lexington, and Paratyphi-B (87). Mostly of human origin, *Salmonella* also causes gastroenteritis, and primarily contaminates seafood during processing (88). This is similar to agricultural meat products, where *Salmonella* is also an important foodborne pathogen. Recent seafood outbreaks include three in 2011 where a total of 168 cases resulted in 48 hospitalizations and 1 death (75). The *Salmonella* isolated in the latter study were all resistant to ampicillin, tetracycline, and amoxicillin/clavulanic acid, all of which are on the WHO list. These data suggest that resistance in zoonotic pathogens isolated from commonly eaten meats and seafoods is prevalent and a growing concern for the food industry.

CONCLUSIONS

Swine, cattle, and poultry agriculture all have relied on antibiotic usage for over half a century, promoting the development and spread of antibiotic resistance. As aquaculture continues to grow, the knowledge gap regarding how antibiotic usage, development of resistance mechanisms, and human health risks connect with each other must be filled with scientific research and results. Here, we present data showing that agriculture and aquaculture share many similarities, from the antibiotics used to the resistance mechanisms shared by the zoonotic pathogens corresponding to these two important food production sectors. The bacteria isolated from both meat and seafood have been reported to display resistance to antibiotics commonly applied in animal production. From the data gathered here, it is concluded that the recent growth of aquaculture is contributing to the development of the same resistance mechanisms also seen in agricultural production. The usage of antibiotics provides selective pressure that can accelerate ARG development and spread. As zoonotic pathogens have been isolated exhibiting resistance mechanisms known to be effective against multiple antibiotics, co-resistance is increasingly becoming a major concern. The lack of data and discrepancies in existing data regarding antibiotic usage contribute to the fact that it is challenging at present to accurately determine the magnitude of influence both aquaculture and agriculture has on resistance development. However, as water provides a constant and facile mechanism for dispersal of drug residues, microbial pathogens, and resistance genes, aquaculture will continue to pose a threat that may increase as the demand for seafood increases.

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Table 1. Total reported US antibiotic usage (in million kg) by animal industry and for human health.

Reporting Source	**Year Reported[a]**	**Total Amt. Sold for Food Production Animals (Million kg)**	**Reported Sub-Therapeutic Usage[b] Million kg (% of Total Animal Amount)**	**Total Human Usage (Million kg)**	**% of Total AB Sold is for Animals**	**Reference**
AHI	2001	8.1	1.4 (18%)	14.6	35%	(48)
UCS	2001	12.5	11 (88%)	3	70%	(48)
USFRA	2007	NR	(13%)	NR	NR	(90)
FDA; Rep. Slaughter	2009	13.1	NR	3.3	80%	(91, 92)
CSPI, NRDC, This Review	2011	13.5	NR	3.3	80%	(72-75)

[a] Year reported does not always correspond to year data was collected/formulated. NR= not reported in publication.

[b] Reported sub-therapeutic usage, does not differentiate between amounts of antibiotics used for prophylaxis, metaphylaxis, growth promotion, or feed efficiency.

Legends to Figures

Figure 1. Animal production values 1950-2011 and top producing countries of cattle, swine, and aquaculture. **A)** 1950-2011 world production of pork (purple), beef (blue), poultry (green), and total for all three (gray). **B)** 1950-2011world production of total seafood (orange), wild-caught seafood (red), and aquacultured seafood (yellow). **C)** Top 5 cattle producing countries in 2013, counting only beginning stocks by head. **D)** Top 5 swine producing countries in 2013, counting only beginning stocks by head. **E)** Top 15 aquaculture producing countries in 2010 by percentage of total world production. (67, 93, 94)

Figure 2. Antibiotic classes sold for use by animal production industries in US and EU (25 countries) in 2011. Total sold in US is approximately 13.5 million kg. Total sold in EU is approximately 8.4 million kg. (73, 95)

