A STUDY OF ANTIBIOTIC RESIDUES IN INDIAN AQUACULTURE SHRIMP (*LITOPENAEUS VANNAMEI*)

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Abstract
Shrimp holds a pivotal role in global aquaculture as a valuable commodity traded extensively across borders. The use of antibiotics in shrimp farming has been a subject of concern and criticism within the aquaculture industry. India stands as one of the prominent contributors to the United States' shrimp supply, playing a substantial role in the global shrimp market. Alongside nations like Indonesia, Thailand, Ecuador, and Vietnam, India has consistently provided substantial quantities of shrimp to the U.S. market. Recent years have witnessed a rise in rejections of Indian shrimp exports to the United States, stemming from various factors, including the presence of prohibited antibiotics. Indian shrimp farmers have faced accusations of employing banned antibiotics in shrimp cultivation, which can subsequently contaminate the shrimp meat. Such antibiotic residues pose a significant health risk to consumers in the United States, leading to the rejection of numerous Indian shrimp shipments. To gain deeper insights into the prevalence of antibiotics in Indian shrimp farming, researchers collected shrimp samples from key aquaculture regions in India, including Andhra Pradesh, Gujarat, Odisha, Tamil Nadu, and West Bengal. These states collectively contribute to over 90% of India's aquaculture shrimp production. In a comprehensive analysis, samples from these regions underwent testing for 37 antibiotic compounds and metabolites at a certified laboratory. a total of 20 composite samples were analysed for 37 antibiotics, chloramphenicol, Nitrofurans, and Oxytetracycline. Remarkably, the study yielded results indicating the absence of detectable antibiotic residues in any of the samples, thus contradicting prior findings in frozen shrimp analyses that often reported low levels of antibiotic prevalence. These findings provide a positive outlook on the safety and quality of aquaculture shrimp produced in India. It suggests that rigorous monitoring and regulations may be contributing to safer shrimp products for consumers. Nonetheless, ongoing vigilance and adherence to best practices remain paramount to maintaining the integrity of the global shrimp industry.
Keywords: Aquaculture, Shrimp, *Litopenaeus vannamei*, Chemical contaminants, Antibiotics.

1. Introduction

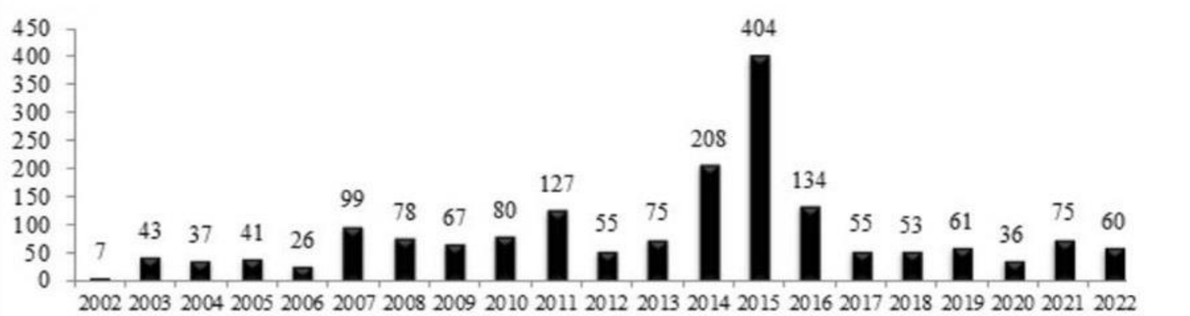
The global population is on a trajectory to reach between 9 and 10 billion people by the year 2050. This population growth, particularly in developing nations, will necessitate a significantly disproportionate increase in protein production to meet the rising standards of living (FAO, 2009). Seafood plays a pivotal role in the world's protein supply, offering not only nourishing proteins but also Omega 3 fatty acids, known for their human health benefits (Boyd and McNevin, 2014). In aggregate, both capture fisheries and aquaculture contribute to approximately 19% of the world's animal protein supply for human consumption (Edwards *et al.,* 2019). However, the production levels from traditional fisheries have remained relatively stagnant over the past three decades, while aquaculture has surged and now constitutes roughly 50% of the global seafood output (FAO, 2018). Given these circumstances, aquaculture emerges as the most viable means to sustainably expand seafood production to meet the growing global demand.

