

Antibiotic-Resistant Bacteria and Aquaculture

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Antibiotic residues originated from aquaculture can select for resistant aquatic bacteria, promoting the spread of antibiotic resistance, even when concentrations were below the minimum inhibitory concentration (MIC) of bacterial strains of the community.

Keywords: aquaculture ; antibiotic-resistance ; Mediterranean Sea

1. Induction of Antibiotic Resistance

Antibiotic residues can select for resistant aquatic bacteria, promoting the spread of antibiotic resistance, even when concentrations were below the minimum inhibitory concentration (MIC) of bacterial strains of the community ^{[1][2][3]}. High frequencies of antibiotic-resistant bacteria have been reported in sites near aquaculture where antibiotics have been used, demonstrating that modified antibiotics in an aquaculture facility have a high potential to exert selective pressure and increase the frequency of antibiotic resistance in other environmental bacteria ^{[4][5]}. In the aquatic environment, 90% of aquatic bacteria show resistance to at least one antibiotic, and approximately 20% were multi antibiotic-resistant. In the case of simultaneous application of different antibiotics in aquaculture, multiresistant bacteria can develop. Bacteria carrying genes coding for novel antibiotic resistance mechanisms were moreover present ^[6]. Antibiotic resistance allows bacteria the survive high concentrations of antibiotics, conferring a selective advantage to members of communities carrying resistances. Resistant bacterial strains prevail over susceptible ones. An important and at the same time worrying aspect is that the antibiotics used in aquaculture include those used in human therapies, thus inducing resistance to these antibiotics ^[7].

In Mediterranean aquaculture facilities, misuse of antibiotics caused an increase in levels of antibiotics in the surrounding sediments and water column ^[8]. The use of antibiotics in the Mediterranean farmed European seabass (*D. labrax*) and gilthead seabream (*S. aurata*), describing oxytetracycline, oxolinic acid, flumequine, and potentiated sulphonamides evidencing methaphylaxis, a control treatment of a group of animals after the diagnosis of infection and/or clinical disease in part of the group, as the best practice for their use ^[9].

2. Transfer of Antibiotic Resistance between Bacteria

Aquaculture sites represent 'hotspots for antibiotic-resistant genes' ^{[10][11][12][13]}. Bacteria harboring different genes of antibiotic resistance can grow according to environmental features, spreading genes in different sites ^[14]. The aquatic environment may also contain human and animal bacterial pathogens, which act as agents in sharing genetic determinants between aquatic and terrestrial bacteria ^[15]. The set of mobile genetic elements in a genome is called a mobilome and can spread among aquatic bacteria. The mobilome comprehends naked DNA, insertion sequences, insertion sequence elements with common regions, integrons mobilized by plasmids, transposons, and integrative and conjugative elements, genomic islands, transposons and conjugative transposons, conjugative and mobilizable plasmids and bacteriophages, including phage-like elements designated gene transfer agents ^[15].

Horizontal gene transfer between aquatic and human pathogens is an important phenomenon involving antibiotic resistance genes. New genetic elements can thus enter into terrestrial bacterial communities, including human pathogens, the latter becoming more difficult to treat ^[16]. The same aquatic environments with aquaculture facilities can present unique conditions allowing horizontal gene transfer. One case is represented by the biofilms of aquatic bacteria attached to organic particulate matter, sediment clays and sands, and fish farm structures, combined with the large concentrations of bacteriophages and gene transfer agents in seawater, also allowing the transfer of horizontal gene and dissemination of antibiotic resistance ^[17]. In aquatic environments, horizontal gene transfer can be mediated by DNA generated by lysis of bacteriophage and by plasmids. Both naked DNA from bacteriophages and plasmids can contain antimicrobial resistance genes that may be expressed after entering bacteria. Aquatic bacteria such as *Vibrio* spp. resulted naturally competent for DNA uptake, allowing transformation to occur in the aquatic environment ^[15]. Antibiotic-resistant genes are characterized by a different persistence in the environment, depending on the plasmid or chromosomal origin. Higher mobility and higher concentrations characterize antibiotic-resistant genes originated from plasmid with respect to those from chromosomes ^[18].

