Review Paper:

Methodologies for determination of antimicrobial resistance in commensal and pathogens of aquaculture

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Abstract

Antimicrobial resistance (AMR) is a serious concern globally in human and animal health sectors and the menace is percolating to the aquaculture too. Since the aquaculture sector is at the receiving end of the terrestrial run-off, the AMR detection strategy would vary. Hence, AMR determination in the aquaculture sector strategy needs triangular approaches viz. pathogens of aquatic animals, commensal bacteria of the aquaculture ponds and food safety pathogens in harvested food fish. Farmed aquatic animals are being transported as food material to distant places of intra, inter-countries and continents. In this context, robust surveillance of AMR in microflora associated with farmed aquatic animals remains an essential tool for identification and control of its spread.

Despite numerous reviews that have been published for determining resistance in pathogens of clinical importance, literature is scant on aquaculture and seafood safety. The present review is oriented to illustrate the different approaches possible for the identification of AMR especially in the aquatic animal pathogen, commensal bacteria and seafood pathogens along with the molecular approach. The need of the hour is rapid identification of resistance in the surveillance platforms, thereby limiting the spread of the resistance and improving food security.

Keyword: Antimicrobial resistance, Aquaculture, Seafood, culture-independent method, Culture-dependent method, Molecular approaches.

Introduction

Antibiotics are an important discovery of the 20th century and still remain indispensable compounds in therapeutics of human and animal health care systems[41]. However, in the past two decades, there has been an alarming trend in the increase of resistance to antimicrobials i.e. antimicrobial resistance (AMR) in bacteria that is driving humanity towards the pre-antibiotic era due to the evolution of multidrug resistance (MDR), pan drug resistance (PDR) and extensive drug resistance (XDR) bacteria[122]. Further, the fact that there was no new class of antibiotic discovery in the last three decades, has worsened the condition and favoured the spread of AMR in the health sector and animal agriculture.

In the livestock and agriculture sector, fisheries contribute immensely to nutritional and food security, both in terms of domestic consumption and export. It has reached the status of the most traded commodity of animal protein source recently in 2018, valued at USD 164 billion and identified as the major driver of economic growth[1]. To cater to the requirements of food preferences and food demand of the growing population, the fisheries sector has been rapidly expanding towards intensive aquaculture practices with limited space which leads to a potential increase in the incidence of infectious diseases in farmed aquatic animals. In many instances, aquaculture farmers are left without a choice and forced to use antimicrobials to avoid crop loss which also favours extensively the development of antimicrobial resistance.

Though the consumption of antibiotics in the fisheries sector is relatively meagre, it has been gradually rising to combat infectious diseases or for prophylactic use[10,11]. In aquaculture, the global annual antimicrobial consumption was determined as 10,259 tons in 2017 and is projected to rise to 13,600 tons in 2030; with the Asia-Pacific region accounting for 93.8% of the global consumption[190]. Aquaculture requires special attention because it is at the receiving end of all the run-off of across different sectors (human, terrestrial animal and terrestrial environment) which includes antibiotics.

Also unlike terrestrial animals, in aquaculture the animals are in very close intimacy with the environment *viz.* water and soil, thereby making individual therapeutic regimen nearly impossible.

Antibiotic usage disrupts the environmental microbiota associated with the pond; hence, it is inevitably facilitating the development of antimicrobial resistance[7,69]. The development of AMR in commensal or pathogenic or seafood safety bacteria may easily spread between each other through horizontal or vertical gene transfer and also the presence of antimicrobials in sub-therapeutic level concentration[30]. After the completion of the aquaculture crop period, the water is generally discharged to the adjoining major aquatic body; hence, the possibility of spreading to other aquatic environments is very high[17].

Earlier, the studies were centred on clinical AMR pathogens which have now expanded to the bacterial populations associated with animals, foodborne, waterborne pathogens, other environmental bacterial populations and since a decade, in pathogens of aquaculture settings like Aeromonads and Vibrios[79,152,158]. Addressing the AMR in aquaculture settings needs a triangular approach encompassing pathogens of aquatic animals, commensal bacteria of the pond, that should be linked to the pathogens of the food fish. Special emphasis needs to be given to bacteria unique to human and terrestrial sectors *viz.* methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL) *Escherichia coli and Klebsiella pneumoniae*, Vancomycin-resistant enterococci (VRE) and Vancomycin-resistant MRSA. In depth understanding is essential to unlock the role of commensal bacteria in the ecology of antimicrobial resistance.

In the present review, the major focus is on bacteria associated with aquaculture ponds relevant to aquatic animal's health (cultured in fresh and brackish water environment) and methods available to determine resistance and molecular approaches in deciphering the mechanism behind the resistance. Emphasis was also laid on the mobile genetic elements (MGEs) implicated in the transfer of resistance. The aim is to provide a one-stop solution to the researchers in the fisheries sector and riverine and reservoir fisheries are dealt elsewhere.

One health in Antimicrobial resistance: World health organization has defined one health as the approach of designing and implementing program, policies and research activity across all the sectors such as human and animal health and environment. It envisages establishing a coordinated multi-sectoral and inter-disciplinary approach for containing the AMR. Public health, animal health, plant health and the environment are the different stakeholders in the one health approach working at the global, regional, national and local levels[14,28,120,232]. The aquaculture environment is an important component of one health as it at the receiving end of all terrestrial discharges and runs-off and involves a complex system for AMR control.

Bacterial population associated with aquaculture settings

Aquatic pathogens: Aquaculture is one of the rapidly growing food production sectors in the world; but the growth of the sector is plagued by diseases of bacteria, viruses, fungi and parasites, of which bacteria can survive well in the aquatic environment independently without the hosts. Moreover, bacterial diseases are a major impediment to aquaculture especially in tropical countries. The important Gram-negative bacteria reported to be pathogenic to finfish and shellfish in the tropical region are: *Vibrio alginolyticus, Vibrio harveyi, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio anguillarum, Photobacterium damselae, Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae, Aeromonas veronii, Edwardsiella ictaluri, Edwardsiella tarda, Pseudomonas fluorescens and Flavobacterium columnare,* while *A. salmonicida* and *E. ictaluri* are very relevant in a temperate climate[86,99,100,110,132,177,196,197] .

The Gram-positive bacteria that cause infections in fish and shellfish include *Mycobacterium marinum, Mycobacterium fortuitum, Nocardia asteroides, Streptococcus iniae, Streptococcus agalactiae, Lactococcus garviea, Streptococcus parauberis, Aerococcus viridans, Renibacterium salmoninarum* and in rare cases *Pasteurella piscicida, Yersinia ruckeri,* or *Piscirickettsia salmonis*[68,70,187,194]. Necrotizing hepatopancreatitis (NHP), caused by alpha-Proteobacteria and recently, acute hepatopancreatic necrosis disease (AHPND) due to binary toxin-producing *V. parahaemolyticus* have been the most important bacterial diseases affecting shrimp farming in recent years[150,212]. Moreover, every year new pathogens are being added across the globe in the development of diseases *viz*. *Vibrio cholerae* O139, *Serratia* sp, *Proteus* sp., *Pseudomonas* sp., *Lactococcus* sp., *Klebsiella* sp. and many more emerging[44,85].

