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Public Health Significance of Antibiotic Resistance Profiles in Bacteria Associated with Fresh, Frozen, and Dried Whiteleg Shrimps *Litopenaeus vannamei* (Boone, 1931)

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Abstract: The use of effective environmental sanitation practices, and appropriate handling techniques may lead to a reduction in bacterial infections present in prawns, hence improving their nutritional value. The present study aimed to study the antibiotic susceptibility in isolates of various bacterial strains from fresh shrimp, frozen shrimp, and dried shrimp samples collected from (i) Pondicherry, (ii) Cuddalore, (iii) Parangipettai, (iv) Nagapatinam, and (v) Karaikal. Fresh samples had an average total culturable heterotrophic bacterial (TCHB) count of 2.5×10^7 cfu/g and a total coliform bacterial (TCB) count of 2.8×10^6 cfu/g. In contrast, frozen samples had an average count of 8.7×10^6 cfu/g for TCHB and 2.4×10^4cfu/g for TCB, and dried shrimp averaged 1.7×10^1 cfu/g for TCHB and 1.0×101 cfu/g for TCB. The density of THCB and TCB in the fresh samples was significantly higher than that obtained for the frozen shrimp and dried fish samples ($p<0.05$). A total of 405 bacteria were isolated, comprising 193 from fresh shrimp samples, 115 from frozen shrimp samples, and 97 from dried shrimp samples. The percentage of bacterial genera in fresh sample is -- *Vibrio* sp. 6.2%, *Salmonella* sp. 8.2%, *Shigella* sp. 9.8%, *Aeromonas* sp. 10.8%, *E.coli* sp. 15.0%, *Staphylococcus* sp. 8.2% *Klebsiella* sp. 5.1%, *Listeria* sp. 4.6%. *Bacillus* sp. 9.3%, *Pseudomonas* sp. 11.0%, Unknown sp. 6.2%. While frozen shrimp had *Pseudomonas* sp. 17.9%, *Bacillus* spp. 11.7%, *Micrococcus* sp. 4.6%, *Enterobacter* sp. 10.1%, *Aeromonas* sp. 13.2%, *Proteus* sp. 7.3%, *Streptococcus* sp. 8.5%, *Flavobacterum* sp. 4.6%, *Lactobacillus* sp. 10.9%, *Acetobacter* sp. 3.9%, Unknown spp. 7.3%; dried shrimp sample had *Pseudomonas* sp. 17.9%, *Bacillus* spp. 11.7%, *Micrococcus* sp. 4.6%, *Enterobacter* sp. 10.1%, *Aeromonas* sp. 13.2%, *Proteus* sp. 7.3%, *Streptococcus* sp. 8.5%, *Flavobacterum* sp. 4.6%, *Lactobacillus* sp. 10.9%, *Acetobacter* 3.9%, and Unknown 7.3%. Consumption of bacterial-contaminated shrimps has been reported to be responsible for gastroenteritis, diarrhea, bacillary dysentery, and typhoid fever in humans. Antibiogram of selected isolates indicated their multiple antibiotic resistances to all antibiotics. The resistance patterns shown by bacterial isolates obtained from fresh shrimp samples showed that the isolates were 100% resistant to four antibiotics: Ampicillin (AMP), Cefpodoxime (CPD), Doxycycline (DO), and Gentamicin (GEN). The antibiotics Amoxcillin (AMC), Cephalexin (CN), Levofloxacin (LE), Ciprofloxacin (CF), Norfloxacin (NX), and Ofloxain (OF) exhibited the lowest levels of resistance, with resistance rates of 85%. 97%, 93%, 81%, 75%, 76%. Frozen shrimp revealed that all isolates resisted several antibiotics, as shown in. AMP, CF, NX, GEN Nevertheless, showed 100% resistance. Dried shrimp revealed that all isolates exhibited resistance to five antibiotics, namely AMP, CXM, OF, CF, and DO. The antibiotics AMC, CPD, GEN, Levofloxacin, and Norfloxacin (NX) exhibited the lowest resistance levels, with resistance rates of 85%. 91%, 98%,

71%, 95%, 68%. The use of effective environmental sanitation practices and appropriate handling techniques may lead to a reduction in bacterial pathogens present in prawns, hence improving their nutritional quality.