Litopenaeus vannamei, also known as the Pacific white shrimp, is the most widely cultured shrimp species in India. It was first introduced in India in 2001 and has since grown to become the dominant species of shrimp farmed in the country. There are a number of reasons for the popularity of *Litopenaeus vannamei* culture in India. First, the shrimp is relatively easy to culture and the cost of production is relatively low. Second, the demand for shrimp is high and the price of shrimp is relatively stable. Third, there are a number of government programs in place to support the shrimp farming industry. The culture of *Litopenaeus vannamei* in India is concentrated in the coastal states of Andhra Pradesh, Tamil Nadu, and Gujarat. These states have the necessary infrastructure, such as hatcheries, feed mills, and processing plants, to support the shrimp farming industry. The culture of *Litopenaeus vannamei* in India is a major contributor to the country's economy. The industry provides employment to millions of people and generates billions of dollars in revenue each year. Shrimp are disproportionally valuable as an aquaculture species compared to their overall contribution to aquaculture production. In 2020, shrimp accounted for only 6% of the total global aquaculture production by weight. However, they accounted for 25% of the total value of aquaculture production. This is because shrimp are a high-value commodity, and they are often exported to high-income countries. There are a few reasons why shrimp are so valuable. First, they are a popular food item. Shrimp are often eaten as a delicacy, and they are a good source of protein and other nutrients. Second, shrimp are relatively easy to farm. They can be grown in a variety of environments, and they are not as susceptible to diseases as some other aquaculture species. Third, the demand for shrimp is growing. The global population is increasing, and people are eating more seafood. The high value of shrimp has led to a boom in aquaculture production. In the past few decades, the global production of shrimp has increased dramatically. This growth has been driven by the demand for shrimp in high-income countries, and by the development of new aquaculture techniques.

India exported 719,357 metric tons of shrimp in 2022-23, valued at $5.48 billion. This was a decrease of 2% in volume and 4% in value from the previous year. The United States was the largest market for Indian shrimp exports, accounting for 275,662 metric tons or 38% of the total. Other major markets were China (145,743 MT), the European Union (95,377 MT), South East Asia (65,466 MT), Japan (40,975 MT), and the Middle East (31,647 MT).

Despite the challenges, India is still the world's second largest exporter of shrimp. The industry is expected to recover in the coming years, as the demand for seafood continues to grow.
According to the Marine Products Export Development Authority (MPEDA), the overall export of frozen shrimps during 2022-23 was pegged at 7,11,099 MT. The United States was the largest market, importing 2,75,662 MT of frozen shrimp. Other major importers of frozen shrimp from India were: China: 1,45,743 MT, European Union: 95,377 MT, South East Asia: 65,466 MT, Japan: 40,975 MT & Middle East: 31,647 MT.

The U.S. Food and Drug Administration (FDA) has rejected shipments of antibiotic-contaminated shrimp from India in recent years. The FDA has a zero-tolerance policy for antibiotic residues in imported food, and any shipments that test positive for banned antibiotics are rejected. The use of antibiotics in shrimp farming is a major concern for the FDA and other health organizations. Antibiotics can be used to treat diseases in shrimp, but they can also be used to promote growth. The use of antibiotics to promote growth is a major contributor to the development of antibiotic resistance, which is a serious public health threat. The FDA has been working with the Indian government to address the issue of antibiotic-contaminated shrimp. The Indian government has banned the use of some antibiotics in shrimp farming, and it is working to improve enforcement of the ban. However, the problem of antibiotic-contaminated shrimp from India persists. The FDA is urging consumers to be aware of the issue of antibiotic-contaminated shrimp. Consumers can reduce their risk of exposure to antibiotic-contaminated shrimp by buying shrimp from reputable sources and by avoiding shrimp that is imported from countries with a history of antibiotic residue problems.



