A Review on Antibiotic Usage and Antibiotic Resistance in Shrimp Culture; Asian, South Asian and Sri Lankan Scenario

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Abstract

Asian and South Asian regions contributed to aquaculture and shrimp farming with a major proportion, with a rapid increase in production during the past two decades. Although production experienced exponential growth, farming practices and production were affected by the occurrence of diseases. Antibiotic drugs in shrimp farming were practiced as an alternative to combat the diseases that have affected shrimp production to a greater extent. However, the misuse and overuse of antibiotic drugs continued to be practiced as a prophylactic or metaphylaxis measure and as a treatment option. Antibiotic resistance has become an alarming global issue owing to the overuse and misuse of therapeutic antibiotics around the world. Due to the irrational use of antibiotics in the shrimp farming, shrimp industry has become one of the most prominent industries that harness antibiotic-resistant genes to human health. Although surveillance and monitoring programmes are being implemented to monitor antibiotic usage in food animal production, the burden of resistant gene occurrence in shrimp farming is still high. The occurrence of resistant genes in food animals can easily be transferred to human bodies causing antibiotic resistant bacteria in human health. Numerous evidence from literature in Asian, South Asian and Sri Lankan studies suggest the presence of antibiotic drug residues and adverse effects of antibiotic resistance at an alarming level for shrimp farming industry, and human health.

Keywords: Antibiotic resistance; antibiotic usage; shrimp farming

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Introduction

Aquaculture is the main contributor to global fish consumption, and more than 70% of such aquaculture production is from the Asian and South Asian regions (FAO, 2019). Shrimp culture has expanded rapidly over the past two decades in South Asia due to favourable environmental conditions in the region. Shrimp culture has given rise to numerous employment opportunities as well as capital for the country; whereas negative impacts on the natural environment have also been detected within the past decade (Henriksson *et al.*, 2018). The brackish water shrimp farming systems have been categorized as traditional extensive, semi-intensive, and intensive farming systems. The major shrimp species involved in the brackish water farming system is the *Penaeus* shrimp (Thuy *et al.,* 2011). Shrimp are heavily traded commodities and represent the second main group of exported species in value terms (FAO, 2018). Shrimp is cultured worldwide in terms of international trade. The world production of farmed shrimp reached six million tons in 2018 (FAO, 2020).

Infectious diseases are a major health concern in shrimp culture due to poor water quality maintenance and the need for antibiotics and other chemical products to manage the disease emergence. Thus it has become a major issue in the culture-based shrimp industry in Asia (Heal *et al.*, 2021).Proper management aspects are needed in shrimp farming practices to mitigate the impact of diseases to the economic benefit and to protect cultured stock from diseases. Management practices to control infectious diseases in shrimp farming include; hygiene maintenance, effective prevention of common infectious diseases, enabling biosecurity measures, supplementation of dietary supplements such as vitamin C to enhance immunity in cultured animals, and probiotics and prebiotic usage to support the healthy growth of stocks (Manage, 2018). The continuous impact of disease risk involves losses of shrimp stocks; at which stage usage of antibiotic agents to treat such infections came into concern. Antibiotic usage is needed to be monitored and surveillance methods are to be established to control the overuse and misuse of recommended antibiotics. As no specific antibiotic agents are specially designed to be used for aquaculture and shrimp farming, veterinary drugs recommended for other livestock are used for shrimp culture practices. However, a few veterinary medicines that are licensed in aquatic animals are allowed for human consumption, and such medicines still need certain withdrawal periods before human consumption (Honda *et al.,* 2016). Permitted antibiotics that are recommended to be used in shrimp culture vary from different geographical areas in the world. However, from a South Asian perspective, India, Bangladesh and Bhutan have established policies and legislations to develop and monitor antibiotics for food animal production (Allcock *et al.,* 2017). These documents and legislations emphasize the procedures for the establishment of permissible Maximum Residue Limits (MRL) of pharmacologically active substances in food commodities of animal origin (Okeke *et al.*, 2005). Governments of each country are responsible for monitoring the MRL levels for each permitted antibiotic or veterinary drug to ensure food safety for human consumption.

Frequent usage of antibiotic agents as a prophylaxis measure or a treatment, in an abusive way, causes major impacts on human health. Cabello, 2006 suggests that the heavy use of antibiotics in aquaculture causes serious problems to human health and the environment. Heavy use of antibiotics in shrimp farming can cause residual effects that may be retained in the sediments and the water bodies; causing antibiotic residue accumulation in food animals and surrounding water bodies. The presence of residual antibiotics even in lower doses in food animals such as shrimp can pass through food chains and bio accumulate within human bodies in larger quantities causing toxic effects on human health (Sarmah *et al.*, 2006). The persistent usage of antibiotics in food animals can cause antibiotic resistance; which is a huge threat to human health (Allcock *et al.,* 2017). The greatest associated risk of the use of inappropriate antibiotics is the development and spread of Antibiotic Resistant Genes (ARG) and antibiotic resistant bacteria (Martínez *et al.,* 2015). Once the antibiotics are being used, the residual antibiotics can remain in shrimp farms affecting the natural microflora of the farm. ARG may either accumulate in aquatic bacteria and food animals that are farming, and they possess the ability to transfer within genes via horizontal gene transfer technique or reach human pathogens causing resistance among zoonoses (Cloeckaert *et al.,* 2017). ARG in human health can lead to significant issues, including reduced effectiveness of existing therapeutic treatments(Martínez *et al.*, 2015).Certain banned antibiotics in shrimp farming, such as chloramphenicol and nitrofurans, and banned dye residues that are used as antimicrobial compounds such as malachite green result in slowdown imports, causing huge economic losses for the producers as well as for the government (Food and Agriculture Organization, 2020).