Genes of antibiotic resistance are present as both intracellular and extracellular fractions of DNA in the environment. Intracellular and extracellular antibiotic resistance genes observe the same pathway in sediments in an aquaculture site and nearby sites, revealing the presence of connections between these different sites [19][20]. Characteristics of DNA molecules also impact the persistence of antibiotic-resistant genes in the extracellular environment [18]. Some extracellular antibiotic-resistant genes are more recalcitrant to DNA degrading enzymes (DNases) than others, likely due to their sequence and structural features [21]. Due to the higher surface charges and higher molecular flexibility, chromosomal DNA is more adsorptive than plasmid DNA [22]. Therefore, in the environment, extracellular chromosomal DNA can persist longer than extracellular plasmid DNA. In sediments, a lower detection frequency of extracellular DNA located on the plasmid is expected with respect to chromosomal DNA [23].

2.1. Conjugation

Intracellular antibiotic-resistant genes may be disseminated via conjugation from cell-to-cell contact, and transduction because of infection of bacterial phages [24]. Conjugation is a horizontal gene transfer mechanism via cell to cell contact through junction due to pili or adhesins, by using a pore [25]. Conjugation is associated with plasmids that can transfer faster than a whole chromosome [26][27]. A percentage higher than 50% of known plasmids can be transferred by using conjugation as a horizontal gene transfer mechanism [28]. The process of conjugation may be present between the same bacterial species, but may also occur between unrelated populations characterized by a taxonomic distance, while at lower frequencies [25][26]. Plasmids and transposons can facilitate the conjugative transfer of antibiotic resistance by collecting antibiotic-resistant genes and carrying them to recipient cells [25][29]. Conjugation of antibiotic-resistant genes has been frequently reported in various environments, including soil, sediments, water, food, plant, animal, and clinical bacteria [26][28][30]. Conjugative transfer of multi-drug resistance among and across bacteria in different environments was showed [23][26] (Figure 1A).

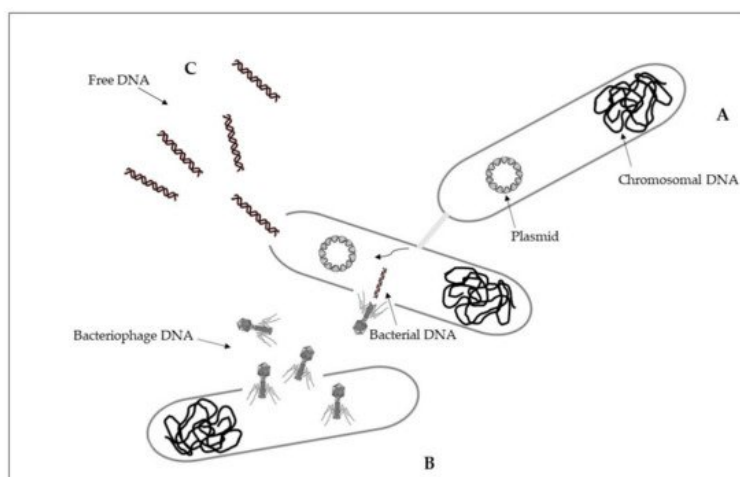


Figure 1. Horizontal gene transfer: (A), conjugation; (B), transduction; (C), transformation. See text for explanations.

2.2. Transduction

Transduction is a horizontal gene transfer mechanism with bacteriophages that act as mediators for intracellular DNA transfer from an infected bacterial cell to a recipient bacterial cell [26][31]. Bacteriophages, or phages, are viruses that infect bacteria and are able to collect and transfer genes to a host bacterial [26][32]. Bacteriophages can transfer both chromosomal DNA and plasmid DNA [31][33]. Once DNA is transferred, it must be incorporated into the recipient chromosome by homologous recombination [31]. Some bacteriophages have a wide range of bacterial hosts and can move across different species [34]. The transfer of antibiotic resistance genes via phages for different bacterial species has been extensively described [35][36][37]. During the first phase, bacteriophages attach to the bacterial host and then inject their genome that has the capability to sequester the molecular machinery of the host bacterial cell to synthesize new phages. New phages can then lyse the host cell and spread to the environment [38] (Figure 1B).

2.3. Transformation

Differently from intracellular DNA, extracellular antibiotic-resistant genes can enter the competent cells of non-resistant bacteria through the mechanism of natural transformation [24]. Extracellular DNA can originate from the lysis of dead cells and the secretion from live cells, representing a dynamic gene pool for natural transformation. Adsorption on sediment colloids, sand particles, clay minerals, and humic substances can protect extracellular DNA against enzyme nuclease attacks [39][40]. Natural transformation represents a direct uptake and integration of extracellular DNA [41]. It is essential that bacterial cells must first develop a regulated physiological state, a defined state of competence, for carrying out natural transformation [25][42]. Specific environmental conditions can stimulate competence development [26][43]. On the basis of the absorption of extracellular DNA by competent cells, there may be reasons such as nutrition, chromosomal DNA repair, and diversification of the genetic material for evolution [42]. Natural transformation requests the persistence of