FDA Refusals of Shrimp Entry Lines for Veterinary Drug Residues

The inappropriate use of antibiotics at the farm level can lead to the development of antibiotic resistance, which is a serious public health threat. Antibiotic resistance occurs when bacteria become resistant to antibiotics that are used to treat them. This can happen when antibiotics are used incorrectly, such as when they are used to treat diseases that do not require antibiotics or when they are used in excessive amounts. When antibiotic-resistant bacteria are present in food, they can be transferred to humans who eat the food. This can lead to infections that are difficult or impossible to treat with antibiotics.

Custom agencies in many countries have strict regulations in place to prevent the import of food that contains antibiotic residues. These regulations are designed to protect public health and to prevent the spread of antibiotic resistance. If food is found to contain antibiotic residues, it will be rejected by custom agencies and will not be allowed to enter the country. This can have a significant impact on the farmers who produce the food, as well as on the businesses that import and sell it.

The objective of the study. It is important to understand the prevalence of antibiotics in shrimp farming in India. The use of antibiotics in shrimp farming can have a number of negative consequences, including:

- The development of antibiotic resistance in bacteria.
- The contamination of the environment with antibiotics.
- The exposure of humans to antibiotics through the consumption of shrimp.

The study that you mentioned was a good first step in understanding the prevalence of antibiotics in shrimp farming in India. However, there are a few limitations to the study. First, the samples were collected haphazardly, which means that they may not be representative of all shrimp farms in India. Second, the study only tested for a limited number of antibiotics. It is possible that other antibiotics are also being used in shrimp farming. Despite these limitations, the study provides valuable information about the prevalence of antibiotics in shrimp farming in India. It is important to continue to study this issue in order to develop strategies to reduce the use of antibiotics in shrimp farming.

2. Methods

2.1 Sampling: Shrimp specimens were systematically collected from aquaculture ponds located across all five major shrimp-producing states in India, spanning the period from August 2019 to

February 2023. The selection of ponds was meticulously executed to encompass a wide spectrum of farms, encompassing those registered with the Coastal Aquaculture Authority (CAA). This comprehensive approach aimed to include various farm types, such as those managed by high-end technically proficient large-scale farmers, mid-range farmers, and small-scale farmers. In total, our sampling efforts covered 20 distinct farms situated in India. A comprehensive list detailing the stores sampled and the specific samples obtained can be found in the supplementary information provided.

Shrimp Farm located Near Kona–Andhra Pradesh

Fresh Shrimp (Vannamei) at the time of Harvest

During each site visit, procurement involved the acquisition of bags containing both private label and farm label raw shrimp from individual farmers, with the identity of the farmers concealed for privacy reasons. To clarify, a total of 20 samples were procured and collected in this manner. The samples were meticulously labeled according to their respective farm locations, encompassing Andhra Pradesh (n = 4), Gujarat (n = 4), Odisha (n = 4), Tamil Nadu (n = 4), and West Bengal (n = 4). Each sample consistently comprised a minimum of 700 grams (equivalent to a 24-ounce bag) of raw shrimp. To maintain the samples in optimal condition, they were promptly refrigerated and kept chilled until the point of processing for subsequent analysis. It is noteworthy that one bag of raw shrimp, regardless of its weight (which ranged between 700 grams and 900 grams), was deemed a complete "sample." These samples were meticulously packed and dispatched on dry ice to the analytical laboratory, enclosed in sealed plastic bags, in preparation for the forthcoming analytical procedures.

2.2. Analytical procedures: The analysis of samples for antibiotic residue was carried out at a commercial analytical laboratory that holds certification in accordance with the International Standards Organization (ISO) standard 17,025, as accredited by an independent third-party certification body specializing in antibiotic testing. The testing procedure adhered to a comprehensive multi-class analytical method, as per the protocol outlined in Adams et al. (2017), aimed at detecting the presence of a diverse array of 37 antibiotics. The LC MS/MS procedure is a sensitive and specific method for detecting and quantifying drugs in shrimp tissue. It can be used to detect a wide range of drugs, including antibiotics, pesticides, and other contaminants.
Here are the specific steps involved in each of the three steps:

2.3 Sample preparation:
1. The shrimp tissue is homogenized in a blender or homogenizer.
2. The homogenized tissue is extracted with a solvent, such as methanol or acetonitrile.
3. The extract is filtered to remove any particulate matter.
4. The extract is concentrated by evaporation or rotary evaporation.