Keywords: Shrimp, Fresh sample, Frozen sample, Dry sample, Total Culturable Heterotrophic Bacteria, Total Coliform Bacteria, Antibiotic resistance test

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Introduction

Shrimp has significant potential as a lucrative income stream and a considerable contributor to the economic sustenance of coastal communities. The rise in the importation of frozen seafood, which is subject to varying temperatures as a result of power disruptions, leading to repeated freezing and thawing cycles, presents a potential health hazard for consumers of such seafood (Banwart, 1989; Ibe, 2008). Consequently, this situation warrants attention and worry. The presence of bacteria in shrimp poses a potential risk to public health when eaten either live with the shrimp or when their toxins are ingested (Nester *et al.*, 1998; Jablonski *et al.*, 2001; Jeyasekaran *et al.*, 2006; Iwamoto *et al.*, 2010; Adedeji *et al.*, 2012). The ingestion of seafood infected with bacteria has been linked to significant seafood-related illnesses, including salmonellosis and gastroenteritis. These infections are characterized by symptoms such as fever, bloody diarrhea, stomach cramps, and vomiting (Hatheway, 1995).

The issue of antimicrobial resistance (AMR) is a matter of worldwide significance, with a particular sense of urgency in poor countries where there is a high prevalence of infectious illnesses, poverty, and hunger (Okeke *et al.*, 2005; Planta, 2007; DACA, 2009; Moges *et al.*, 2014). Studies have shown that infections resulting from bacteria that are resistant to treatment are more often linked to higher rates of illness and death compared to infections produced by bacteria that are sensitive to treatment (Helms *et al.*, 2002; Travers and Barza, 2002).

In settings characterized by high levels of activity, such as hospitals, the emergence of antibiotic resistance has been associated with prolonged hospitalization, escalated healthcare expenditures, and, in severe instances, the occurrence of untreatable diseases (Byarugaba, 2004). Because there are not enough clinical microbiology labs that can find the exact etiological agents and test antibiotic susceptibility, doctors have to rely more on trial-and-error treatments. This has led to the development of antimicrobial resistance. Abera *et al.* (2014) found that self-prescribing antibiotics, a lack of local data on antimicrobial resistance patterns, and lack of knowledge about AMR among prescribers are the main local factors that contribute to the development of antimicrobial resistance (AMR) in Ethiopia.

Research has shown that the antimicrobial resistance profile of bacteria varies between populations due to differences in geography, local antimicrobial prescribing practices, and the number of resistant bacterial strains (Alemu *et al.*, 2012; Kabew *et al.*, 2013; Abejew and Denboba, 2014; Tadesse *et al.*, 2014). The aforementioned variations are inherently unstable and prone to quick fluctuations, particularly in regions where the improper use of antibiotics is prevalent, notably in poor nations. According to a systematic review conducted in Ethiopia, there is evidence

suggesting a rise in resistance rates among various pathogens, including *Escherichia coli*, *Shigella* spp., *Salmonella* spp., and *Staphylococcus aureus*, towards commonly prescribed antibiotics such as ampicillin, amoxicillin, penicillin, tetracycline, and trimethoprim/sulfamethoxazole (Moges *et al.*, 2014).

Pathogenic bacteria commonly associated with shrimps include *Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Vibrio cholerae*, *V. parahaemolyticus*, *Campylobacter jejuni*, *Pseudomonas* sp., *Alcaligenes* sp., *Acinetobacter* sp., *Yersinia enterocolitica*, *Moraxella* spp., *Achromobacter* spp., *Staphylococcus aureus*, *Flavobacterium* sp., *Bacillus cereus*, *Clostridium botulinum*, and *Listeria monocytogenes* (Aribisala, 1975, Elliott and Michener, 2003; Iwamato et al., 2010 and Mukilan et al.2022) These bacteria, which are of public health concern, are commonly found in aquaculture shrimp products and are primarily derived from the environment rather than poor hygiene and sanitation practices (Anthonio, 1995; Jeyasekaran *et al.*, 2006; Ekpo *et al.*, 2010; Adedeji *et al.*, 2012).