DNA in the extracellular environment, and the ability to resist degradation in environmental conditions. Higher efficiency of natural transformation is evident in the presence of longer extracellular DNA fragments with respect to the results obtained with smaller extracellular DNA fragments [44][45]. Once in the recipient cell, the new DNA must be integrated into the recipient bacterial genome in the case of chromosomal DNA, and be integrated or recircularized into a self-replicating plasmid, in the case of plasmid DNA [42]. Natural transformation through chromosomal DNA is thus more efficient than in the presence of plasmid DNA [23][25] (Figure 1C).

3. Transfer of Antibiotic Resistance from Aquaculture Bacteria to Human Pathogens

3.1. Quinolones

Antibiotics of the quinolones class are widely used in aquaculture, with traces found in aquaculture effluents, the water column, and sediments near aquaculture facilities. The quinolone concentrations found in these compartments are high enough to exert selective pressure on aquatic bacterial species. The latter can mix with species of different origins, favoring gene exchange and spreading resistance to antibiotics [46]. Quinolones are antibiotics with a broad spectrum against both Gram-positive and Gram-negative bacteria, including mycobacteria and anaerobes. They exert their actions by inhibiting bacterial nucleic acid synthesis through disrupting the enzymes topoisomerase IV and DNA gyrase, and by causing breakage of bacterial chromosomes. Mechanisms of resistance to quinolones provide mutations in the bacteria genes, as the mutation in genes encoding the DNA gyrase and topoisomerase IV targets, or other genomic alterations which alter topoisomerase targets, modify quinolone, or reduce drug accumulation by decreasing drug absorption or increasing drug outflow. Resistance to quinolones may result from the uptake of the plasmid gene from the environment or from other resistant strains [47]. Genes of quinolone resistance included in plasmid-mediated quinolone resistance (PMQR) are the followings: six *qnr* genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qnrVC*) encoding gyrase-protection repetitive peptides; *oqxAB*, *qepA*, and *qaqBIII* encoding efflux pumps; and *aac(6')-Ib-cr* encoding an aminoglycoside and quinolone inactivating acetyl-transferase. Moreover, these genes can synergize with chromosomal *gyrA* and *parA* mutations conferring quinolone resistance [48]. The water-borne bacterial species *Shewanella algae* and *Vibrio splendidus* comprehend bacterial strains carrying *qnrA* and *qnrS* genes, respectively, and gene *qnrS* were detected in another water-borne strain, *Aeromonas* sp., the role of aquatic environments in the diffusion of such resistance determinants has acquired more and more importance [49]. Strains of both the aquatic bacterial species *Aeromonas punctata* subsp. *punctata* and *A. media* evidenced the presence of the *qnrS2* gene. The *qnrS2* gene was located on IncU-type plasmids in both isolates. When these plasmids were transferred into bacteria of the species *Escherichia coli*, they became highly resistant to quinolones and fluoroquinolones. The identification of plasmid-mediated *qnr* genes outside *Enterobacteriaceae* evidence a possible diffusion of those resistance determinants within Gram-negative bacteria [49]. The genes *qnrA*, *qnrB*, and *qnrS* for resistance to quinolones were found in the chromosome of marine bacteria isolated from an aquaculture facility in the Región de Los Lagos, Chile, and the same genes were detected in human pathogenic bacteria. The *qnrA* gene was, in fact, also found in the chromosome of two uropathogenic clinical strains of *E. coli* resistant to quinolones isolated from patients in a coastal area, bordering the same aquaculture region. The *qnrB* and *qnrS* genes were located in plasmids in two other *E. coli* strains isolated from the same clinical context [50]. Further investigations by sequencing *qnrA1*, *qnrB1*, and *qnrS1* genes in quinolone-resistant *E. coli* and in marine bacteria, both from Chile, were identical. A horizontal gene transfer between antibiotic-resistant marine bacteria and human pathogens was confirmed [23]. Concerning genetic elements of marine bacteria and uropathogenic *E. coli*, both evidenced class 1 integron with similar co-linear structures, identical gene cassettes, and similarities in their flanking regions. Investigations in a *Marinobacter* sp. marine isolate and in an *E. coli* clinical isolated strain, highlight sequences immediately upstream of the *qnrS* gene evidencing homology to comparable sequences of numerous plasmid-located *qnrS* genes. These investigations confirm that horizontal gene transfer between bacteria in diverse ecological locations is facilitated [50]. PMQR can be transferred horizontally among distantly related lineages and might play a role in maintaining resistance levels in bacterial populations in the presence of sub-inhibitory concentrations of antibiotics [51][52]. The same plasmid has been shown to play an important role for the spread of resistance genes not only quinolones but also for other antibiotics such as β -lactams and aminoglycosides. In fact, *qnr* genes are frequently carried along with β -lactamase determinants on the same plasmids. Moreover, it was evidenced that the prevalent *qnrA*, *qnrB*, *qnrS*, and *aac(6')-Ib-cr* genes among quinolone and cephalosporin-resistant clinical isolates of *Klebsiella pneumoniae*, are in the association between PMQR genes with resistance to quinolones, cephalosporins, and aminoglycosides [53]. Aquaculture is a possible source of *aac(6')-Ib-cr* and *qnrB2* in aquatic environments and *Enterobacteriaceae* were important hosts of these two genes. The ubiquitous bacteria, *Aeromonas* spp., served as vectors for *qnrS2* by means of IncQ-type plasmids. A water-human transmission by and via *Aeromonas* species was evidenced [54], and a *qnrS*-containing plasmid was identified in an *Aeromonas* sp. clinical isolate [55]. Before, genes *qnr* have only been reported in *Enterobacteriaceae* [56][57], with the one exception of a *qnrS*-containing plasmid found in environmental *A. punctata* subsp. *punctata* and *A. media* isolates [49]. A plasmid containing the *qnrS* was detected in an *Aeromonas* sp. clinically isolated strain for the first time [55], evidencing that a *qnrS2* gene was identified in a clinical isolate that was not within the *Enterobacteriaceae* family. The versatility of these determinants to spread among the different bacterial species with the consequent potential risk for human health became strongly