The worldwide situation of antibiotic use in shrimp farming is worrisome. Shrimp aquaculture, heavily involved in international trade and closely linked to aquatic ecosystems, may play a role in spreading Anti-Microbial Resistance (AMR). Despite efforts to decrease antibiotic usage, significant challenges remain, particularly in major shrimp-producing nations such as China, India, Indonesia, Thailand, and Vietnam. Ongoing monitoring and action are crucial to maintaining sustainable and safe shrimp production (Thornber *et al.,* 2020; Ibarra *et al.,* 2022).

The Joint Food and Agriculture Organization (FAO)/World Organization for Animal Health (OIE)/World Health Organization (WHO) Expert Meeting on Antibiotic Use and Antibiotic Resistance in Aquaculture identified that the two hazards to be considered are antibiotic residues and antibiotic resistance (AMR) (FAO/OIE/WHO, 2006). Measuring the usage of antibiotic data in food animal production can address different objectives; to monitor Anti-Microbial Usage (AMU) over time, to settle benchmarks to promote the reduction of AMU, and to assess possible associations between AMU and AMR (Cuong *et al.*, 2018).

Antibiotic agents usually lose their effectiveness over time due to the emergence of resistant pathogens. This phenomenon, when a particular species is resistant to almost all classes of persistent antibiotics, they are known as superbugs (Krishnasamy *et al.*, 2015). They can be either extreme drug-resistant or completely drug resistant. However, regulation and monitoring of veterinary drug usage over food animals requires sensitive and selective methods to analyze the present antibiotic residues and to ensure food safety among the general public. To date, there is no globally widespread system available to monitor the usage and circulation of antibiotic agents in shrimp farming.

Antibiotic usage in Asian countries

Farmed shrimp production has always been dominated by Asia, which contributed 85% of the world's production in 2018. Shrimp production in the Asian Region is mainly concentrated in countries such as China, Indonesia, Vietnam and India in 2018, and mainly targeted the export market (FAO, 2020). Due to the elevated shrimp production in the Asian region, antibiotic agents are considered high in these regions to prevent diseases and maintain healthy brooder stocks (Tollefson and Miller, 2000). The most common route of administration of antibiotic agents in shrimp culture is via the oral route. However, they may also be administered via injection or as a bath to the species reared. Common antibiotics such as aminopenicillins (amoxicillin, ampicillin), sulphonamides, and quinolones (oxolinic acid) are usually administered via the oral route. Tetracyclines (tetracycline, oxytetracycline), and macrolides (erythromycin) are administered during an infection orally or as a bath (O'Neil, 2014; WHO, 2014).

According to the literature, a considerable fraction of antibiotic residues are found in water resources from aquaculture in several studies conducted in India, Bangladesh, Indonesia, Vietnam and Thailand (Tollefson and Miller, 2000; Sandu *et al.,* 2017). Erythromycin and tetracycline were detected in water bodies of aquaculture up to 180 ng/L from Thailand (Von Baum and Marre, 2005), and fluoroquinolones were detected from fish and shrimp samples in Vietnam (~12 ng/L) (WHO, 2014).

A study conducted in Vietnam on shrimp culture in mangrove areas suggests the presence of norfloxacin and oxolinic acid, trimethoprim and sulphamethoxazole, which are named critically important and highly important antibiotic agents for human health by WHO (Le *et al.,* 2005). Antibiotics in human medicine have been categorized into three categories; as critically important, highly important and important based on their importance to human medicine (WHO, 2018). The WHO list of medically significant antimicrobials for human health (WHO MIA list) serves as a risk management instrument in making decisions to reduce the antimicrobial usage in sectors other than human on the development of antimicrobial resistance in humans (WHO, 2024).

Another study conducted in Vietnam discusses the availability of trimethoprim (TMP), sulfamethoxazole (SMX), norfloxacin (NFXC) and oxolinic acid (OXLA) in water and sediment samples in shrimp ponds in mangrove areas. Results emphasize the availability of the above antibiotics in all samples of both shrimp ponds and surrounding water canals. The highest concentrations of TMP, SMX, NFXC and OXLA are 1.04, 2.39, 6.06, and 2.50 ppm in water samples; and 734.61, 820.49, 2615.96, 426.31 ppm (based on wet mud weight), in sediment samples respectively (Le and Munekage, 2004) as shown in Table 1. Among the above antibiotic agents, TMP and SMX are known as highly important and NFXC and OXLA are quinolones, and critically important antibiotics to human health, according to WHO standards, they are not recommended for non-human use such as food, fish and aquaculture production (WHO, 2005).