Commensal organisms: Bacterial isolates belonging to 15 genera were identified as commensal or opportunistic pathogens in the aquaculture system *viz*. *Bacillus* sp., *Psychrobacter* sp., *Plesiomonas* sp., *Aeromonas* sp., *Vagococcus* sp., *Enterobacter* sp., *Kurthia* sp., *Aerococcus* sp., *Corynebacterium* sp., *Pseudoclavibacter* sp., *Lactobacillus* sp., *Kocuria* sp., *Enterococcus* sp. and *Staphylococcus* sp[79]. Bacterial species such as *A. hydrop*hila, *Corynebacterium* sp., *Corynebacterium urealyticum*, *Edwardsiella* sp., *Micrococcus* sp., *Pantoea* sp., *Pasteurella pneumotropica*, *Shewanella putrefaciens*, *Staphylococcus* sp., *Streptococcus* sp., *Vibrio alginolyticus*, *V. cholerae*, *V. vulnificus* and Unidentified G−ve rods were also identified as the commensal population in aquatic animals. Bacteria such as *Vibrio* sp., *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Edwardsiella tarda* and *Myxobacteria* are the most common bacterial flora of the water, which are also associated with the fish under ailing condition[209,213].

The potential role of commensals along with beneficial flora to harbour transmissible resistance genes and their vulnerability to acquire resistance generally cannot be disregarded[79,124]. A very important observation to note in the bacterial populations associated with aquaculture and its environment is the high frequency of *Vibrio* sp and *Aeromonas* sp. in shellfish and finfish aquaculture settings. They occur as commensal bacteria, aquatic animal pathogens as well as human health hazard bacteria.

Hence, the possibility of gene transmission between these two genera of bacteria is quite high. These antibiotic-resistant genes are transferred to zoonotic pathogens either through vertical or horizontal gene transfer mechanisms mediated by mobile genetic elements[69,129]. In this context, the resistance genes present in the pathogenic flora or

commensal flora across the sectors finally enter the aquatic environment and can readily proliferate in the commensal flora of the aquatic environment and are stable in the commensal[45,69,81]. From one health perspective, important bacteria for addressing the AMR in the aquaculture settings are *Vibrio* sp. and *Aeromonas* sp. (Figure 1).

Seafood borne pathogens: The fish and fishery products are documented with the contamination of several foodborne pathogens namely *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, *Shigella* sp., *V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, *Yersinia enterocolitica*, *Campylobacter jejuni and Escherichia coli*[16,39,101,102,105,121,137,138,141,142,145,149,172,173,226].

Comprehensive approaches for determination of antimicrobial resistance: Aquaculture farms consist of aquatic animals and associated environment (water, sediment) and the determination of AMR in aquaculture settings is based on both culture-dependent and independent approaches (Table 1). To decipher the role of various microflora in the development of AMR in the complex diversity of microbes in the environment, a single method cannot serve the purpose and culture-dependent method should be used along with one of the culture- independent methods.

Culture dependent approach: In this approach, live bacterial isolates are recovered from the animal, water, or environment and assessed for their antimicrobial resistance profile. Direct assessment of antimicrobial resistance in the aquaculture pathogens is the first and foremost approach for infection control and treatment of aquatic animals.

Additionally, assessing the antimicrobial resistance in the commensal and/or opportunistic pathogens is also the need of the hour for aquatic animal health management. The aquaculture produce is intended for human consumption either in the domestic market or for export and hence, the assessment of antimicrobial resistance needs to be extended to aquatic food safety pathogens.

Once the bacteria are isolated and identified, there are several methods available for the determination of AMR viz. qualitative phenotypic method and qualitative genotypic method and the same in quantitative methods either as phenotypic or genotypic. The bacteria isolated from either of the three culture based approach are subjected to disk diffusion assay for measurement of zone of resistance with the recommended antibiotics in qualitative phenotypic method.

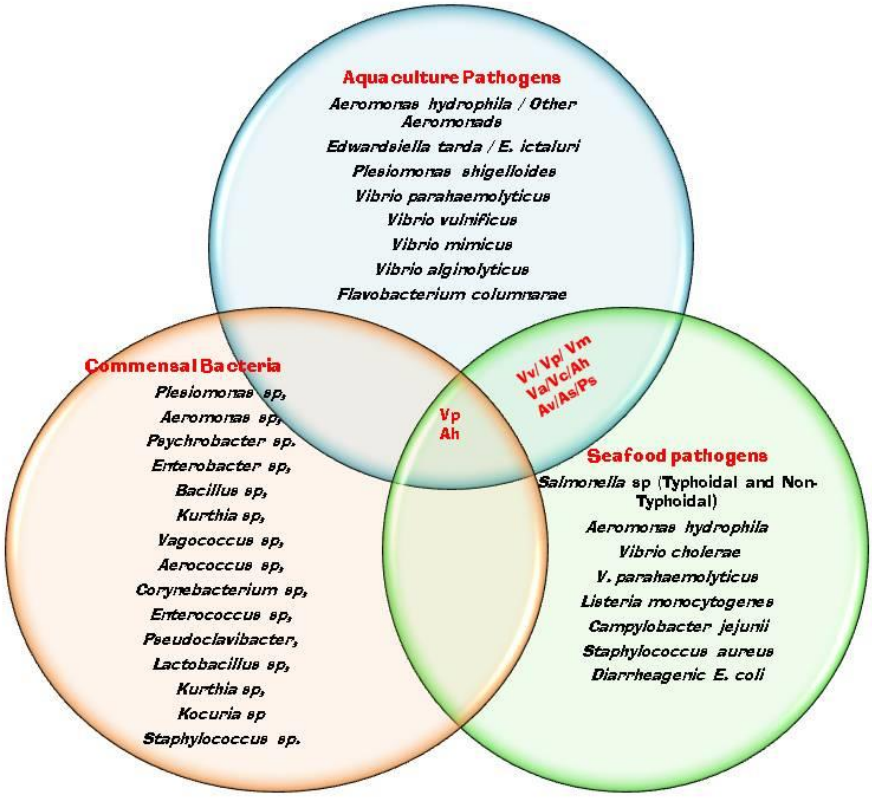

Figure 1: Bacteria of commensal, pathogenic and seafood-borne importance in aquaculture settings.
The blue circle denotes aquatic animal pathogens; the Green circle denotes human health hazard seafood pathogens; the Orange circle denotes the commensal bacteria of aquaculture importance.
Vv: *V. vulnificus*, Vp: *V. parahaemolyticus*, Vm: *V. mimicus*, Va: *V. alginolyticus*; Vc: *V. cholerae*, Ah: *Aeromonas hydrophila*, Av: *A. veronii*, As *A. salmonicida*, Ps: *Plesiomonasshigelloides*

Table 1
Methods available for analyzing AMR in bacteria associated with aquaculture