There exists a public health issue whereby certain bacteria, which previously lacked resistance, are now developing resistance to antibiotics. This resistance is likely due to the acquisition of additional DNA known as R plasmids or transposons, cellular mutation and efflux, alteration of the target cell structure to render antibiotics ineffective, the prevention of antibiotics from reaching their intended target, and the inactivation of antibiotics by microbial enzymes (Jeljaszewicz *et al.*, 2000). According to Khachtourians (1998), the presence of antibiotic-resistant bacterial species may lead to infections that are unresponsive to standard antibiotic treatments. The shrimp-associated pathogens possess virulence characteristics that facilitate their ability to induce illnesses (Adedeji *et al.*, 2012).

Seafood-borne diseases and intoxications:

Seafood-borne diseases and intoxications are illnesses that can result from consuming contaminated seafood, such as fish, shellfish, and other aquatic organisms. These contaminants can include various bacteria, viruses, parasites, and toxins that can cause gastrointestinal, neurological, or other health issues. Here are some common examples:

1. *Vibrio Infections*: *Vibrio* bacteria are commonly found in marine environments and can cause infections when consuming raw or undercooked seafood. *Vibrio parahaemolyticus* and *Vibrio vulnificus* are two species known to cause seafood-related illnesses, often resulting in symptoms like diarrhea, vomiting, abdominal pain, and fever.

2. *Norovirus*: Norovirus is a highly contagious virus that can be transmitted through contaminated water and food, including seafood. It can lead to symptoms like nausea, vomiting, diarrhea, and stomach cramps. Shellfish, especially oysters, are often associated with norovirus outbreaks due to their filter-feeding behavior in potentially polluted waters.

3. *Hepatitis A*: Hepatitis A virus can be transmitted through contaminated seafood, particularly shellfish. Symptoms may include fatigue, jaundice, abdominal pain, and nausea. Proper cooking and hygiene practices can help prevent its transmission.

4. *Ciguatera Poisoning*: This type of food poisoning occurs from consuming certain types of reef fish that have accumulated toxins produced by microscopic marine algae. Symptoms can vary widely and may include gastrointestinal symptoms, neurological symptoms (such as numbness, tingling, and reversal of hot and cold sensations), and cardiovascular effects.

5. *Amnesic Shellfish Poisoning*: This is caused by consuming shellfish that have accumulated domoic acid, a naturally occurring toxin produced by certain types of algae. It can lead to symptoms such as vomiting, diarrhea,

confusion, memory loss, and even neurological damage in severe cases.

6. *Parasitic Infections*: Some types of fish and shellfish can harbor parasites that can cause infections in humans when consumed raw or undercooked. Anisakiasis, caused by the larvae of Anisakis worms, is an example of such an infection. It can lead to symptoms like abdominal pain, nausea, and vomiting.

To reduce the risk of seafood-borne diseases and intoxications, it is important to follow proper food safety practices, including:

- Cooking seafood to appropriate temperatures to kill harmful pathogens.
- Avoiding consumption of raw or undercooked seafood, especially in a high-risk group (e.g., pregnant women, young children, the elderly, and individuals with weakened immune systems).
- Choosing seafood from reputable sources that follow good quality and safety practices.
- Avoiding consumption of seafood from areas with known pollution problems.
- Practicing good hygiene, such as washing hands thoroughly before and after handling seafood and other food items.

If someone consumed contaminated seafood and experiencing severe symptoms, it is important to seek medical attention promptly.

Materials and Methods

Chemicals:

All the chemicals used in the present study were purchased from the Himedia laboratories, India.

Study areas and sample collection:

In the present study, antibiotic resistance microbes from the fresh shrimp, frozen shrimp and dried shrimp White leg shrimps (*L. vannamei*-Boone, 1931) samples which were purchased from (i) Pondicherry, (ii) Cuddalore, (iii) Parangipettai, (iv) Nagapatinam and (v) Karaikal. 100 g of each product were purchased, wrapped in sterile aluminium foil and placed into clean polythene bags and then transported to the laboratory in an ice box within 4 h of collection for microbiological analyses and antibiotic susceptibility tests.