Table 1. Range of antibiotics concentration (ppm) (Le and Munekage, 2004)

Specimen	Type of antibiotic	Minimum level (ppm)	Maximum level (ppm)
Water from the surface layer	TMP	0.08	1.04
	SMX	0.04	2.39
	NFXC	0.06	6.06
	OXLA	0.01	2.50
Water from the bottom layer	TMP	0.08	2.03
	SMX	0.04	5.57
	NFXC	0.08	4.04
	OXLA	0.01	2.31
Wet bottom layer mud (5cm depth in the upper layer)	TMP	9.02	734.61
	SMX	4.77	820.49
	NFXC	6.51	2615.96
	OXLA	1.81	426.31

Another study conducted in Vietnam by Tran *et al.,* (2017) discusses the use of up to 20 different antibiotics in shrimp farming and fish culture in Vietnam, including tetracycline, oxytetracycline, ampicillin, rifampin, chloramphenicol, enrofloxacin, linomycin, ciprofloxacin and sulfamethoxazole/trimethoprim. However, the authors declare that only one shrimp farmer was reported to be using antibiotics; and the reported antibiotic was oxytetracycline. Authors also states that tetracyclines are antibiotics that farmers often use in shrimp culture for disease prevention and treatment, and is a highly important antibiotic for human health according to WHO antibiotic classification (WHO, 2018). Tetracycline has been used extensively in aquaculture for chemotherapy against fin rot and skin ulcers (Mortazavi, 2014).

A study conducted based on shrimp and fish-related aquatic environments in rural areas of Vietnam shows that 53 of the total 362 samples were positive for antibiotic residues. Thirty-nine out of total 362 (10.8%) samples tested, for antibiotic residues showed positive results for enrofloxacin. Enrofloxacin is considered to be banned in Vietnam shrimp culture since 2012, and authors describe the presence of enrofloxacin as well as ciprofloxacin; often considered as a metabolite of enrofloxacin (Nguyen *et al.,* 2015). Authors further described that trimethoprim and sulfamethazine or sulfamethoxazole are normally mixed in commercial aquatic feed, and are often detected as combinations (Uchida *et al.,* 2016).

Another study associated with antibiotic residues in retail shrimp purchases in Vietnam states that 22.5% (9/40) of total samples were positive for antibiotic usage. Antibiotics; tetracyclines (7.5%), fluoroquinolones (7.5%), sulfonamides (2.5%), and macrolides (2.5%) were detected and the shrimp purchased at supermarkets showed a higher AMU (50%) compared to shrimp purchased at street markets (13.3%) (Yen *et al.,* 2020).

Thuy *et al.*, 2011 describe the usage of antibiotics in several shrimp farming sites in Vietnam. Authors express that although oxytetracycline is the most widely used antibiotic for many years, however, quinolones and the combinations of sulfadiazine and trimethoprim have become more popular in Asian shrimp farming (Thuy *et al.*, 2011; Holmström *et al.,* 2003). For Vietnam, enrofloxacin was the most used antibiotic (43%), and norfloxacin (25%) of the farmers in the Mekong delta. In addition, they have also used sulfamethoxazole, Co-trimoxazole and trimethoprim (Thuy *et al.*, 2011; Nga, 2004). Authors divide the most common antibiotics that are used in Vietnam into five categories; (1) Fluoroquinolones (enrofloxacin, norfloxacin, ciprofloxacin, and oxolinic acid), (2) sulfonamides (sulphamethoxazole, sulfadiazine), (3) tetracyclines (oxytetracycline), (4) diaminopyramidines (trimethoprim, ormethoprim), and (5) unclassified (griseofulvin and rifampicin) (Thuy *et al.*, 2011).

Data from the Can Duoc District show the most commonly used antibiotics are sulfonamides in combination with trimethoprim. 73% of the interviewed farmers were using trimethoprim and sulfadiazine. The next most commonly used antibiotics are composed of sulfadimethoxine and ormetoprim as well as trimethoprim and sulfamethoxazole. In the Can Duoc District, the most commonly associated disease for shrimp is white spots and white faeces; norfloxacin and trimethoprim have been used to treat such diseases. More seriously, in the Can Duoc District, besides ciprofloxacin, rifampicin and griseofulvin are used for shrimp larvae; which are considered antibiotics for human use (Thuy *et al.*, 2011); Table 2.

A study conducted by Holmström *et al.*, 2003 on shrimp culture in Thailand shows that shrimp farmers use antibiotics as both therapeutic as well as prophylactic means. Authors also emphasize that the antibiotics are available in powder form, and the farmers either mix them with feed or throw them into water. The most common antibiotics the farmers used in shrimp ponds are; norfloxacin, oxytetracycline, enrofloxacin and different sulphonamides (Holmström *et al.*, 2003). Norfloxacin and enrofloxacin are quinolones and fluoroquinolones, and tetracyclines and sulphonamides are named critically important and highly important antibiotics respectively by WHO (WHO, 2005). The same study reveals farmers use up to 0.5-6 g/Kg of antibiotics, feeding three times a day per week (Holmström *et al.*, 2003).