2.4 Liquid chromatography (LC):
1. The extract is injected into the LC system.
2. The LC system separates the extract into its component drugs by passing it through a column packed with a stationary phase.

3. The drugs are eluted from the column by a mobile phase.
4. The drugs are detected by a detector, such as a UV detector or a mass spectrometer.

2.5 Mass spectrometry (MS):
1. The drugs are ionized in the MS.
2. The ions are separated by their mass-to-charge ratio (m/z).
3. The ions are detected and quantified.

In summary, the sample processing involved homogenizing the samples and then mixing them with a combination of acetic acid and acetonitrile (in a 1:4 ratio), followed by centrifugation. The upper layer of the separated mixture was subsequently decanted and employed for the ensuing analysis. For the detection of analytes, this methodology employed ion chromatography coupled with tandem mass spectrometry. All samples underwent an extensive multi-class antibiotics test to screen for a wide range of antibiotics. The selection of samples for this subset was designed to maintain a reasonably balanced representation of major production states within the overall sample pool. Specifically, this subset comprised five samples each from Andhra Pradesh, Gujarat, Odisha, Tamil Nadu, and West Bengal. For the analysis of Antibiotics, a method developed by the United States Food and Drug Administration (US FDA 2006) was employed. This method utilized liquid chromatography tandem mass spectroscopy (LC-MS-MS) and involved a hydrochloric acid digestion process. All reported results include the detection limit for each specific analyte.

Target Testing Level (TTL)/ Regulatory Action Level (RAL) The following values are the current Target Testing Levels (TTL) or tolerance level (TL) for each chemotherapeutic agent. These levels are also considered as Regulatory Action Levels (RAL). However, TTL is not and should not be interpreted as a safe concentration or a tolerance level and it does not imply that an approval exists for that drug [21CFR530.3(g)].

Animal Drug Residue	Target Testing Level or Tolerance Level (ppb)
Chloramphenicol[1]	0.15
Nitrofurans[1]	
AOZ metabolite of Furazolidone	0.5
AMOZ metabolite of Furaltadone	0.5
SEM metabolite of Nitrofurazone	0.5
AHD metabolite of Nitrofurantoin	0.5
DSH metabolite of Nifursol	0.5
Ampicillin	10.0
Amoxicillin	10.0
Cloxacillin	10.0
Avermectins:	
Ivermectin	10.0
Abamectin	10.0
Doramectin	10.0
Azamethiphos[5]	20.0
Emamectin[2]	100.0
Benzimidazoles:	
Sum of Mebendazole + Mebendazole Amine + Hydroxy Mebendazole	5.0
Thiabendazole	100.0
Erythromycin	100.0
Florfenicol Amine[3]	1,000.0
Fluoroquinolones:	
Sum of Enrofloxacin and Ciprofloxacin	5.0

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Danofloxacin Difloxacin Norfloxacin Ofloxacin Sarafloxacin	5.0 5.0 5.0 5.0 5.0
Isoeugenol	200.0
Lincomycin	10.0
Methyltestosterone	0.8
Quinolones: Oxolinic Acid Flumequine Nalidixic Acid	 10.0 10.0 10.0
Sulfonamides: Sulfamerazine Sulfadiazine Sulfachloropyridazine Sulfathiazole Sulfaquinoxaline Sulfamethazine Sulfadimethoxine Sulfacetamide Sulfadoxine Sulfaethoxypyridazine Sulfamethoxypyridazine Sulfamethoxazole Sulfapyridine Sulfamonomethoxine	 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0
Stilbenes: Diethylstilbesterol Dienesterol Hexestrol	 0.25 0.25 0.25
Tetracyclines:[4] Sum of Oxytetracycline + Tetracycline +Chlortetracycline	 2,000.0
Thiamphenicol	1.0
Trimethoprim	10.0
Triphenylmethane Dyes: Sum of Malachite Green and LeucomalachiteGreen Sum of Crystal Violet and Leucocrystal VioletBrilliant Green	 0.5 0.5 0.5