Isolation of Total Cultivable Heterotrophic Bacteria (TCHB) and Total Coliform Bacteria (*TCB*):

For the selective isolation of Total Culturable Heterotrophic Bacteria (TCHB) from the homogenized five sample, 1 g of each sample was aseptically weighed and transferred to a sterile conical flask containing 99 ml of sterile 50% aged seawater which was used as a diluent. Samples were serially diluted up to 10^{-5} with sterilized 50% aged seawater. 0.1 ml of serially diluted sample was plated using spread plate technique on Zobell marine agar medium (Zobell, 1941) (Hi-media, Mumbai) for analyzing the total heterotrophic bacteria. The plates were incubated at 28±2^{o}C for 24-48 hrs. The microbial load (density) in the given sample was calculated using the given formula and expressed in terms of Colony Forming Units per gram of the sample. Screening of *E. coli, Staphylococcus aureus, Salmonella* and *Shigella* spp*, Vibrio, Listeria* was done using Esoin Methylene Blue (EMB) agar, Manitol Salt Agar (MSA), Salmonella Shigella agar, Thiosulfate-Citrate-Bilesalt-Sucrose agar, and PALCAM agar

The total microbial load in the given sample (CFU.g^{-1}) = Total number of colonies/Total volume of the sample x Volume of the sample plated (0.1 ml) x dilution factor. Each morphologically different colony was isolated and streaked on Zobell Marine agar slants and were maintained at 4^{o}C.

Antibiotic susceptibility studies:

Eleven antibiotics tested were studied. Antibiotic susceptibilities of the isolates were determined by both well diffusion and disk diffusion method using Muller Hinton agar and eleven antibiotics namely Ampicillin (AMP) – 25 µg, Cefuroxime (CXM) – 30 µg, Amoxcillin (AMC) – 30 µg, Cefpodoxime (CPD) – 10 µg, Cephalexin (CN) – 30 µg, Doxycycline (DO) – 30 µg, Levofloxacin (LE) – 5

µg, Gentamicin (GEN) – 10 µg, Ciprofloxacin (CF) – 5 µg, Norfloxacin (NX) – 10 µg and Ofloxin (OF) – 5 µg.

The Clinical and Laboratory Standards Institute (CLSI, 2006) established standards for the interpretation of the data. The antibiotic susceptibility tests of the chosen isolates were conducted using the conventional disc diffusion technique, as described by Bauer *et al*. (1999). The bacterial isolates were cultured overnight and then transferred to peptone water. Subsequently, the cultures were incubated at a temperature of 37°C for a duration of 3 to 4 h. The antibiotics were chosen based on their established efficacy in the treatment of prevalent sea food-borne illnesses and intoxications.

The density of the bacterial culture used for the experiment was modified to 0.5 McFarland standards (1.0 x 10^8 cfu/ml) and thereafter employed to cover (100µl) previously desiccated sterile nutrient agar plates by the implementation of the spread plate technique. The plates underwent a 15 min drying period prior to their use in the susceptibility test. Using sterile forceps, paper discs impregnated with antibiotics were aseptically positioned on the surface of the nutritional agar medium, ensuring equal spacing between each disc. The plates were then incubated overnight at a temperature of 37°C in an incubator. The zones of inhibition surrounding the antibiotic disc were measured to identify and interpret the degree of sensitivity of the test organism to each antibiotic. This was done by categorizing the results as either sensitive (S) or resistant (R) (Jeljaszewicz *et al*., 2000; Akubuenyi *et al*.,2011). The Kirby-Bauer (1999) zone size interpretation chart was used to determine *in vitro* bacterial resistance to different antibiotics.