Table 2. The most commonly used antibiotics in Vietnamese shrimp farming (Thuy *et al.,* 2011)

S/N	Commercial Name	Composition	Percentage of farmers	Usage
1	Ciprofloxacin 500mg	Ciprofloxacin	100%	Larvae
2	Cotrim	Sulfamethoxazole	8.7%	Post larvae- adult shrimp
3	Cotrim-La	Sulfamethoxazole, trimethoprim	N/A	Post larvae- adult shrimp
4	Daitrim	Sulfamethoxazole 10%, trimethoprim 2%	N/A	Post larvae- adult shrimp
5	Griseofulvin 500mg	Griseofulvin	100%	Larvae
6	N300	Norfloxacin, hydrochloride 30%	N/A	Post larvae- adult shrimp
7	Osamet	Sulfadimethoxine 25%, ormetoprim 5%	11.2%	Post larvae- adult shrimp
8	Prawnox	Oxolinic acid 25%	N/A	Post larvae- adult shrimp
9	Rifampicin 300mg	Rifampicin	100%	larvae
10	Romet 30	Sulfadimethoxin 25%	N/A	Post larvae- adult shrimp
11	Silva 54	Sulfadiazine, trimethoprim	N/A	Post larvae- adult shrimp
12	Sulfa-prim	Sulfadiazine, trimethoprim	21.74%	Post larvae- adult shrimp
13	TA-1 oxytetracycline	oxytetracycline	100%	Larvae
14	TMT	Sulfadiazine, trimethoprim	15.9%	Post larvae- adult shrimp

Antibiotic usage in Chinese fish and shrimp farming is studied by Liu *et al.*, 2017 and the authors describe 13 antibiotics are authorized to be used in China; doxycycline, enrofloxacin, florfenicol, flumequine, neomycin, norfloxacin, oxolinic acid, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, thiamphenicol, and trimethoprim. Authors have identified that 12 antibiotics that are used in fish and shrimp farming are not authorized; amoxicillin, chloramphenicol, chlortetracycline, ciprofloxacin, erythromycin, furazolidone, gentamycin S, oxytetracycline, penicillin G, streptomycin, sulfamerazine S, and sulfisoxazole(Liu *et al.*, 2017). Some antibiotics are used occasionally although they have been banned for usage over the years. For example, erythromycin was banned in 2002, but its usage was reported in 2012 (Bondad-Reantaso *et al.*, 2012).

However, compared to the antibiotic usage in other food animals, antibiotic usage in shrimp farming is considerably lower even in developed countries. In Vietnam, antibiotic usage was 1.44 g/ton of production, and it was 1.67 and 4.53 g/tons in China and Thailand respectively (FAO, 2020).

Antibiotic usage in South Asia

A study conducted based on shrimp culture in Kerala, Tamil Nadu, Karnataka and Andhra Pradesh of India describes the presence of antibiotic residues in collected samples. Residual Levels of antibiotics viz: chloramphenicol, sulphonamide, tetracycline, erythromycin, streptomycin and β-Lactams were determined, and authors were able to identify sulfonamides (35.017 to 97.81 ppb with an average of 56.91 ppb) and erythromycin (49.46 to 77.49 ppb and an average value of 61.12 ppb) in levels. The presence of chloramphenicol was detected at <1 ppb levels. The reported chloramphenicol residue concentrations were ranging from 0.1134 to 0.2398 ppb with an average of 0.1761 ppb. Streptomycin, tetracycline and β- lactam antibiotics were not detected in any of the collected samples (Swapna *et al.*, 2012).

Chloramphenicol was detected as an antibiotic agent in fish farms in Bangladesh (~5ng/L), and in shrimp farms in India (~32ng/L) and Indonesia (~45ng/L) (Allcock *et al.,* 2017, Von Baum and Marre, 2005, WHO, 2014) .

Another study conducted in India shows oxytetracycline and erythromycin residues up to 49 µg/L and 1.6 µg/L concentrations respectively from fish and shrimp farms. The same study reveals oxytetracycline was frequently detected in sediments in shrimp farms with concentrations up to 6908 µg/Kg (Manage, 2018;Koeypudsa *et al.,* 2010).

Antibiotic usage in Sri Lanka

According to the authors' knowledge, there is limited data available on antibiotic usage in aquatic food animal farming in Sri Lanka. This may be due to the unavailability of proper monitoring and documentation system to track the usage levels of antibiotic agents in fish and shrimp culture.