evident [55]. From this evidence, the need to control the antibiotic resistance supervision of both clinical and environmental *Aeromonas* isolates has emerged [58][59]. Plasmid-encoded quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*, and *aac* [6]-1b-cr) were found in *E. coli* and in *Klebsiella* [60]. Genes for quinolone resistance were detected in the aquatic genera *Vibrio*, *Shewanella*, and *Aeromonas* and then those genes were detected in human and animal pathogens [15]. Bacterial strains belonging to the water-borne bacterial species *S. algae* and *V. splendidus*, evidenced the presence of *qnrA* and *qnrS* genes, respectively. The gene *qnrS* was identified in another water-borne strain *Aeromonas* sp., increasing evidence of the role of aquatic environments in spreading those resistance determinants [49]. Gram-negative bacterial species of the aquatic environment may be the reservoir of plasmid-mediated Qnr-like determinants, that seem closely related to the species *V. splendidus* [61]. The World Health Organization (WHO) designated *E. coli* that are resistant to fluoroquinolones as one of the nine pathogens of international concern [62]. A description of strains of *E. coli* in countries in the Mediterranean area is reported in Table 1. Values of percentages of *E. coli* resistance to fluoroquinolones originated mostly from hospitals, nevertheless, their origin with aquatic bacteria could be a real problem for both human and animal health, and a concern for the environment.

Table 1. *Escherichia coli* isolated strains resistant to fluoroquinolones in countries of the Mediterranean area.

Country	Mean Percentage (%)	Range Percentage (%)	Year	Isolated Strains	Source
Bosnia and Herzegovina	29	23–35	2016	215	CAESAR
Croatia	29	26–32	2017	1150	EARS-Net
France	17	16–18	2017	13,328	EARS-Net
Greece	34	32–36	2017	1464	EARS-Net
Italy	47	46–48	2017	6945	EARS-Net
Lebanon	45	33–57	2016	65	GLASS
Portugal	30	29–31	2017	6424	EARS-Net
Spain	33	32–34	2017	5557	EARS-Net
Tunisia	19	13–29	2017	78	GLASS
Turkey	55	53–57	2016	3670	CAESAR