Method	Aquaculture pathogen	Commensal Bacteria	Seafood safety bacteria (Includes Indicator bacteria)
Culture- dependent method (Prevalence or surveillance method; Diversity approach; Resistance population analysis screening method)			
Phenotypic Method: Qualitative			
1. Disk Diffusion assay	+	+	+
2. Linear Gradient plating method	_*	+	_**
Phenotypic Quantitative method (MIC)			
Broth dilution method	+	+	+
Agar dilution method	+	+	+
Culture-independent method			
Genotypic Method			
Metagenomic approach (Sequence-based) – NGS based resistome	-	+	-
Microarray-based approach - Resistome	+	+	+
PCR based approach – Cloning and sanger sequencing	+	+	+

* Linear Gradient Plating method is not used in selective screening in aquaculture pathogen. ** Seafood safety pathogens *viz*. MRSA, this method has been employed for selective isolation of the resistant population

In the quantitative phenotypic method, the level of antibiotics was estimated using minimum inhibitory concentration with agar or broth dilution methods and recently Epsilometry paper strip based method. Automated AMR identification systems are available for quick culture based identification of AMR viz. sensititre (Thermofisher Scientific), VITEK-2 (Biomerieux), Phoenix (BD) relies on MIC integrated with the interpretation guidelines. The culture-dependent approach may be combined with the molecular tools used for culture-independent approaches viz. microarray-based resistance genes profiling, whole genome sequencing (WGS) based resistance profiling and other qPCR and PCR based profiling.

Molecular methods viz. PCR, qPCR, microarray genotyping and whole genome sequencing are also applicable to isolated bacteria from culture dependent approach. Culture based phenotypic qualitative and quantitative methods are recommended for database development for guidelines framework, selection of dosage and monitoring the development of AMR.

Surveillance based approach: This is one of the culture based approach. The surveillance-based approach for AMR has two ways *viz*. active and passive surveillance. Active surveillance or targeted surveillance is beleaguered on desired bacterial pathogens of local/national/ regional or global importance. Here, the samples are collected at a regular interval in the population; but, the limitation of the approach is both time-consuming and laborious[125]. The passive surveillance is carried out on bacterial pathogens isolated during a disease outbreak. In this, the etiology for the disease outbreak is of varied reasons and hence, it is relatively less time-consuming, but the bacterial pathogens identified are diverse[225].

A detailed stepwise guideline for setting the surveillance program on aquatic animal diseases in aquaculture setting can be integrated to both active and passive surveillance program on AMR [20]. Samples of target for this study are gills, either internal of external lesions of the disease from organs. Surveillance based approach is very essential for drafting national policy framework for controlling the antimicrobial resistance based on the pattern and trend over a period of time. Guidelines should be followed for the interpretation of data on antimicrobial resistance based on standard described in figure 2. The studies conducted in aquaculture farms based on passive surveillance are described in table 2.

Diversity approach: In this approach, the total culturable bacterial population of the farm is assessed i.e. heterotrophic population of the pond. AMR pattern obtained in this culture dependent approach reflects the current status of AMR in the aquaculture pond. The selection of bacteriological media and incubation conditions vary with the farming practices, fish species and salinity of the water. Analyzing samples from freshwater aquaculture requires the use of basal brain heart infusion or trypticase soy agar or nutrient agar along with one blood agar plate. Samples from brackish water aquaculture require media supplemented with at least 1% NaCl or artificial seawater media or Marine agar (Zobell Marine agar) along with one blood agar plate. In this method, commensal, aquatic pathogens, or seafood safety pathogens

which are non-fastidious and heterotrophic nature will be included.

The samples of the target are aquatic animals, animal gut, water, sediment, feed including live feed, etc. which are serially diluted ten-fold up to 6 or 7 dilutions. Morphologically distinct bacterial populations will be screened for antimicrobial resistance. The critical factor involved in this approach is the selection of the antibiotic panels for the bacterial population. In general, the antibiotics are selected on the nature of the bacteria e.g. Gram-positive or Gram-negative coccus or Bacillus. Interpreting guidelines are based on the same principle, but can be taken from CLSI guideline available for aquatic animals, terrestrial animals or human clinical cut-off. This study is very much essential to understand AMR status of the pond and deals with wide variety of culturable bacteria. Studies that are carried out in aquaculture farms based on diversity approach on heterotrophic populations are depicted in table 2.

Resistant population approach: Once the selection of the antibiotic is completed for the study, a modified agar dilution method is carried out by employing the Minimum Inhibitory Concentration (MIC) for all the antibiotics. The method is also known as the "Linear Gradient plating method" which is employed to recover the organism which has received MIC level of antibiotics in a particular environment[13,113]. The gradient plating method is generally employed in a culturable total bacterial population. However, it can be adapted to both aquaculture and seafood borne pathogens *viz*. Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolation 2µg/mL concentration of oxacillin are added commonly and for isolation of Vancomycin-resistant *S. aureus* (VRSA) 6µg/mL[22,214].

These can be extrapolated to the aquaculture pathogens with their pre-determined MIC values. Care is necessitated for avoiding the bacterial cultures with known intrinsic resistance for the particular antibiotic e.g. penicillin for Gram-negative bacteria. This approach can be adapted for the selective plating media combinations for the target population or the entire non-fastidious population. Non-selective enrichment media may be adapted based on the salinity requirement. Resistant bacterial population prevalence across the ecosystem of aquaculture and its products has been reported[244].