The higher usage of tetracycline and Oxy-Tetra-Cycline (OTC) in a study conducted using 16 selected aquaculture farms including shrimp farming. The effluent water in all farming sites except Dambulla, Muthupanthiya and Udappuwa was positive for the usage of tetracycline and oxytetracycline. Detected OTC levels in shrimp hatcheries were 0.056±0.001 µg/mL - 0.234 ± 0.014 µg/mL (Manage, 2018). Authors suggest that high tetracycline levels were detected in shrimp hatcheries (0.012±0.019 µg/mL - 0.112± 0.017 µg/mL). WHO recommendation for tetracycline in aquatic environments is less than 0.001 µg/mL and less than 0.1 µg/mL in soil (Liyanage and Manage, 2019; O'Connor and Aga, 2007). The study found lower levels of erythromycin concentrations (~0.001µg/mL) whereas penicillin and sulfonamides were not detected (Liyanage and Manage 2016). Another study conducted by Munasinghe *et al*., (2012) describes the usage of oxytetracycline in 15% of the total shrimp farms (92/603) in Puttlam District, Sri Lanka.

Being a country that exports cultured fish, shrimp and fishery products to the international market, especially to the EU, the Ministry of Fisheries and Aquatic Resources Development has a gazette of the Aquaculture (Monitoring of Residues) Regulations 2002" under the Fisheries and Aquatic Resources Act No 2 of 1996. Since then, an "Annual Residual Monitoring Plan" for exports of cultured fish, shrimp and fishery products has been under implementation by the Department of Fisheries and Aquatic Resources which is the competent authority for the export of fishery products from Sri Lanka.

Development of antibiotic resistance

Shrimp farms once operated in intensive farming systems with elevated stocking densities, extended farming periods, poor sanitation and poor biosecurity measures enhance the ability of a farming system to acquire diseases and spread rapidly over populations (Walsh, 2000). Rapid disease spread causes the entire shrimp farm to contaminate, and once disease outbreaks happen, the requirement for antibiotic agents to treat the prevailing disease condition and prevent the spread of the current outbreak arises. Antibiotic agents are used in shrimp farming and aquaculture both as a treatment method, as well as a prophylaxis measure. Not all the administered antibiotics can be absorbed and utilized by shrimps and fish in the farm. Studies suggest that up to 80% of applied antibiotic agents are excreted through the urine and faecal matter of such animals without complete decomposition (Blair *et al*., 2014). Accumulation of such excretory matter, sometimes along with feed components if they were fortified with antibiotic agents as a therapeutic or prophylactic measure, can give rise to aquatic microorganisms which are resistant to the exposed antibiotics over an extended period (Allcock *et al*., 2017). AMR in microorganisms has primarily developed due to the selective pressure exerted on aquatic microbial populations with the presence of antibiotic agents. Antibiotic agents act on microbes in several ways; they interrupt the cell wall biosynthesis of microorganisms, and they either interfere with bacterial protein synthesis, DNA replication and repair or other cellular mechanism parameters (Walsh, 2000). However, microorganisms develop mechanisms to withstand the selective pressure of antibiotics and evolve into a more resistant community to antibiotic agents when they are utilized frequently. These developed mechanisms are; enzyme production to inactivate antibiotics, alter the target site of action for antibiotics, alteration of metabolic pathways, change of permeability of antibiotic to outer cell membrane and presence of effluent pumps (Walsh, 2000). Resistant microorganisms may exhibit one or more mechanisms to overcome antibiotic activity on them and may be resistant to one or more classes of antibiotics (Blair *et al*., 2014). Once the AMR gene is acquainted within the bacterium with a single mutation, the mutant gene starts to withstand the selective pressure by antibiotics, by surviving while all the sensitive bacteria are killed; which makes the mutant gene survive successfully and replicate throughout the population and become dominant. This replication causes the bacterial strains to evolve gradually and emerge as AMR bacteria with time (Blair *et al*., 2014).

Microorganisms in a shrimp farming system act as an enormous pool of genes in ecosystems where they can acquire resistant genes with prolonged antibiotic exposure. This acquisition of resistant genes is occurred mainly via mobile platforms such as plasmids, transposons and integrons and can spread over different ecosystems (Davies, 1994; Courvalin and Arthur, 1993). Further, certain genes can be located on plasmids that can segregate within transposons. Such genes can cut their genes from one strand of DNA and transfer and fit into another locus of a DNA strand. This mechanism can transfer the AMR genes within a gene pool (Courvalin and Arthur, 1993). It takes a period from months to years for the AMR genes to emerge and clinically significant resistance to appear (Allcock *et al*., 2017).

The most important impact that occurred due to multiple antibiotic resistance genes is the reduction of effectiveness of existing antibiotic agents. Reduced effectiveness to the infectious disease-causing pathogens makes it brings the requirement of new effective antibiotics that would meet the ready effectiveness; however, the main problem is that there are no new classes of antibiotics reported in the recent past. What is currently being done is making alterations to existing classes of antibiotics (Silver, 2011), which may also continue to use their effectiveness unless they were used deliberately.