Figure 3. Common antibiotics used in aquaculture, agriculture, and included in the 2011 WHO antimicrobials list. Displayed as number of antibiotics followed by antibiotic class. Aquaculture antibiotics include the ones reported to be used by top 15 aquaculture-producing countries. Agricultural antibiotics include the ones used in cattle, swine, and poultry farming. WHO antibiotics are ones on the antimicrobial list in all three labels: “critically important”, “highly important”, and “important”. (16, 34, 39, 50, 54, 96)

Aquaculture: qui-sarafloxacin; other- miloxacin.
WHO: excludes antibiotics used solely for veterinary use. See reference 41 for full list.
Agriculture: ami- apramycin*, neomycin; ceph- cefquinome*, ceftiofur*; ion- monensin; qui-marbofloxacin*; other- virginiamycin*, narasin.
Agriculture and Aquaculture: other- tiamulin, ormetoprim.
Agriculture and WHO: mac- kanamycin, oleandomycin, spectinomycin, streptomycin; pen-cloxacillin, dicloxacillin, oxacillin; lin- lincomycin; sul- sulfamethazine, sulfathiazole; other-tylosin
Aquaculture and WHO: qui- norfloxacin, ciprofloxacin, pefloxacin, oxolinic acid, nalidixic acid, flumequine; sul- sulfadiazine, sulfamerazine, sulfamethoxazole; other- chloramphenicol, colistin, florfenicol, furazolidone, thiamphenicol.
Aquaculture, Agriculture, and WHO: ami- gentamicin; mac- spiramycin, erythromycin; pen-amoxicillin, ampicillin, penicillin G; qui- enrofloxacin; sul- sulfadimethoxine, sulfadimidine, sulfapyridine; tet- chlortetracycline, oxytetracycline, tetracycline; other- trimethoprim.
* These agriculture antibiotics are included in the WHO list but are reserved for veterinary use only.

Figure 4. Resistance mechanism development in agriculture and aquaculture. Top panel explains how each row exhibits a resistance mechanism. Each row in chart is an example via a different resistance mechanism. Each resistance mechanism can allow bacteria to be resistant to many classes of antibiotics (leftmost column). Antibiotics reported to be used in agriculture and aquaculture (column 1) can select for resistance mechanisms (column 2) that are sometimes expressed by common pathogens listed here are examples (column 3). Column 4 shows bacterial isolates reported in literature that are resistant to the stated antibiotics *and* have been genetically shown to express the resistance mechanism in that row. AG= isolate from agriculture; AQ=

isolate from aquaculture. Reference numbers for the publications are noted with the bacterial strain. Strain genera are as follows: P = *Pseudomonas*, E = *Escherichia*, S = *Streptococcus pneumoniae*/*pyogenes* or *Staphylococcus aureus*, N = *Neisseria*, E = *Enterococcus*, H = *Haemophilus*, K = *Klebsiella*, M = *Moraxella*, and B = *Bacillus*. Resistance mechanisms from Giedraitiene et al., 2011 (58).

Figure 5. 2007 density maps of cattle, swine, poultry, and combined values of production and 2005 number of aquaculture farms in US. 2007 US density of **A)** cattle, **B)** swine, **C)** poultry, and **D)** combined production. Maps A-C show animal density by county. For map A cattle density level: very high = > 17,400; high = 7,300-17,400; moderate = 2,175-7,299; some = < 2,175; none = 0. For map B swine density level: very high = > 48,500; high = 19,000-48,500; moderate = 9,500-18,999; some = < 9,500; none = 0. For map C poultry density level: very high = > 2.75 million; high = 1-2.75 million; moderate = 350-999 thousand; some = < 350 thousand; none = 0. For map D combined production, the total number of livestock across different animals types was calculated using the US Department of Agriculture definition of a livestock unit, which is 1000 pounds (454 kg) of live weight. Map D county density level (in livestock units): very high = > 13,200; high = 5,200-13,200, moderate = 2,000-5,199; some = < 2,000; none = 0. **E)** 2005 US density of aquaculture production by number of reported farms, with percentage of farm being freshwater or saltwater indicated in blue pie charts. States without a pie chart contain fully freshwater operations. (99,100)