Biochemical identification and characterization of bacterial isolates:

The bacterial isolates underwent characterization and identification using various methods, including assessment of their motility, microscopic morphology, colonial morphology, and biochemical characteristics. These procedures were carried out following the guidelines provided in the Medical Laboratory Manual for Tropical Countries (Cheesbrough, 2005), as well as referencing Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1994) and the Manual of Microbiology: Tools and Techniques (Kanika, 2011).

Results and Discussion

The Total Culturable Heterotrophic Bacterial counts of fresh shrimp samples ranges from 1.8×10^7 cfu/g To 3.1×10^7 cfu/g with an average count of 2.5×10^7 cfu/g while, their Total Coliform Bacterial count ranged from 2.7×10^5 cfu/g to 3.5×10^6 with an average count of 2.8×10^6. Total Culturable Heterotrophic Bacterial counts of frozen shrimp samples ranges from 8.2×10^6 cfu/g To 9.3×10^6 cfu/g with an average count of 8.7×10^6 cfu/g while, their Total Coliform Bacterial count ranged from 6.9×10^4 cfu/g to 5.7×10^5 with an average count of 2.4×10^4. The Total Culturable Heterotrophic Bacterial counts of dried shrimp samples ranges from 2.2×10^1cfu/g To 3.9×10^1 cfu/g with an average count of 1.7×10^1 cfu/g while, their Total Coliform Bacterial count ranged from 1.1×10^1 cfu/g to 2.2×10^1 with an average count of 1.0×10^1.

The density of THCB and TCB in the fresh shrimp samples exhibited a statistically significant increase compared to the frozen and dried shrimp samples ($p<0.05$). This disparity in density might perhaps be attributable to the low temperature (-21^0C) at which the frozen shrimp samples were maintained, as well as the complete drying process of the dried shrimp samples under direct sunlight. This finding further supports the argument put forth by Ibe (2008) that traditional markets exhibit more unclean and worse food handling practises compared to retailers. A comprehensive collection of 193 bacterial isolates was acquired from a sample of fresh shrimp, whereas 128 isolates were obtained from frozen shrimp and 97 were derived from dried shrimp. The majority of these isolates were found to be associated with the Enterobacteriaceae family (Table 1). The values in

Table 1: Total Heterotrophic Cultivatable Bacterial count and Total Coliform Bacterial count of Fresh shrimp

Place Name	Fresh shrimp		Place Name	Frozen shrimp		Place Name	Dried shrimp	
	TCHB	TCB		TCHB	TCB		TCHB	TCB
A1	2.9×10^{6}	2.2×10^{6}	B1	8.5×10^{6}	8.3×10^{4}	C1	2.2×10^{1}	1.1×10^{1}
A2	1.8×10^{7}	2.7×10^{5}	B2	9.1×10^{6}	5.7×10^{5}	C2	2.5×10^{1}	1.3×10^{1}
A3	1.9×10^{7}	3.5×10^{6}	B3	8.8×10^{6}	8.1×10^{4}	C3	3.5×10^{1}	2.2×10^{1}
A4	3.1×10^{7}	2.3×10^{6}	B4	8.2×10^{6}	8.6×10^{4}	C4	3.0×10^{1}	2.0×10^{1}
A5	2.8×10^{7}	3.1×10^{6}	B5	9.3×10^{6}	6.9×10^{4}	C5	3.9×10^{1}	1.7×10^{1}
*AV.TVC	2.5×10^{7}	2.8×10^{6}	*AV.TVC	8.7×10^{6}	2.4×10^{4}	*AV.TVC	1.7×10^{1}	1.0×10^{1}

Table 2: Isolated bacterial genus of fresh shrimp, frozen shrimp and dried shrimp samples