AMR in Asian and South Asian scenarios

Although AMR is a globally recognized concern, the burden of the problem in Asian and South Asian regions shows an upward trend with the excessive and irrational use of antibiotic agents in food animal production. Countries such as China, India, Thailand, Vietnam, the Philippines and Sri Lanka have demonstrated increasing trends in AMR problems (Bhatia, 2019). When a single bacterial strain become resistant to more than one antibiotic agent, it is called multiple antibiotic resistance (MAR). Numerous studies prove the presence of MAR in common bacteria associated with shrimp farming in Asia indicating the usage of antibiotic agents as prophylactic, metalactic and therapeutic agents. However, the alarming concern with antibiotic usage is that discovering the evidence to prove that the drugs that are not generally permissible for food animal production, such as chloramphenicol and trimethoprim have also been used in shrimp culture in Asia (Changkaew *et al.,* 2014). According to literature, numerous evidence has been found that MAR in the shrimp industry in Asian and South Asian scenarios. Examples of the evidence for antibiotic resistance in shrimp culture in Asian and South Asian regions are categorized in Table 3.

Table 3. Presence of multiple antibiotic resistance in Asian and South Asian Regions

Year	Country/ Origin of AMR	Species associated	Description	Reference
1973	Japan	*Vibrio anguillarum*	Sulfonamides,streptomycin, chloramphenicol, tetracycline resistance	Aoki *et al.*, 1974
1994	India	*Vibrio harveyi*	Hatchery strains were resistant to streptomycin, chloramphenicol, co-trimaxazole	Karunasagar *et al.*, 1994
1995	Thailand	*Streptococcus* and *Vibrio*	All vibrio strains were susceptible to sulfadimethoxine/ormetoprim and moderately susceptible to sulfadiazine/trimethoprim and oxytetracycline. Resistant to amoxicillin.	Maisak *et al.*, 1995
1999-2002	India	*Vibrio spp.* and *Aeromonas spp*	100% of the samples resistant to ampicillin. Lower resistance was detected in chlortetracycline and erythromycin. Vibriosshowed high resistance to the antibiotics compared to *Aeromonas* isolated from the same sample.	Vaseeharan *et al.*, 2005

2001	Philippines	*Vibrio harveyi*	Oxytetracycline, oxolinic acid, chloramphenicol and furazolidone resistance in pond cultured shrimps	Tendencia and Pena, 2001
2004	Thailand and India	*E.coli*, *Salmonella*, *Bacillus* spp and *Vibrio* spp	Samples from Thailand and India shows the presence of multiple drug resistance (MDR) associated with erythromycin, tetracycline, chloramphenicol, nalidixic acid and trimethoprim	Duran and Marshall, 2005
2005	India	*Vibrio* spp	30.3% of isolates were MDR; Out of total MDR isolates, resistance to 4–10 antibiotics in 55.5% of isolates; resistance to >10 antibiotics was observed in 14.14% of isolates	Manjusha *et al.*, 2005
2006-2007	India	*Vibrio* spp	100% resistant of all the *Vibrio* samples to ampicillin, cloxacillin, oxacillin, erythromycin, vancomycin,penicillin G and furazolidone	Srinivasan and Ramasamy, 2009
2007-2008	Thailand	*E. coli*	Presence of 29.1% of MAR in shrimp farms, with tetracycline (14.4%), ampicillin (8%) and trimethoprim (6.7%)	Changkaew *et al.*, 2014
2010-2011	India	*Vibrio* spp	The highest incidence of antibiotic resistance was evident against ampicillin and colistin followed by amoxicillin, carbenicillin, ceftazidime andcephalothin	Sudha *et al.*, 2014
2011	Malaysia	*Salmonella* and *Vibrio*	Presence of MAR for ampicillin and tetracycline, followed by doxycycline in *Salmonella* and *Vibio spp*	Banerjee *et al.*, 2012

2011	China	*E.coli* and *S. aureus*	Resistance to antibiotics such as ampicillin, trimethoprim, tetracycline, chloramphenicol and cefazolin	Zhang *et al.*, 2011
2011	India	*Vibrio* spp.	78% of isolates were MDR. The highest incidence of antibiotic resistance against amoxicillin (94%), followed by Ampicillin and carbenicillin (90%); cefuroxime and streptomycin (65%) followed by neomycin and amikacin (59.57%); rifampicin (58.00%); furazolidone (42%) and meropenem (35%)	Manjusha & Sarita, 2011
2011-2013	Malaysia	*Vibrio parahaemolyticus*	High resistance to ampicillin, cefalexin and ciprofloxacin	Al-Othrubi *et al.*, 2014
2012	China	*Vibrio vulnificus*	Resistance or intermediate resistance to cefepime (3.03%), tetracycline (6.06%), aztreonam (24.24%), streptomycin (45.45%), gentamicin (93.94%), tobramycin (100%), and cefazolin(100%).	Pan *et al.*, 2013
2013-2014	India	*Vibrio harveyi*	Resistance to ciprofloxacin, penicillin, rifampicin, and vancomycin out of 15 antibiotics tested	Stalin and Srinivasan, 2016
2014-	Thailand	*Aeromonas veronii* and *A. aquariorum*	Resistance to cefotaxime and imipenem in two *A. aquaritheorum* and in three *A. veronii* isolates; the resistance of tetracycline and ampicillin	Yano *et al.*,2015
2014	China	*Vibrio parahaemolyticus*	High level of resistance to the antibiotics ampicillin (94.2%), rifampin (93.3%), and streptomycin (77.9%)	Hu and Chen, 2016