3.2. Tetracyclines

Tetracyclines are antibiotics inhibiting bacterial protein synthesis by preventing the association of aminoacyl tRNA with the bacterial ribosome [63]. Bacteria could use three strategies to become resistant to tetracycline: limiting the access of tetracycline to the ribosomes, altering the ribosome to prevent effective binding of tetracycline, and producing tetracycline-inactivating enzymes [64]. Tetracyclines are commonly used in human and veterinary treatment, with oxytetracycline that is permitted to be mixed with feed for fish [65]. In Japan, bacterial strains from aquaculture fish and bacteria from a close clinical facility exhibited a high similarity for genes of tetracycline resistance, suggesting that they may have originated from the same source. Laboratory experiments in which tetracycline resistance from marine strains of genera *Photobacterium*, *Vibrio*, *Alteromonas*, and *Pseudomonas* were transferred to *E. coli* by conjugation, confirmed the possibility of a transfer of resistance determinants from marine bacteria to bacteria inhabiting the human gut. Moreover, the same resistance gene profile in aquatic bacteria and in human clinical isolates was evidenced. The antibiotic-resistant genes for tetracycline resistance identified in fish pathogenic bacteria are common to those identified in human pathogens, and that bacteria from different environments, such as aquatic and clinical, can share the same antibiotic-resistant genes [66][65][67]. Tetracycline resistance determinants revealed in *Salmonella* spp. strains were detected in fish pathogens of the species *Vibrio anguillarum* [68][69][70]. Moreover, the DNA sequence of these antibiotic resistance determinants has an important DNA sequence similarity to a plasmid of *Pasteurella piscicida*, which is also a fish pathogen [69][70]. The independently evolved tetracycline-resistance determinant *tetG* was first discovered in aquatic bacteria [69]. In the genome of the animal pathogen *Chlamydia suis*, the *tetC* gene probably may have originated from the genome of the aquatic bacterium *Aeromonas salmonicida*, a pathogen of salmon [71]. The independently evolved tetracycline-resistance determinant *tetG* was first discovered in aquatic bacteria [69]. Tetracycline resistance genes were identified in marine sediments from resistance genes in bacterial plasmids from marine sediments that shared high identity with transposons or plasmids from human pathogens, indicating that the sediment bacteria can spread resistance genes from pathogens [72][51]. The same resistance gene profile has been described in both fish bacteria and human clinical isolates. About half of the antibiotic resistance genes identified in fish pathogens are common to those identified in human pathogens [65][67].

3.3. β -Lactams, Macrolides, Fosfomycin, Chloramphenicol, Colistin, Florfenicol

Commercial fish and seafood may act as a reservoir for multiresistant bacteria, facilitating the dissemination of antibiotic resistance genes. Broad-spectrum β -lactamase resistance genes, including *bla*TEM-52, *bla*SHV-12, as well as *cmlA*, *tetA*,

aadA, *sul1*, *sul2*, and *sul3* were recovered in the faecal matter from *S. aurata* (Gilthead seabream) [73]. The β -lactam antibiotics inhibit the last step in peptidoglycan synthesis by acylating the transpeptidase involved in cross-linking peptides to form peptidoglycan. The targets for the actions of β -lactam antibiotics are known as penicillin-binding proteins. This binding, in turn, interrupts the terminal transpeptidation process and induces loss of viability and lysis, also through autolytic processes within the bacterial cell [74]. Although bacterial resistance to β -lactams mostly expresses through the production of β -lactamases, other mechanisms are involved. Following are the mechanisms of resistance: (i) inactivation by the production of β -lactamases; (ii) decreased penetration to the target site as resistance in *Pseudomonas aeruginosa*; (iii) alteration of target site penicillin-binding proteins as penicillin resistance in pneumococci; (iv) efflux from the periplasmic space through specific pumping mechanisms [75]. Other examples of resistances with aquatic origin include the gene of fosfomycin resistance isolated from the aquatic environment, the widely disseminated emerging *floR* gene of human pathogens, and the chloramphenicol resistance genes *catIII*, *catB9* and *catB2*, originating respectively from aquatic bacteria of the genera *Photobacterium*, *Vibrio*, and *Shewanella* [45]. Additionally, the plasmid-associated colistin resistance mediated by the *mcr-1* gene appears to be another transmissible antibiotic resistance determinant that might have originated in the aquaculture facilities [72][76][77]. The macrolide resistance genes *mef(C)* and *mef(G)* in *Vibrio* spp. and *Photobacterium* spp. strains appear to have an aquatic origin [78]. Indeed, resistance genes have been found on transferable plasmids and integrons in pathogenic bacterial species of the genera *Aeromonas*, *Yersinia*, *Photobacterium*, *Edwardsiella*, and *Vibrio*. Class 1 integrons and IncA/C plasmids have been widely identified in important fish pathogens (*Aeromonas* spp., *Yersinia* spp., *Photobacterium* spp., *Edwardsiella* spp., and *Vibrio* spp.) and are thought to play a major role in the transmission of antimicrobial resistance determinants in the aquatic environment. The identification of plasmids in terrestrial pathogens (*Salmonella enterica* serotypes, *E. coli*, and others) which have considerable homology to plasmid backbone DNA from aquatic pathogens suggests that the plasmid profiles of fish pathogens are extremely plastic and mobile and constitute a considerable reservoir for antimicrobial resistance genes for pathogens in diverse environments [79]. The florfenicol determinant, *floR*, was detected for the first time in the fish pathogen *Vibrio damsela* [80]. This molecular evidence strongly suggests that there was a horizontal transmission of antibiotic resistance determinants from bacteria in the aquaculture environment to a human and terrestrial veterinary pathogen [69][70]. The epidemiology of the dissemination of *S. typhimurium* DT104 also suggests this pathogen could have been spread by fish meal as has happened with the *Salmonella* Agona that originated in Peru several years ago [69][81]. This process illustrates the potential role of the transport of antibiotic-resistant bacteria as an alternative mechanism responsible for the spread of antibiotic resistance determinants from the aquatic environment to the terrestrial environment [70][82]. As another example, *V. cholerae* of the Latin American epidemic of cholera that started in 1992, appeared to have acquired antibiotic resistance as a result of coming into contact with antibiotic-resistant bacteria selected through the heavy use of antibiotics in the Ecuadorian shrimp industry [69][83]. Multiresistant bacteria were evidenced in aquaculture contexts, including potential human pathogens strains of the genera *Vibrio*, *Pseudomonas*, and *Salmonella*, evidencing the possibility to transfer genetic determinants of resistance to pathogenic bacteria, both in water and in sediments [84]. Pathogens bacterial strains in aquaculture, belonging to the genera *Aeromonas*, *Edwardsiella*, *Flavobacterium*, *Lactococcus*, *Photobacterium*, *Pseudomonas*, *Renibacterium*, *Streptococcus*, *Vibrio*, *Yersinia* were pointed out [85].