3. Results

In the context of this report, a grand total of 20 samples were gathered and subjected to analysis. These samples were meticulously collected across five different states within India. The sample collection process involved visiting a total of 20 distinct farms, with a notable observation being that the majority, precisely 60% of these samples, originated from high-range farmers cultivating over 10 hectares of land. The remaining samples were sourced from mid-range and low-range farmers whose landholdings were less than 5 hectares. Further categorization of the samples reveals that out of the total collected, 15 samples were obtained from farmers who held certifications through the BAP (Best Aquaculture Practices) certification scheme. Additionally, only 10 samples bore the label of being registered with the CAA (Coastal Aquaculture Authority). In all examined samples, there were no traces of antibiotics detected, as indicated in Table 1. It's worth noting that all the samples subjected to testing were Pacific whiteleg shrimp, scientifically known as *Litopenaeus vannamei*.

Table-1							
Sl. No.	**Test Parameters**	**Detection Limit**	**Andhra Pradesh**	**Gujarat**	**Odisha**	**Tamil Nadu**	**West Bengal**
1	Chloramphenicol	0.07	ND	ND	ND	ND	ND
2	AMOZ (3-amino-5-orpholinomethyl- 2-oxazolidinone	0.1	ND	ND	ND	ND	ND
3	AOZ (3-amino-2-oxazolidinone)	0.1	ND	ND	ND	ND	ND
4	AHD (1-aminohydantoin)	0.1	ND	ND	ND	ND	ND
5	SEM(semicarbazide)	0.1	ND	ND	ND	ND	ND
6	DNSH (3,5 -dinitrosalicylic acid hydrazide)	0.1	ND	ND	ND	ND	ND
7	Furazolidone	0.1	ND	ND	ND	ND	ND
8	Furaltadone	0.1	ND	ND	ND	ND	ND
9	Nitrofurantoin	0.1	ND	ND	ND	ND	ND
10	Nitrofurazone	0.1	ND	ND	ND	ND	ND
11	Nifursol	0.1	ND	ND	ND	ND	ND
12	Sum of Chlortetracycline and 4-epi-Chlortetracycline	5	ND	ND	ND	ND	ND
13	Sum of Oxytetracycline and 4-epi Oxytetracycline	5	ND	ND	ND	ND	ND
14	Sum of Tetracycline and 4-epi-Tetracycline	5	ND	ND	ND	ND	ND
15	Sulfamethazine/ Sulfadimidine	5	ND	ND	ND	ND	ND
16	Sulfadiazine	5	ND	ND	ND	ND	ND
17	Sulfadimethoxine	5	ND	ND	ND	ND	ND
18	Sulfamerazine	5	ND	ND	ND	ND	ND
19	Sulfamethoxypyridine	5	ND	ND	ND	ND	ND
20	Sulfamethiazole	5	ND	ND	ND	ND	ND
21	Sulfathiazole	5	ND	ND	ND	ND	ND
22	Sulfachlorpyridazine	5	ND	ND	ND	ND	ND
23	Sulfapyridine	5	ND	ND	ND	ND	ND
24	Sulfamethoxazole	5	ND	ND	ND	ND	ND
25	Ciprofloxacin	1	ND	ND	ND	ND	ND
26	Danofloxacin	1	ND	ND	ND	ND	ND
27	Difloxacin	1	ND	ND	ND	ND	ND
28	Enrofloxacin	1	ND	ND	ND	ND	ND
29	Flumequine	1	ND	ND	ND	ND	ND
30	Norfloxacin	1	ND	ND	ND	ND	ND
31	Sarafloxacin	1	ND	ND	ND	ND	ND
32	Nalidixic acid	1	ND	ND	ND	ND	ND
33	Oxolinic acid	1	ND	ND	ND	ND	ND
34	Florfenicol	0.5	ND	ND	ND	ND	ND