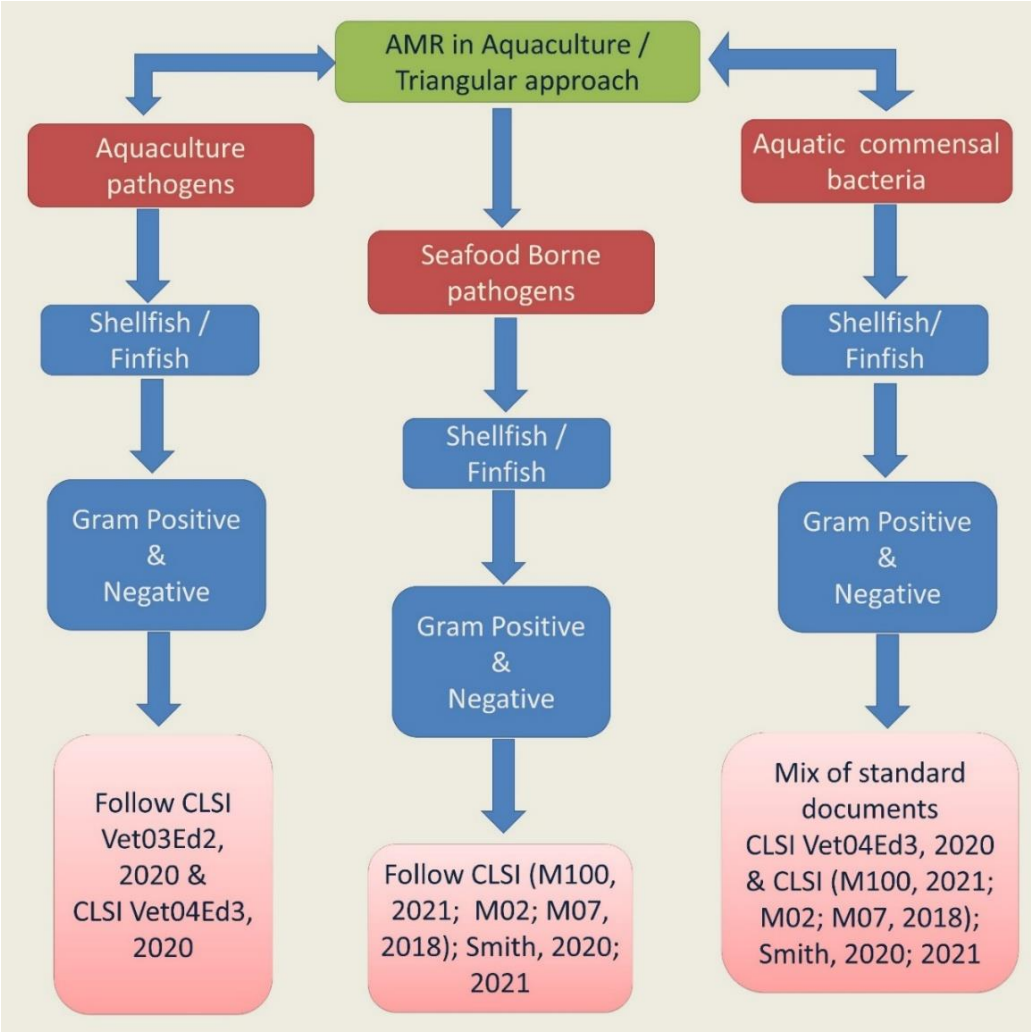

Figure 2: AMR determination in triangular approach for aquaculture

Table 2
Culture dependent and independent methodologies employed for AMR in finfish and shrimp aquaculture

S.N.	Culture dependent method	Culture independent method	Aquaculture farm type and Country	Methodologies	Type of resistance
1	Resistance population approach/ Gram negative, oxytetracycline resistant bacteria[228]	-	Freshwater salmon farm Chile	Phenotypic-DDA,	Tetracycline, amoxicillin, ampicillin. erythromycin, furazolidone, florfenicol, chloramphenicol, cefotaxime and trimethoprim-sulfamethoxazole
2	Passive surveillance/ *Aeromonas* sp[50]	*Tet* gene, *qacEDelta*1, *sulI, ant(3'')Ia, aac(6')Ia, dhfr1, oxa2a* and *pse1*	Tilapia, trout and koi aquaculture system South Africa	PCR, Phenotypic	Tetracycline, erythromycin nalidixic acid resistance
3	*Heterotrophic / Commensal* and *Salmonella* spp and *Vibrio vulnificus*[19]	-	Farmed and wild caught shrimp South Carolina	Phenotypic, DDA	Ceftriaxone tetracycline ampicillin ceftriaxone, gentamicin, streptomycin, trimethoprim nalidixic acid trimethoprim
4	Commensal / *Pseudomonas fluorescens, Aeromonas hydrophila, Stenotrophomonas maltophilia*[128]	-	Chilean freshwater salmon farms Chile	Phenotypic	Amoxicillin, ampicillin, erythromycin, furazolidone, florfenicol, chloramphenicol, cefotaxime and trimethoprim-sulfamethoxazole
5	Resistant population method-*Florfenicol resistant* bacteria[53]	*floR*	Chilean freshwater salmon farms Chile	Phenotypic	Florfenicol, streptomycin, chloramphenicol and oxytetracycline.
6	Active surveillance/ *Vibrio* sp[182]	-	Shrimp farms Brazil	Phenotypic - MIC	Ampicillin tetracycline
7	Active surveillance/ *Photobacterium damsela ssp. piscicida, Vibrio fluvialis, Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio metschnikovii*[104]	-	Aquaculture (fish, shellfish and crustaceans) Italian	Phenotypic – DDA and MIC	Sulfadiazine-trimethoprim, ampicillin, carbenicillin, kanamycin, cefalothin
8	Heterotrophic / Commensal Bacillus	ARGs – *sul1, sul2, tetM, tetO, tetT, tetW*[59]	Aquaculture farm – Commensal flora China	ARGs- qPCR	Sulfonamide tetracycline
9	Heterotrophic / Commensal bacteria	*tetA, tetG, dfrA1, dfrA5, dfrA12, sul1, sul2, bla$_{TEM}$, strA-strB, intl1, aad9* and *intl2*[195]	Chilean salmon aquaculture Norway	DNA hybridization and PCR, amplicon sequencing	Tetracycline, sulfonamide, β-lactamase

10	-	ARGs – *sul1, sul2, sul3, tetM, tetO, tetQ, tetW, tetX, tetB/P,* PMQR- *qepA, oqxA, aac-16, qnrS*[238]	Aquaculture farms China	Metagenomics (16s rDNA); ARGs -qPCR;	Sulfonamide, tetracycline and quinolone resistance detected
11	Active surveillance / *V. parahaemolyticus*[205]	-	Farmed shrimp Ecuador		Ampicillin resistance and intermediate resistance to tetracycline and amikacin
12	Active surveillance/ *Aeromonas* sp[155]	ARGs- *sul1, tetA* and *intI1*	Aquaculture farm- fish – Culture method Silver Carp Israel (*Hypophthalmichthys molitrix*)	Phenotypic and PCR	Sulfonamide and tetracycline resistance detected
13	Heterotrophic / Commensal bacteria[112]	ARGs- *blaTEM, tetC, sulI, aadA, floR* and *qnrB*; *int1, int2*; Int-1 associated with *qacEΔ1/sul1* gene; 14 cassette array detected *dfrB4-catB3-blaOXA-10-aadA1, dfrA12-orfF-aadA2*	Aquaculture farms eels China.	PCR	Aminoglycoside Sulfonamide tetracycline, quinolone resistances also detected.
14	-	*tet(32), tetM, tetO, tetS, tetW, tetA, tetE, tetG, tetH, sul2, dfrA1, aadA, aadA1, aadA2, strB, tnpA, qacEΔ1, mexF, oprD, oprJ, pncA, yecL, blaOXY, blaCTX, blaSHV, acrA, vanC, aacC*[139]	Fish farms Finland	qPCR metagenomics – Bacterial diversity	Tetracycline Sulfonamide Aminoglycosides Beta-lactam resistance Transposons Efflux pump mechanism
15	-	*sul1, dfrA1, tet(32), tetM, tetO, tetW, aadA1, aadA2, catA, emrB, matA, mefA, msrAe intI1, qacEΔ, tnpA*[81]	Fish Farm Finland	WaferGen qPCR array Metagenomics- bacterial diversity	Tetracycline Sulfonamide Trimethoprim Chloramphenicol Macrolides Integron and transposons
16	-	*tetA, tetB, tetD, tetE, tetG, tetH, tetM, tetQ, tetX, tetZ, sul1 and IntI1*[84]	Fish farm & effluents South Korea.	Metagenomics (16s rDNA); ARGs -qPCR;	Tetracycline Sulfonamide Integron
17	-	*flo*R, *sul*II, *sul*I, *str*B, *str*A, *aad*A, *tetS and tet*S[74]	Aquaculture farms China	Metagenomics (16s rDNA); ARGs -qPCR;	Florfenicol, Sulfonamide Aminoglycoside Tetracyclines
18	Diversity/ Fish gut microbiota	ARGs to florfenicol and oxytetracycline also detected[72]	Chilean freshwater salmon farms Chile	Phenotypic, Resistance population approach and PCR.	Florfenicol oxytetracycline
19	-	*sul1, sul2, sul3, tetA, tetM, tetT, tetW, aac(6')-Ib, floR, qnrB, qnrA, qnrS, fexA, fexB, qepA, bla$_{SHV}$, cmlA, cfr*[249]	Integrated polyculture aquaculture farm China	Metagenomics (16s rDNA); ARGs -qPCR; shotgun sequencing - Resistomes	Tetracycline, sulphonamides, quinolones, chloramphenicol and β-lactamases
20	Heterotrophic / Commensal (*Pseudomonas* sp)	*gyrB* mutations[33]	Chilean salmon farms Chile	PCR, Phenotypic	Flumequine, florfenicol and oxytetracycline