4. Antibiotic Resistance in the Mediterranean Basin

In the Mediterranean area, fish farms of European seabass (*D. labrax*) and Mediterranean gilthead seabream (*S. aurata*) are mostly present, and antibiotics were added by feed amendments [9]. In Western Mediterranean coastal sediments from the vicinity of Ligurian Sea coastal fish facilities, Gram-negative bacterial strains have been found showing high resistance to the antibiotics ampicillin and streptomycin. Multiple resistances were found in the same strains. Antibiotic resistance patterns close to fish farming showed high incidences of quinolone, tetracycline, and penicillin-resistant bacterial populations [86]. In the Mediterranean area, antibiotic-resistant bacterial pathogens isolated from sediments near a fish farm in Greece, were compared with bacterial isolates in Italy and France, including *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., and *Pasteurella piscicida*. Greek, Italian and French aquaculture sites allowed isolation of bacterial strains evidencing similar antibiotic sensitivity pathways, with resistance to erythromycin, kanamycin, and streptomycin, and sensitivity to most of the other antibiotics tested. Resistance was also demonstrated for some isolates towards the potentiated sulphonamides [87]. Bacterial strains *Vibrio* spp., *Pseudomonas* spp., and *Photobacterium damsela* ssp. *piscicida* isolated from gilthead seabream (*S. aurata*) from an aquaculture facility in southwestern Spain, in the Mediterranean region, evidenced high levels of antibiotic resistance [88]. Bacterial strains of *V. anguillarum* isolated from Greek fish farms evidenced multiple antibiotic resistance [89]. Multiple antibiotic resistance pathways have been highlighted in native marine bacterial strains isolated from fish farms located along the Adriatic Sea (Italy) and identified as *Vibrio* spp. Resistance to tetracycline (17%), trimethoprim-sulfadiazine (7%), and trimethoprim (2%) resulted in the most frequently obtained patterns [90][91]. Pathogenic halophilic strains *Vibrio parahaemolyticus* and *V. vulnificus* evidencing resistance to lincomycin, were moreover isolated from seafood in Italy [92]. High frequencies of *Aeromonas* spp. contamination in *S. aurata* from the Italian coast was pointed out, with elevated biodiversity among isolated bacterial strains. The bacterial strains showed high resistance to sulfadiazine, amoxicillin, ampicillin, erythromycin, cephalotin, streptomycin, and trimethoprim antibiotics. It was evidenced