2014	Parangipettai, India	*Bacillus pumilus* and *Bacillus flexus*	Presence of novobiocin, ciprofloxacin and vancomycin resistance	Sundaramanickam *et al.*, 2015
2015-2016	India	*Vibro* spp.	Highest resistance (50.4%) against ampicillin. Very high intermediate resistance (87.4%) against erythromycin. 20% of *Vibrio* isolates were resistant to two or more antibiotic classes with MAR index value of ≥0.28.	Singh *et al.*, 2018
2016-2018	China	*Vibrio parahaemolyticus*	High resistance to chloramphenicol, sulfamethoxazole-trimethoprim,trimethoprim, rifampicin, ampicillin, spectinomycin, kanamycin,	He *et al.*, 2019
2020	India	*Vibrio parahaemolyticus*	One pathogenic isolate was identified as MDR and 59% exhibited a MAR index of 0.2 or above. 100% resistance to ampicillin, 74.1% to cefotaxime, 48.1% to cefoxitin, 44.4% to cefepime, 29.6% to ceftazidime, 7.4% to amoxicillin/clavulanic acid, 3.7% to ciprofloxacin, gentamicin and meropenem	Narayanan *et al.*, 2020

Numerous studies suggest the presence of multidrug resistance associated with shrimp culture in the Asian and South Asian regions. The most common reason for such high resistance to persist within shrimp farms can be suggested as the irrational use of antibiotic drugs in shrimp farming practices.

In Asian and South Asian cuisine, farmed shrimp constitutes a significant portion of the human diet. However, consuming these shrimps can lead to the transfer of antibiotic-resistant genes from shrimp to humans. These acquired genes alter the natural microflora in humans, reducing the effectiveness of existing therapeutic drugs (Palaniappan and Holley, 2010). Unfortunately, new antibiotics are not emerging rapidly enough to keep pace with the generation of resistant genes and bacteria (Laws *et al.*, 2019). Critically important antibiotics, as outlined by the WHO's 2018 guidelines, are the only treatment options for certain medical conditions (WHO, 2018). If resistance develops to these critical antibiotics in human medicine, patients may face a lack of effective treatment options, potentially resulting in fatalities. While highly important and less critical antibiotics exist, frequent exposure to them through food animals, antibiotic misuse, and overuse in food animal farming can lead to resistance even to these less critical antibiotics. Over time, this could render all therapeutic options ineffective, pushing humanity toward a pre-antibiotic era. To address this critical situation, better controls on antibiotic usage in food animal production, such as shrimp farming, are essential.

AMR in Sri Lankan Scenario

Although Sri Lanka has plenty of natural and brackish water resources for fish and shrimp farming, Sri Lanka did not have a traditional aquaculture system until the beginning of 1980 (Heenatigala and Fernando, 2016). Since aquaculture and marine shrimp culture have reached their maximum commercial dimensions, brackish water ponds for shrimp culture and cage culture have gradually emerged (NAQDA, 2015). The major limiting factor to Sri Lankan shrimp farming was the incidence of infectious diseases such as white spot syndrome virus (WSSV) and Vibriosis. *Vibrio* spp. is a part of the natural microflora in wild and cultured shrimp (Heenatigala and Fernando, 2016), and the members of the family Vibrionaceae contribute up to 60% of the total bacterial population in shrimp aquaculture (Heenatigala and Fernando, 2016; Simidu and Tsukamoto, 1985). *Vibrio* spp. are one of the major pathogenic bacterial organisms which cause high mortality in shrimp culture and act as a primary pathogen spreading WSSV (Priya *et al.*, 2009). Maintaining proper water quality is an important aspect of shrimp culture as it considerably reduces the pathogenic *Vibrio* spp. population in the water system (Ganesh et al., 2010).

Management of infectious diseases in the Sri Lankan shrimp farming is mainly based on the management of aquatic microflora with the aid of probiotics and antibiotics (Heenatigala and Fernando, 2016; Havenaar *et al*.,1992). Misuse and overuse of antibiotics for disease management in shrimp farms have caused multiple antibiotic resistance among the bacterial population in water bodies and bacteria in sediments of shrimp culture systems; there they can act as a vehicle that delivers MAR aquatic pathogens to humans from one country to another (Zanetti *et al*., 2001).

According to research conducted by Heenatigala, 2013, 96 pathogenic bacterial strains such as *Vibrio alginolyticus*, *V. fluvialis* and *Pseudomonas aeruginosa* were isolated and subjected to antibiotic sensitivity test. None of the pathogenic bacterial strains were highly resistant to erythromycin, and only 29% showed intermediate sensitivity; while only 20% of the total isolates were sensitive to oxytetracycline, and 80% showed intermediate sensitivity to oxytetracycline (Heenatigala, 2013).