21	Heterotrophic / Commensal (*Pseudomonas* sp)	*sul1*, *sul2*, *sul*3, *intI1*, int2, *dfrA1*, *dfrA12* and *dfrA14*[47]	Chilean salmon farming Chile	PCR, phenotypic, Resistance population approach	Florfenicol, erythromycin, furazolidone, amoxicillin, sulfisoxazole and trimethoprim.
22	Active surveillance / Columnaris diseases	Oxazolidinone/phenicol-resistant gene optrA[246]	Salmon fish China	Phenotypic, metagenome, resistome analysis	Florfenicol linezolid and chloramphenicol
23	-	ARGs- *Sul1*, *sul2*, *dfrA1*, *tetA*, bla_{TEM}, *Int-1* Sulfonamide and tetracycline resistant genes[156]	Aquaculture farms – Silver carp Israel	Metagenomics (16s rDNA); ARGs -qPCR; shotgun sequencing - Resistomes	Tetracycline sulfonamides β-lactamase
24	-	*sul*1, *sul*2, *flo*R, *str*A and *gyr*A[250]	shrimp hepatopancreas China	qPCR	Sulfonamide Quinolones
25	Active surveillance / *V. parahaemolyticus*[199]	-	*Oreochromis niloticus* (tilapia), *Labeo rohita* (rui) and *Penaeus monodon* (shrimp) Bangladesh		Ampicillin amoxicillin cefotaxime ceftriaxone
26	Heterotrophic / Commensal[185]	-	*common carp* Lithuania	Metagenome; Phenotypic	Tetracycline

This method is very useful for determination of resistant population among the heterotrophic population in the aquaculture ponds, which in turn, will be useful for the selection of antibiotics for therapeutic purpose or in-directly to interpret the usage of antimicrobials in the ponds. Resistance population approach on tetracycline, sulfonamide and florfenicol resistance carried out aquaculture ponds are depicted in table 2.

Culture-independent approach: The advancement in metagenomics helped in unravelling the role of the microbiome in a complex environment[224]. The culture-independent approach provides valuable information on the abundant diversity of the microbial populations that are non-culturable but play a vital role in the particular niche. The commonly used culture-independent approach is the detection of antimicrobial resistance genes (ARGs) by collecting the environment sample of gut micro-flora or sediment or water and these directly extracted meta-DNA from the sample are processed for the presence of ARGs. Determination of ARGs using a culture-independent approach from this meta-DNA can be assessed by PCR, PCR-cloning approach, qPCR approach, a meta-genomic approach using next-generation sequencing and microarrays.

PCR based AMR determination: PCR-based assays are simple and are being used in most of laboratories to detect the ARGs. Single or multi-plex PCR assays were developed to detect ARGs such as ESBL, CRE, aminoglycosides, chloramphenicol, quinolones or macrolides[37,51,78,133,147,148,151,157,167,168,169,211,220,227,234,235]. However, new resistance determinants are being discovered every year with variations in the mechanism within the classes of resistance and to date over 923 ARG sequences have been reported [87, 126]. The method is highly encouraging on targeted list of ARGs from the meta-DNA or bacteria isolated from aquatic environment. However, the method becomes cumbersome for screening an array of ARGs which limits its usage in environmental DNA. Relatively easier to start and cost effective, but time constraint and positive control DNA are required for each confirmative step. Studies carried out in aquaculture ponds based on PCR confirmation of ARGs are depicted in table 2.

In PCR cloning approach, the target genes were amplified, cloned and sequenced based on the Sanger sequencing method. This approach also has same advantages and limitation like conventional PCR based method. This method is time-consuming and costly in terms of optimization, however, it is confirmatory as it involves sequencing. Real-time/qPCR-based approach with the target genes with probes or sans probes method is used for the quantification of copy numbers of ARGs in the sample and has various other applications such as spatio-temporal and time series changes in ARGs.

This method has the advantage of no requirement of positive control in probe based assessment, however, has the limitation in terms of the cost involved for processing the sample, establishment of infrastructure and for analyzing each gene. Studies carried out in aquaculture farms based on qPCR are depicted in table 2. Generally, qPCR studies are used for estimation of the abundance of ARGs, temporal-spatial variations in the ARGs, detection of ARGs, time series degradation of ARGs, comparison of ARGs diversity

between antibiotics treated/untreated ponds and between organs and so on. Several revisions were made in qPCR platforms with 96 wells to 384 wells for detecting over 50 ARGs and since 2016 it has been expanded to over 295/360 as array of genes of ARGs. Recently a smart chip was developed which can perform over 5000 reactions in an array for which ARGs were developed and are used in aquaculture ponds[140]. The same PCR-based method can be adapted for any cultured population of bacteria from aquaculture pond water, sediment and animal.

Microarray-based AMR determination: The second culture-independent method is the microarray-based genotyping of resistome. This approach can be used for genotyping of single culture with array of resistant genes or from the meta-genome. This approach is very rapid and the cost per sample of DNA is relatively low. Microarray slides are available as default or customized platforms. In 2005, 90 genes were customized on DNA microarrays[160]. Aminoglycosides (64 types), penicillin beta-lactamases (50 types), other -lactamases (17 types), amphenicols (40 types), trimethoprim (22 types), macrolide-lincosamide-streptogramin (MLSB) (52 types), sulfonamides (3 types) and tetracyclines (40 types) were identified using a personalised microarray developed by Roche NimbleGen[118].

Microarray based analysis also has wide applications viz. time trend, spatio-temporal assessment, however, the limitation of this method is infrastructure requirement for microarray scanner and analysing softwore for more targets, dependency of microarray slides developed for ARGs. The method has the possibility of expanding up to 2000 odd genes in a single run and also quantification.

Sequencing based AMR determination: The diversity analysis for taxonomic assessments was initially carried out by PCR-cloning and sequencing-based approach wherein the targeted resistant genes were selectively amplified, cloned and sequenced as amplicon sequencing. The limitation of this method for specific primer requirements for the large variety of resistant genes was overcome by whole metagenome analysis[42]. A recent breakthrough in sequencing strategies is the next-generation sequencer with advanced resistome analysis using meta-genomic data. Here, the meta-genome extracted from the sediment or animal gut microbiota is readily sequenced for the determination of the diversity of resistance genes as well as microbiome/ diversity/bacterial community structure.