that almost all *Aeromonas* spp. strains showed multiple antibiotic resistance and potentially pathogenic species for humans were included, evidencing the capability to transfer via the food chain the genes responsible for antibiotic resistance to human pathogens [93]. More than one hundred bacterial strains were isolated from samples of *S. aurata* in an aquaculture site in Portugal. Included in the isolates, *Enterobacter* spp. and *Pseudomonas* spp. strains resulted in resistance to ertapenem and meropenem, antibiotics used in serious clinical infections. Several antibiotics for which resistance was found in these isolates appear in the World Health Organization list of “critically important antimicrobials” and “highly important antimicrobials” for human medicine [94]. In Mediterranean aquaculture, and in particular the Greek fish farm facilities, several registered antibiotics are currently available against bacterial infections, including tetracyclines, quinolones, and fluoroquinolones, potential sulfa, penicillin, and chloramphenicol derivatives. Oxytetracycline and quinolone drugs, with oxolinic acid and flumequine, are the most widely used in Mediterranean aquaculture [8]. A comparison was made of the distribution of tetracycline resistance genes in neighboring Greek fish farms and coastal environments. The *tetA* and *tetK* genes were detected in both habitats, while the *tetC* and *tetE* genes were present in fish farms and in wastewater, and *tetM* was found in fish farms and coastal sites. Some isolates were obtained, highlighting the presence of resistance genes *tetH*, *tetC*, *tetK*, and *tetM* in the main part. The isolates were assigned to the genera *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, and *Staphylococcus*. Isolated strains showed the presence of IncP plasmids, harboring tetracycline resistance genes (*i.e.*, *tetH*, *tetC*, *tetE*, and *tetK*), and the dissemination of IncP plasmids was evidenced. Based on these results, it has been shown that tetracyclines resistance genes from seawater sites have spread in bacterial communities, via broad-spectrum host plasmids [95]. The onset of antibiotic resistance in the environment and in non-clinical environments, highlight the importance of the investigation of antibiotic resistance in environmental contexts [96]. The use of antibiotics in aquaculture can lead to the emergence of antibiotic resistance in bacteria that are pathogenic to humans, posing a serious threat to public health [97]. In 2015, antibiotic-resistant bacteria increased in the European area, causing over 33,000 human deaths. Furthermore, this number is projected to increase by 2030, due to rapid socioeconomic growth and population expansion [98][99]. The emergence of antibiotic resistance in bacteria poses a serious threat, with a reduction in the use of antibiotics in aquaculture feed that is absolutely required by 2030 [100]. In Table 3, a description of antibiotic-resistant bacteria, and eventual genes for antibiotic resistance, in aquaculture sites of different countries of the Mediterranean basin was reported. The presence of antibiotic-resistant bacteria from aquaculture facilities in the Mediterranean area, highlighting serious concerns for human and animal health and for the environment, is evidenced.

Table 2. Bacteria isolated from aquaculture in the Mediterranean area, antibiotic resistance and antibiotic-resistance genes.

Country/Area	Isolated Bacteria Species/Genus/Family/Order/Class	Antibiotic-Resistance	Antibiotic-Resistant Genes	References
Algeria	<i>Vibrio alginolyticus</i> , <i>V. cholerae</i> , <i>V. fluvialis</i> , <i>V. hollisae</i>			[133]
Croatia	<i>Aeromonas</i> spp.			[134]
Eastern Adriatic	<i>Vibrio</i> spp.	flumequinone, chloramphenicol, oxytetracycline		[135]
Egypt	<i>Aeromonas</i> spp.	chloramphenicol, kanamycin, azithromycin		[136]
Egypt	<i>Pseudomonas anguilliseptica</i>			[137]
Egypt	<i>Aeromonas hydrophila</i>	penicillin, erythromycin		[138]
Egypt	<i>Enterobacteriaceae</i>	cephalosporins, carbapenem	<i>blaKPC</i> (<i>blaCTX-M-15</i> , <i>blaSHV</i> , <i>blaTEM</i> , <i>blaPER-1</i>)	[139]
France	<i>Flavobacterium psychrophilum</i>			[140]
France	<i>Yersinia ruckeri</i>			[141]
Greece	<i>Acinetobacter</i> spp., <i>Bacillus</i> spp., <i>Pseudomonas</i> spp., <i>Staphylococcus</i> spp., <i>Stenotrophomonas</i> spp.	tetracycline	<i>tetA</i> , <i>tetK</i> , <i>tetC</i> , <i>tetE</i> , <i>tetM</i>	[127]
Italy	Enterococci	ampicillin, gentamicin	<i>tetM</i> , <i>tetL</i> , <i>tetO</i> , <i>ermB</i> , <i>mef</i>	[142]
Italy	<i>Aeromonas</i> spp., <i>Photobacterium</i> spp., <i>Shewanella</i> spp., <i>Vibrio</i> spp.	tetracycline, flumequine, trimethoprim		[122]