Fresh shrimp			Frozen shrimp			Dried shrimp		
Bacterial Genus	No. of Positive	%	Bacterial Genus	No. of Positive	%	Bacterial Genus	No. of Positive	%
Vibrio sp.	12	6.2%	*Pseudomonas* sp.	23	17.9%	*E. coli*	13	13.4%
Salmonella sp.	16	8.2%	*Bacillus* sp.	15	11.7%	*Staphylococcus* sp.	09	09.2%
Shigella sp.	19	9.8%	*Micrococcus* sp.	06	4.6%	*Bacillus* sp.	06	06.1%
Aeromonas sp.	21	10.8%	*Enterobacter* sp.	13	10.1%	*Vibrio* sp.	10	10.3%
E.coli	29	15.0%	*Aeromonas* sp.	17	13.2%	*Lactobacillus* sp.	11	11.3%
Staphylococcus sp.	16	8.2%	*Proteus* sp.	09	7.3%	*Salmonella* sp.	08	08.2%
Klebsiella sp.	10	5.1%	*Streptococcus* sp.	11	8.5%	*Pseudomonas* sp.	13	13.4%
Listeria sp.	09	4.6%	*Flavobacterum* sp.	06	4.6%	*Aeromonas* sp.	10	10.3%
Bacillus spp.	18	9.3%	*Lactobacillus* sp.	14	10.9%	*Listeria* sp.	04	04.1%
Pseudomonas sp.	20	11.0%	*Acetobacter* sp.	05	3.9%	*Enterobacter* sp.	08	08.2%
Unknown	23	11.8%	Unknown	09	7.3%	Unknown	05	05.01%

Leera Solomon *et al.* (2013) study exhibit comparable variability. Likewise, bacterial species were isolated from frozen shrimp and dried shrimp samples irrespective of previous frozen storage. Some of the identified bacterial isolates (like *Pseudomonas* sp, *Streptococcus* sp and *Alcaligenes* sp) from frozen shrimp (Table 2, Figs. 1- 3) are known psychrophiles (Prescott *et al*., 1999; Cheesbrough, 2004; Dhinesh *et al*., 2020; Mukilan *et al*., 2022). The results obtained on the cultural, motility, microscopic and biochemical characterizations of the bacterial isolates from fresh, frozen and dried shrimp samples.

In a previous investigation conducted by Jeyasekaran *et al*. (2006), the presence of *Salmonella* sp. in marine fish products that were fresh, frozen, canned, and sun-dried had been identified. Salmonella is known to be present in fresh prawns and other seafood items, particularly when they have been cultured or washed in water