In contrast to the microbial community's analysis using culture-dependent methods, metagenome analysis using high throughput sequencing-based analysis offers a detailed community structure that includes dominant flora as well as the diversity flora (HTS) and the data can be statistically compared between the samples. Even though, this method offers the better understanding on the resistome targeted as well as emerging or novel ARGs. The biggest limitation of this method is involvement of millions of dollars for the establishment of the infrastructure and comparative analysis/ storing of data requires expertise and space. There is a considerable reduction in the analysis charge in the last decade and establishment of numerous private laboratories for outsourcing the analysis may outcompete the limitation soon.

Fifty-eight resistant genes for 11 antibiotics in aquaculture sediments were determined using the metagenomic method[241]. Location of the majority of these resistance genes was identified on Mobile Genetic Elements (MGE) *viz*. transposons and plasmids of human pathogens attributing to the mobility of the resistance genes[29]. Further research is needed to establish the direction of resistance transmission whether it is from an aquatic environment to humans or vice versa[67,223].

Beneficial bacteria bear transmissible ARGs and the resistance has been observed in them as well [50, 228]. Metagenomics is a valuable method for figuring out the intricate processes at work in the host-environment relationship. AMR bacteria and ARGs are studied using both targeted (culture-based and quantitative PCR) and non-targeted methods (metagenomics)[204,248]. However, depending on the environment, only around 1–10% of bacteria can be cultivated[223]. As a result, using a combination of culture-based approaches and a culture-independent approach to investigate the environmental resistome may be the most effective tool[69,107,171,242].

ARGs are primarily located on plasmids and integrons[3,27,56,89,163-165]. A combination of the metagenomics and meta-transcriptomic analyses may unravel the active population carrying these resistance genes in the environment and may provide evidence for the hypothesis that the presence of antibiotics at sub-MIC concentration favours the development of resistance in the environment[115]. Detailed investigations on efflux pump mediated resistance and enzymatic degradation of antibiotics in individual bacteria of human health importance are available elsewhere[109,236]. Forty-four environmental samples from two monoculture freshwater aquaculture farms and four integrated farms (two duck and fish farms, two laying duck and fish farms) in Guangdong, were China, analyzed using high-throughput sequencing-based metagenomics and network analyses revealed that relative abundance of ARG and MGE subtypes in the integrated (fish and duck/laying duck) farm samples was significantly higher than those in samples from monoculture freshwater aquaculture farms. In particular, integrated farms had higher overall relative abundance of both mobile colistin resistance genes *mcr* variants and tigecycline resistance gene *tetX* variants than monoculture farms[239].

The effect of feed-administered antibiotics on the composition and metabolic ability of the gut microbiome in the European seabass was studied using quantitative PCR (to calculate bacterial copy numbers and amplicon sequencing

of the 16S rRNA gene). Antibiotics had a different effect around the gut, demonstrating distinct impacts on these microbial niches. Despite the antibiotic intake, a large portion of the European seabass gut microbiome remained, suggesting high stability to perturbations[94]. Using a metagenomic approach, *Piaractus mesopotamicus* was used as a model to assess the impact of the antimicrobial florfenicol on the diversity of the gut microbiome, as well as ARGs and MGEs and it was discovered that ARGs and MGEs significantly increased during antibiotic exposure and that the pathogen profile in the fish's gut shifted[134].

The possible transmission of ARGs between *Acinetobacter baumanii* to *Klebsiella* and *Pseudomonas* was established and the requirement of framework on resistome based global epidemiology was indicated [71]. Functional metagenomics is a recent approach that has the advancement of determining unknown antibiotic-resistant genes in the environments having the ability to unravel thousands of resistances genes that have a similarity of more than 65% to the existing database[54,129].

Many platforms are now available for analyzing antitbiotic resistance genes and one such platform is Antibiotic Resistance Genes Database (ARDB)[114]. Other tools and software available for the determination of antimicrobial resistance from sequencing analysis data are sraX, ResistoXplorer, MEGARes, SARG, DeepARG, PRAP: Pan Resistome analysis pipeline[5,43]. These tools offer a resistome diversity analysis from a variety of sequence data output.

Considering the importance that the majority of the aquaculture ponds and or animals and its environment have been identified with tetracycline and sulfonamides as a major resistant determinant along with minor variations in the class of the antimicrobial resistance genes identified as depicted in table 2, any study concentrating in the aquaculture farms and their environment should focus on resistant determinants for tetracycline, sulfonamides, aminoglycoside, quinolones, macrolides and β-lactams antibiotics. In particular, the studies concentrated on finfish aquaculture should look for tetracycline, sulfonamides and florfenicol resistances.

Molecular mechanism of antimicrobial resistance: Resistance to antimicrobial agents in the bacterial population is an evolving response to protect themselves from killing or inhibition. The main mechanisms of action of antibiotics in bacteria are inhibition of cell wall synthesis, protein synthesis, DNA synthesis and metabolic pathways.

In general, resistance to antimicrobials in bacteria arises out of three major mechanisms i.e. preventing the entry of the antibiotics to the bacterial cell, efflux the entered drug outside of the bacterial cell by pump mechanism and directly acting on the antimicrobial compounds either by degradation or modification of cell membrane for permeability by mutation and thereby making them inactive to the target[105]. These resistance mechanisms in bacteria may be intrinsic/natural or due to acquiring resistant genetic materials by horizontal or vertical transmission.

Intrinsic resistance refers to the inherent properties of a microorganism that limit the action of antimicrobials e.g. the permeability barrier of the outer membrane (OM) and constitutively expressed drug efflux pumps[9]. Intrinsic resistance arises due to the presence of resistant genes in chromosome, however, in acquired resistances, the genetic material for resistance is transferred through different mobile genetic elements such as plasmids, phages, transposons and others like integrons, insertion sequences between species of genera and at a lower frequency between different genera[6]. In general, Gram-negative bacteria are more intrinsically resistant than Gram-positive due to the presence of outer membrane, lipopolysaccharide (LPS) and modified lipo-oligosaccharide (LOS).

Because of their hydrophobic nature which prevents macrolides from entering their outer membranes, Gram-negative bacteria are naturally immune to them. Anaerobic bacteria are naturally immune to aminoglycosides because the transfer of aminoglycosides into the cell requires energy from oxidative metabolism, which these bacteria lack.

In general, the resistance of Gram-negative bacteria was common for tetracycline, aminoglycosides and cotrimoxazole; and recently towards the β-lactams and fluoroquinolones[161]. However, in the Gram-positive bacteria, the resistance to β-lactams is very minimal compared to the Gram-negatives where the ESBL and CRE are increasing globally.

There is a knowledge gap regarding the prevalence of antimicrobial-resistant bacteria in various aquaculture settings, AMR in the fish value chain, the transmission of resistance between aquaculture bacteria and clinically relevant bacteria under field conditions and the epidemiology of AMR in different countries' intensive aquaculture zones[131]. Even the integrated aquaculture systems are reported to be reservoirs for AMR genes[73,143,153,161].