Athurupana *et al*., 2020 conducted a study to test the antibiotic resistance of *Escherichia coli* isolated from pond water, bottom sediments and shrimps (*Penaeus monodon*) from shrimp farms, in Puttalam District, Sri Lanka. In this study, antibiogram against *E. coli* to antibiotics belonging to different families, ß-Lactams: amoxicillin (30 µg); tetracycline: tetracycline (30 µg) oxytetracycline (30 µg); macrolides: erythromycin (15 µg) and chloramphenicol (30 µg) was studied. A total of 67 *E. coli* bacteria were isolated and 48 (71.64%) were resistant to at least one drug out of the total number. A high index of resistance to erythromycin (15 µg) 70.15% was reported. In contrast, none of the *E. coli* strains was resistant to chloramphenicol (30 µg). Multidrug resistance to two or more antibiotics was observed in 24 isolates. Multiple Antibiotic Resistance Index varied within the range of 0 to 0.8 for the antibiotics used.

Gallage *et al*., 2019 has reported a study consisting of 146 isolates belonging to the family Vibrionaceae were recorded and identified as, *Aeromonas hydrophila*, *Vibrio metschnikovii*, *V. anguillarum*, *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, *V.*

damsela, *V. mimicus* and *V. fluvialis*. *Vibrio* isolates were found to be resistant to amoxicillin (68.5%), nitrofurantoin (25.2%), nalidixic acid (21%), tetracycline (5.18%) and chloramphenicol (4.48%). This study showed that *Vibrio* species are more resistant to amoxicillin in comparison with other antibiotics used in this study. Results also indicated that the application of antibiotics for the control of vibriosis in shrimp farms has limited effectiveness due to the development of resistant bacterial strains.

Another study conducted by Heenatigala and Fernando, 2016 discusses the alterations of the antibiotic sensitivity of Vibrio spp. isolated from disease-infected P. monodon. Ariyawansa *et al*., 1999 reported that *Vibrio* spp. from *P. monodon* is highly sensitive to chloramphenicol (10µg and 30µg), and tetracycline (30µg); and moderately sensitive to streptomycin (25µg); when compared to furazolidone (50µg), erythromycin (15µg) and sulphafurazole (30µg). However, the study by Heenatigala and Fernando, 2016 reveals the bacterial sensitivity of chloramphenicol has changed from sensitive to moderately sensitive and/ or resistant, and oxytetracycline has changed from moderately sensitive to resistant; and the study suggests the alteration may be due to the continuous use of the antibiotics in shrimp farming.

A study conducted by Liyanage and Manage, 2016 shows *Bacillus* sp., *Acinetobacter* sp., *Achromabacter* sp. *Staphylococcus* sp., and *Micrococcus* sp., are the most abundant resistant genera for tetracycline and oxytetracycline in Sri Lankan aquaculture farms (Liyanage and Manage 2016). Maximum Inhibitory Concentrations (MIC) values for OTC-resistant bacteria ranged from 360-840 µg/mL and the highest MIC was recorded for *Pseudomonas aeroginosa* and the lowest MIC was detected for *Bacillus* sp. MIC values for tetracycline resistance varied from 320-780 µg/mL, and the highest MIC was obtained by *S. haemolyticus* and the lowest MIC was reported for *B. pumilus* (Manage, 2018; Liyanage and Manage 2016).

AMR surveillance systems and its importance

Although monitoring of AMR is required to understand the burden of AMR and to find solutions to the problem of potential gaps in the distribution of AMR within populations and individuals, the factors that contribute to AMR are needed to be understood. Large-scale epidemiological surveys are required to determine the relationships between AMR and antibiotic prescription and consumption (Allcock *et al*., 2017). This sort of surveillance system needs to access the geographical regions, different communities, and potential zoonotic transmission pathways (Van *et al*., 2012). Enabling real-time monitoring of resistance patterns and the spread of AMR genes within populations is required to monitor AMR successfully (Van *et al*., 2012). Due to the continuous usage of banned antibiotics in shrimp culture, tightened guidelines and national regulations to monitor antibiotic usage and residual level monitoring are currently available in many countries. Due to the rejections in exports and due to the reduction of profits from seafood marketing as a result of residual antibiotics, Maximum Residual Levels (MRL) accepted by Codex Alimentarius Commission is currently available for a limited number of antibiotics. However, these MRLs are not consistent among different countries. Therefore, the need for Codex regulations for all the drugs acceptable to be used in food animal production persists around the world (FAO, 2020).

Sri Lanka is in combating AMR with multisectoral programs. The development of the National Strategic Plan (NSP) 2017-2022 provides the roadmap to combat AMR. The NSP is developed under five key strategies which are aligned with a global action plan. These strategies cover all aspects of combating AMR involving human, animal, agriculture, fisheries and environment sectors. The five strategies are expressed with specific objectives and short and long term (2 years and 5 years) including improving awareness and understanding of antibiotic resistance through effective communication, strengthening the knowledge and evidence base through surveillance and research, reducing the incidence of infection through effective sanitation, hygiene and infection prevention measures, optimize the use of antibiotic medicines in human and animal health and prepare the economic case for sustainable investment and increase investment in new medicines, diagnostic tools, vaccines and other interventions.

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