Country/Area	Isolated Bacteria Species/Genus/Family/Order/Class	Antibiotic-Resistance	Antibiotic-Resistant Genes	References
Italy	<i>Photobacterium damsela</i> ssp. <i>piscicida</i> , <i>Vibrio fluvialis</i> , <i>V. alginolyticus</i> , <i>V. parahaemolyticus</i> , <i>V. metschnikovii</i>	ampicillin, carbenicillin, kanamycin, cefalothin		[143]
Italy	<i>Aeromonas</i> spp.	ampicillin, amoxicillin, cephalothin, erythromycin, streptomycin, sulfadiazine, trimethoprim		[125]
Italy	<i>Shewanella algae</i> , <i>Vibrio</i> spp.	beta-lactams, quinolones, tetracyclines, macrolides, polymyxins, chloramphenicol, fosfomycin, erythromycin	<i>blaOXA-55-like</i> , <i>blaAmpC</i> , <i>mexB-OprM</i> , <i>mdtG</i> , <i>mdlB</i> , <i>tet34</i> , <i>tet35</i> , <i>tetR</i> , <i>eptA</i> , <i>cat</i> , <i>mdtL</i>	[46]
Lebanon	<i>Streptococcus pneumoniae</i>	polymyxins, chloramphenicol, fosfomycin, erythromycin		[144]
Spain	<i>Aeromonas</i> spp., <i>Salmonella</i> spp., <i>Vibrio mimicus</i> , <i>V. furnissii</i> .	oxytetracycline, nitrofurantoin, oxacillin, sulfomethoxazole/trimethoprim		[145]
Spain	<i>Flavobacterium psychrophilum</i>	oxytetracycline, florfenicol		[146]
Spain	<i>Aeromonas salmonicida</i>	nalidixic acid, oxytetracycline		[147]
Tunisia	<i>Vibrio alginolyticus</i>	ampicillin, erythromycin, kanamycin, cefataxime, streptomycin, trimetoprim		[148]
Tunisia	<i>Escherichia coli</i>	tertracycline, streptomycin, ampicillin, trimethoprim, sulfamethoxazole	<i>tetA-tetB</i>	[149]
Turkey	<i>E. coli</i> , coliforms, fish pathogens	sulfamethoxazole, ampicillin, sulfamethoxazole, imipenem, aztreonam	<i>ampC</i> , <i>blaCTX-M1</i> , <i>tetA</i> , <i>sul2</i> , <i>blaTEM</i>	[150]
Turkey	<i>Y. ruckeri</i>		<i>floR</i> , <i>sulI</i> , <i>tetC</i> , <i>tetD</i> , <i>tetE</i>	[151]
Turkey	<i>Y. ruckeri</i>	erythromycin, florfenicol, sulfonamide, tetracycline, trimetophrin	<i>ermB</i> , <i>ermY</i> , <i>floR</i> , <i>sulI</i> , <i>suffII</i> , <i>tetA-tetG</i>	[152]
Turkey	<i>Aeromonas media</i> , <i>A. rivipollensis</i> , <i>A. salmonicida</i> , <i>Bacillus pumilus</i> , <i>B. zhangzhouensis</i> , <i>Hafnia alvei</i> , <i>Kluyvera intermedia</i> , <i>Pantoea</i> spp., <i>Pseudomonas</i> spp., <i>P. protegens</i> , <i>Staphylococcus</i> spp., Gammaproteobacteria, Betaproteobacteria, Enterobacteriales, Burkholderiales	sulfamethacin, sulfamerazine, erythromycin, tetracycline		[153]

5. Conclusions

In the face of undoubted advantages in economic and social terms, aquaculture in the Mediterranean basin involves some critical issues, one of which is represented by resistance to antibiotics. A link between antibiotics utilization in animals, the emergence of resistant bacterial strains, and the transfer of resistance to human pathogens have been highlighted. New insights and new research directions could include the use of new antibiotics, for instance from marine microorganisms, to be applied in aquaculture, avoiding the utilization of the same antibiotics deputed to human therapies. As for recommendations, it is important to decrease the use of antibiotics in aquaculture, improving the care of fish farms, and promoting the hygiene of the facilities.

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