4. Discussion & Conclusion

Global production of *Litopenaeus vannamei*, also known as the Pacific white shrimp, is expected to reach 5.011 million metric tons in 2022, a significant increase over the 4.569 million metric tons grown in 2021, which itself was an increase over the 4.086 million MT produced in 2020. The production of *Litopenaeus vannamei* in India in 2022 is estimated to be 340,000 metric tons. This is a decrease from the 400,000 metric tons produced in 2021. Crustaceans are disproportionally valuable as an aquaculture commodity. In 2020, the global production of crustaceans was valued at US$100 billion, which is about 16% of the total value of aquaculture production. Shrimp is the most valuable crustacean species, accounting for about 60% of the global value of crustacean production. There are a number of reasons why crustaceans are so valuable as an aquaculture commodity. First, they are a popular seafood dish and the demand for them is increasing. Second, crustaceans are relatively easy to culture and the cost of production is relatively low. Third, there are a number of technologies available to help farmers culture crustaceans, such as controlled-environment aquaculture (CEA) systems. Shrimp are grown mainly for export. In 2020, about 85% of the global production of shrimp was exported. The main export markets for shrimp are the United States, the European Union, and Japan. The use of antibiotics in shrimp farming is a well-known and documented practice. Antibiotics are used to treat and prevent diseases in shrimp, but they can also have negative consequences for human health and the environment.

Antibiotic residues have been found in shrimp from farms around the world, including India. This is a concern because antibiotic residues can make people sick and can also contribute to the development of antibiotic resistance.

Antibiotic resistance is a serious problem that is becoming increasingly common. When bacteria become resistant to antibiotics, they are more difficult to treat, which can lead to more serious infections and even death. We will delve into the key findings and implications of our study, which focused on investigating the presence of antibiotic residues in Indian aquaculture shrimp, specifically the *Litopenaeus vannamei* species.

1. Absence of Antibiotic Residues: Our study's most significant and reassuring finding is the absence of detectable antibiotic residues in the sampled Indian aquaculture shrimp. These results indicate that the shrimp samples collected from major farming states in India did not contain any significant traces of antibiotics, as confirmed by rigorous testing procedures.

2. Compliance with Regulations: The absence of antibiotic residues in the sampled shrimp is a positive indicator of compliance with antibiotic usage regulations in Indian aquaculture. It suggests that, in general, farmers are following responsible antibiotic practices and adhering to regulatory guidelines, which is essential for food safety and consumer confidence.

3. Global Consumer Assurance: This study carries important implications for international consumers of Indian shrimp products, particularly in the United States, a major importer of Indian shrimp. The absence of antibiotic residues provides assurance to consumers regarding the safety and quality of Indian shrimp exports.

4. Sustainability of Indian Aquaculture: Sustainable aquaculture practices are essential for the long-term viability of the industry. The absence of antibiotic residues aligns with the principles of sustainable farming, reducing the environmental impact associated with antibiotic use and promoting healthier aquatic ecosystems.

5. Continued Monitoring and Education: While our findings are encouraging, it is crucial to continue monitoring antibiotic usage in Indian aquaculture to maintain these positive outcomes. Additionally, ongoing education and awareness initiatives among shrimp farmers can further promote responsible antibiotic practices.

6. Future Research Directions: This study opens avenues for future research, including more extensive surveys and in-depth investigations into antibiotic usage patterns in different regions of India. Understanding regional variations and specific antibiotic practices can provide valuable insights for enhancing the sustainability of Indian aquaculture.

7. Collaboration and Knowledge Sharing: Collaboration between government agencies, industry stakeholders, and research institutions is crucial for the effective regulation and promotion of responsible antibiotic use in aquaculture. Knowledge sharing and best practice dissemination can benefit all stakeholders in the industry.

In conclusion, our study's findings are a positive testament to the state of antibiotic residues in Indian aquaculture shrimp, indicating responsible antibiotic usage practices and adherence to regulatory standards. However, continued vigilance, research, and education efforts are essential to maintain and further improve the sustainability and safety of the Indian aquaculture industry.

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