Molecular targets for cell wall synthesis resistance: β-Lactam antibiotics and glycopeptide are bacterial cell wall synthesis inhibitors which typically exert their action on the peptidoglycan layer[48]. The enzymatic degradation is very common in Gram-negative bacteria and the modification of PBP is more common in Gram-positive cocci[48]. β-lactamases has been characterized into 4 molecular classes (C, A, D and B and their sub-classes)[24-26,176,184]. Screening of β-lactamases, extended-spectrum β-lactamases and carbapenemases producers in both Gram-positive and negative bacteria is performed by disk diffusion assay using penicillin, cefoxitin, cephalosporins, extended-spectrum cephalosporins, monobactams and carbapenem discs along with clavulanic acid, EDTA, tazobactam and boronic acid.

This is further confirmed by performing a double-disk synergy assay and quantifying the MIC for ceftazidime and cefotaxime with and without clavulanic acid. Carbapenem resistance is confirmed by performing MIC with carbapenems as per CLSI or EUCAST guidelines (CLSI M100 document in comparison to M2 and M7 for performing DDA and MIC and interpretation). The antimicrobial resistance in *Enterobacteriaceae* in gold fish was reported without ciprofloxacin resistance[174].

Among the glycopeptide antibiotics, only vancomycin and teicoplanin are used in the treatment of Gram-positive infections. The glycopeptide antibiotics bind to d-alanyl–d-alanine (d-Ala–d-Ala) cell wall peptidoglycan precursors and inhibit cell wall synthesis[92]. Genes for glycopeptide resistance are present either in the plasmids or on the chromosome. For detection of vancomycin resistance, only MIC method is recommended, however, for teicoplanin both DDA and MIC are in rule.

Molecular targets for protein synthesis resistance: Antibiotics target the prokaryotic ribosome and interfere with protein synthesis. The 30S ribosomal subunit that monitors the correct base pairing between the mRNA codon and the anticodon of the aa-tRNA is the target for tetracyclines and aminoglycosides whereas macrolides, lincosamides, oxazolidinones and streptogramins exert their action on the 50S ribosomal subunit[111].

Resistance to amino-glycosides is mediated by four mechanisms[123,166,198,210,221,240]. More than 300 Aminoglycoside-modifying enzymes (AMEs) are involved in modification of aminoglycosides at amino or hydroxyl groups, thereby reducing their binding activity at the ribosome. These enzymes are categorized into AG *N*-acetyltransferases, AG *O*-nucleotidyl transferases, AG *O*-phospho transferases and are highly mobile through MGEs[178,179]. The next group is the Ribosomal methyltransferases (RMTases)[36,46,58].

Bacteria acquire tetracycline resistance by way of acquiring genes responsible for tetracycline-specific efflux pump, ribosome protection protein and tetracycline degrading enzymes. The protein responsible for ribosome protections dislodges tetracyclines from the binding site. Several *tet* genes were reported in tetracycline-resistant bacteria isolated from Chilean salmon, fish farms in Korea, cultured yellowtail in Japan, Danish fish farms, Vietnam catfish farms, fish farms in Thailand, fish farms in China[3,49,60,88,91,130,146,207]. Class1 integrons harbouring resistance gene cassettes *ant(3")Ia*, *aac(6")Ia*, *dhfr1*, *oxa2a*, and/or *pse1* and *tet* genes were detected in *Aeromonas* sp isolated from fish farms[82].

The Macrolide – Lincosamide – Streptogramin and Ketolide (MLS_BK) antibiotics have a shared binding position in the 50S unit of the prokaryotic ribosome; hence the resistance developed against any one of the antibiotics quickly confers cross-resistance to others. MLSB resistance occurs due to 3 mechanisms viz. enzymatic modification, efflux pump and enzymatic degradation[208]. Based on the sequences upstream to *erm* genes, they are constitutive or inducible by erythromycin in nature[57]. Another form of cfr methyltransferase mediated methylation in 23S rRNA produces a broad spectrum of resistance to phenicols, lincosamides and oxazolidinones. Over 38 *erm* genes were identified[57,230].

Chloramphenicol inhibits protein synthesis by binding to peptidyl transferase of the 50S ribosomal subunit of 70S ribosomes of bacteria[192]. Hence, resistance occurs by enzymatic inactivation, efflux pump systems, resistance to permeability of the drug and mutation of the target site[18,21,136]. *floR* gene was detected in tetracycline-resistant bacteria isolated from aquaculture farms in Asia and fish farms in Africa[38,80]. Plasmid-mediated florfenicol resistance was reported in bacteria isolated from salmon fish farms and catfish[66,231].

Molecular targets for Folate pathway resistance: Antimicrobials sulfonamide and trimethoprim interfere in the folate pathway and hence, resistance to sulfonamide occurs due to mutations in the enzyme and acquiring alternative genes of DHPS resulting in low-affinity for sulfonamides[90,159,181,201,202,233].

Molecular targets for Nucleic acid synthesis: Quinolone resistance occurs by different mechanisms mostly encoded within mobile genetic elements and to a lesser extent within chromosome viz. mutation in target site topoisomerase II, altered expression of porins, efflux pump and target protection[77]. The major resistance mechanisms identified in Gram-negative and positive bacterial species were *gyrA*, *grlA*, *gyrB*, *parC*, *qnr* alleles, *qepA*, *oxqAB*, aac(60)Ib-cr 4)[52,83,116,162,183,237] and were reported in bacteria from aquaculture[75,76,243].

Rifampicin or rifampin is the antibiotic that acts on RNA synthesis and its resistance has been reported in *Mycobacterium tuberculosis*, *E. coli and S. aureus* due to mutation in the RNA polymerase and hence the reduction in the binding ability of the antibiotic [64, 200, 247]. Efflux of antibiotics by the bacteria is mediated mainly by 5 transporters or efflux pumps *viz*. Major Facilitator superfamily (MFS), Small Multidrug Resistance (MDR) family, ATP-Binding Cassette superfamily (ABC), Resistance-Nodulation-Cell Division superfamily (RND) and multidrug and Toxic Compound Extrusion family (MATE)[108]. These efflux pump systems are not only used for the effluxing of anti-bacterial but are also involved in other functions *viz*. the influx of ions and nutrients, effluxing of metabolic end-products and toxic compounds and communication between cells and the environment. The transporters ATP and MFS are large family single-component transporters across the cytoplasmic membrane / inner membrane.

The MATE, SMR and RND are very small family transporters or multi-component transport across the cytoplasmic membrane, outer membrane channel protein and periplasmic fusion proteins. In general, Gram-negative bacteria are inherently more resistant to antibiotics due to the presence of outer membrane barrier and possession of broad-specificity multidrug efflux pumps[144].

MATE – *BexA, NorM, YdhE, VcmA, MepA* - fluoroquinolones, polymixins, kanamycin and streptomycin (Aminoglycosides and Fluoroquinolones);
MFS – *BcrA, EmrAB-TolC, EmrKY-TolC, EmrD, Dep, MdfA/Cmr/CmlA, SmvA-OmpD, YddG-OmpD, VceAB, NorA, QacA/QacB, Bmr and Blt, LmrP* - nalidixic acid, tetracyclines, thiolactomycin, bicyclomycin, chloramphenicol, puromycin, rifamycin;
ABC - *MacAB-TolC, LmrA* pumps - macrolides; MDR transporters -*EbrAB, QacC/Smr, SepA, EmrE, SugE, TehAB* - disinfectants and antiseptics; and RND transporter - *AcrAB-TolC, FarE, MexAB-OprM* systems - β-lactams, aminoglycosides, chloramphenicols, fluoroquinolones, tetracyclines, sulfonamides and others[23,93,134,170,188,191,245].

The outer membrane porins in Gram-negative bacteria favour the passage of hydrophilic antibiotics into the bacteria and are very specific to a group of bacteria (*OmpF, OmpC, OprP, OmpF and OmpE*). Loss or modification in the porins facilitates the resistance to antibiotics called "porin mediated resistance" viz. *OprD* in *P. aeruginosa* to imipenem/ meropenem; *OmpF* for multidrug-resistance[65,95,117].

Mobile Genetic Elements (MGE) in resistance development: A segment of DNA which encodes for proteins mediating the transfer of DNA intracellularly within the genome and intercellular between bacterial cells along with the enzymes are known as "Mobile genetic elements (MGEs)"[55]. The transfer of DNA between bacterial cells occurs in three forms in prokaryotes viz. transduction, conjugation and transformation whereas the transfer or mobility of the DNA within the cell happens by transposons, which randomly recombine or 'jump' between replicons and also 'hop'onto phages or plasmids. When the transfer happens onto the phage DNA or plasmid, then once again the probability of intercellular mechanism of transfer arises. MGE has transposases and site-specific recombinases which involve in catalyzing within the cell movement of MGEs which favors the homologous recombination in the host genome and also deletion or insertion or rearrangements[2,106,154].

Integrons is a fragment of DNA with an integrase gene (*intI*) and a proximal primary recombination site (*attI*). Based on the amino acid variation, the *intI* gene is classified as *intI1, intI2, intI3 and intI4.* The *intI* favours or catalyzes the recombination of the genes between *attI* and *attC* sites. These carry the resistance genes for antibiotics, antiseptics and disinfectants. Tn21 type integron were highly linked to *sul1* gene-mediated resistance, however, the *sul2* mediated resistance was by small plasmids of the *IncQ* family (RSF1010) and also on plasmids of another type represented by pBP1[222]. The possible role of class I integrons (Tn21-like transposons) in the spread of the antimicrobial resistance through food was established and the aquatic food products mostly from aquatic environment role generally cannot be disregarded[61].

Soil integron / cassette richness was elaborated in diversity study in Australia, over 4000 cassettes were estimated[62]. The role of integrons in the transmission of resistance as well as virulence and their possible benefits of using as biotechnological tools were elucidated[63].

Guidelines to be followed for phenotypic determination of AMR: AMR determination in aquaculture settings by susceptibility testing was being carried out since 1990's. However, there exists a huge variation in the employment of standard operating procedure in determination of AMR [127]. Shortcomings need to be avoided due to the adverse impact of antimicrobial resistance in terrestrial and aquatic animal pathogens[193,203,217]. No significant variation was occurring in susceptibility testing measurements for *V. vulnificus* and *V. anguillarum* at 24-28h and 44-48h as a result of inter-laboratory validation[15]. The steps involved in the harmonization of procedure by CLSI are elaborative [229]. Approaching AMR in aquaculture settings is a triangular approach which can be addressed as per figure 2.

CLSI Vet04Ed3, 2020 deals with the aquatic animal pathogens such as *Aeromonas salmonicida, Aeromonas hydrophila, Flavobacterium columnare, Flavobacterium psychrophilum*. Other pathogens viz. *Vibrio* sp., *Photobacterium damselae*, Gliding bacteria, *Streptococci* and other few fastidious bacteria shall be taken from CLSI, Vet03A, 2006 and commensal flora can be adapted either from CLSI M100, M02, M07 and CLSI M45 for the infrequently isolated or fastidious pathogens CLSI Vet04A, 2006.

The recent interpretation guideline is based on Normalized Resistance Interpretation based on disk diffusion or minimum inhibitory concentration for the determination of epidemiological cut-off for the environmental bacteria which is more appropriate instead of using the Clinical standard breakpoints based on hospital pahogens[96-98]. Increasing the data on these epidemiological cut-off for the environmental bacteria will accumulate useful data on bringing guideline separately on epidemiological cut-off value.

To improve the quality of the AMR data from the aquaculture settings and the reproducibility of the data across the laboratories, certain guiding principles were proposed[31,32,203]. The following points are necessary: adherence to the standard protocol (SOP); uniformity of interpretative criteria followed for particular group or species of bacteria to avoid ambiguity in the data generated;

compulsory use of quality control strains, strict adherence to incubation time and temperature; data generation of epidemiological cut-off values for aquatic animal pathogens from local laboratories across the globe for arriving at a consensus epidemiological cut-off distinguishing wild type from non-wild type bacteria; appropriate use of terminologies; sharing raw data of the antibiotic susceptibility test in the public domain for easy comparison and arrival of breakpoints for aquatic animal pathogens and interpretation using non-clinical breakpoints

Trends in the determination of antimicrobial resistance across the sector: The first detection of AMR in the human health sector was in the 1940's after penicillin and sulfonamide release into the therapy[40]. Likewise, the first detection of AMR in food animals was reported in the 1950's for streptomycin and tetracyclines in Turkey[119,206].

In aquaculture settings and environment, the first detection of AMR was in the 1980's[4,8]. The infrastructure required for the determination of AMR in the human health sector was more significant compared to the terrestrial and aquatic animal food production sectors.

Recently, more attention has been given to animal, food and environment as an important component of the development of antimicrobial resistance and in the next decade due to the advancement of testing infrastructure in these sectors may show more detection rate of antimicrobial resistance that has the potential to surpass the human health sector due to the increase in the projected use of antibiotics in terrestrial food-producing animals (174,549 tons) and aquaculture (13,600 tons) by 2030. Detailed informations on AMR in each sectors including fisheries, environment, food and molecular fingerprinting strategies and individual organism wise AMR informations may be sought elsewhere[12,103,135,175,180,189,215,216,218,219].

Conclusion

There is increasing evidence of AMR emergence in the aquatic environment that includes aquaculture and thereby acts as a sink or mixing vessel for further transmission to the human or animal health or food chain. Hence, foregoing the aquatic environment in tackling the AMR menace is not appropriate. AMR surveillance in aquaculture has to be approached using a triangular strategy focusing on commensal flora, aquatic animal pathogens and human pathogens. Molecular tools remain very indispensable tools for understanding such a complex system, but should always be used in tandem with the conventional population-based approach, thereby deciphering the real-time picture of antimicrobial resistance.

However, understanding the direction of transmission of resistance between populations of bacteria in the environment can only be possible after establishing the real-time measurements over some time. In the coming year, advancement in the sequencing tools will provide a very fine-tuned picture to devise and adapt better AMR control strategies. This is very important in meeting the food and nutritional security needs of the increasing population and also for a healthy environment.

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