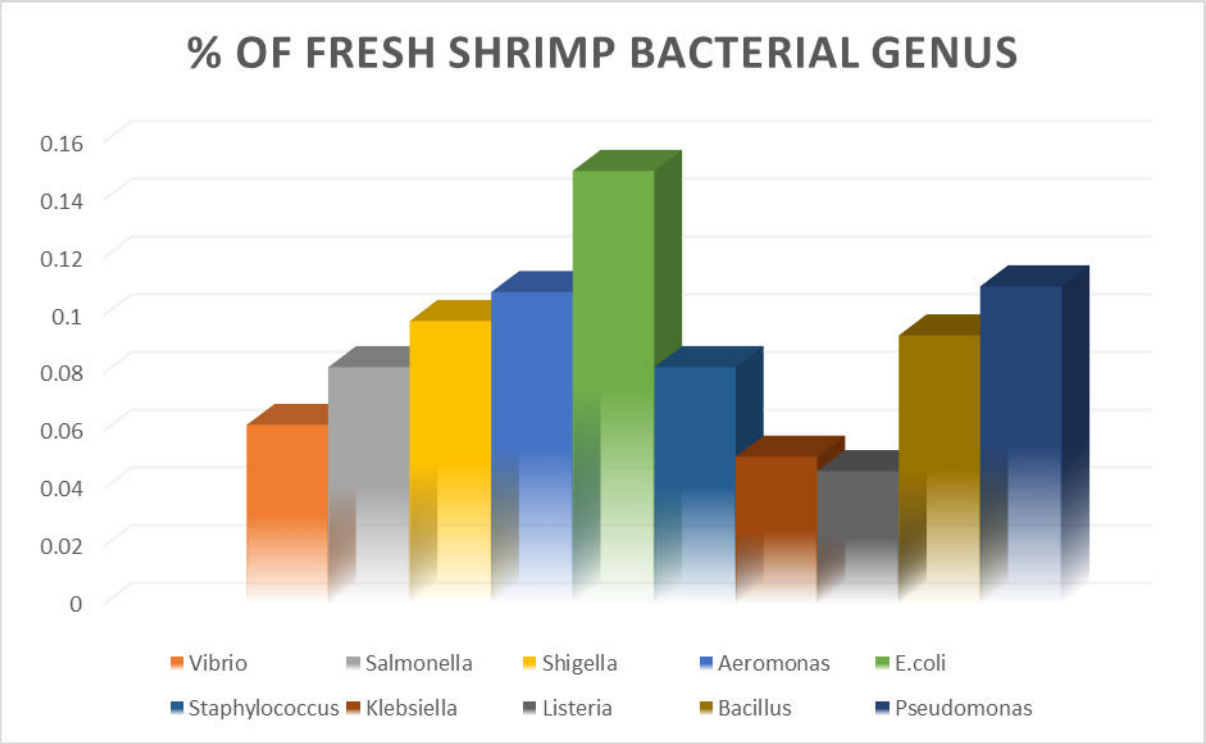

Fig. 1: Percentage of Fresh shrimp bacterial genus.

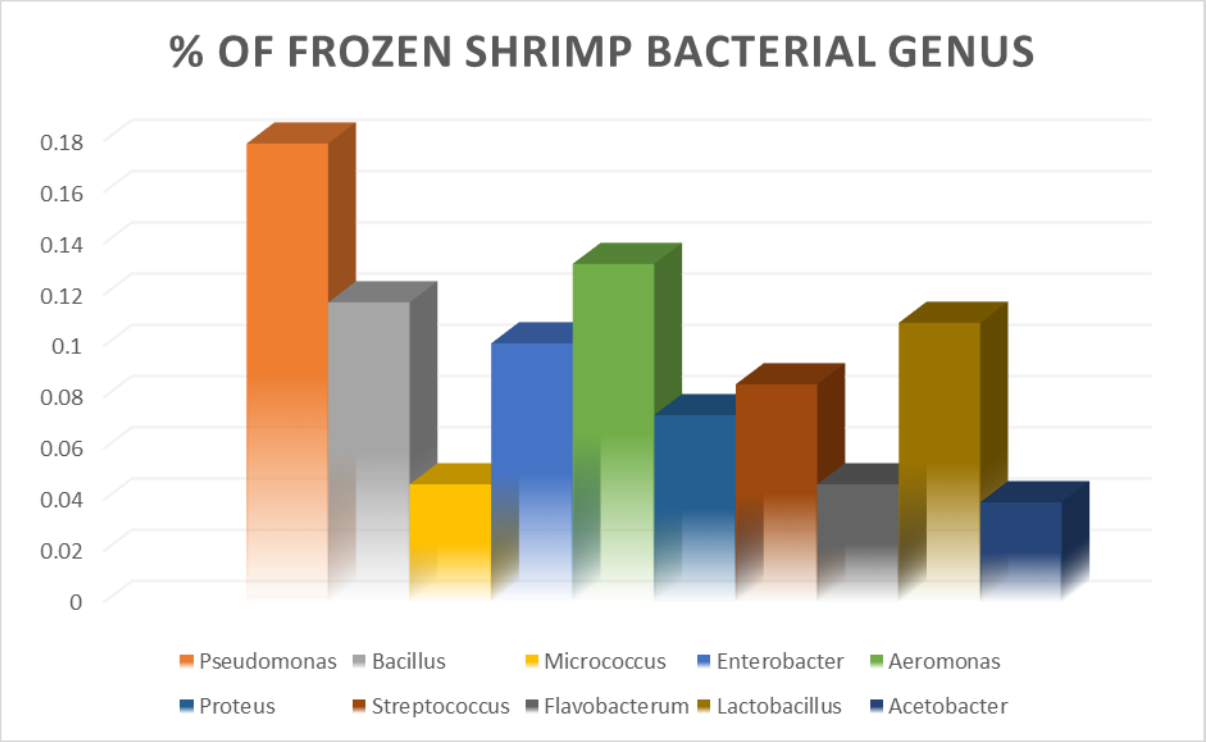

Fig. 2: Percentage of Frozen shrimp bacterial genus.

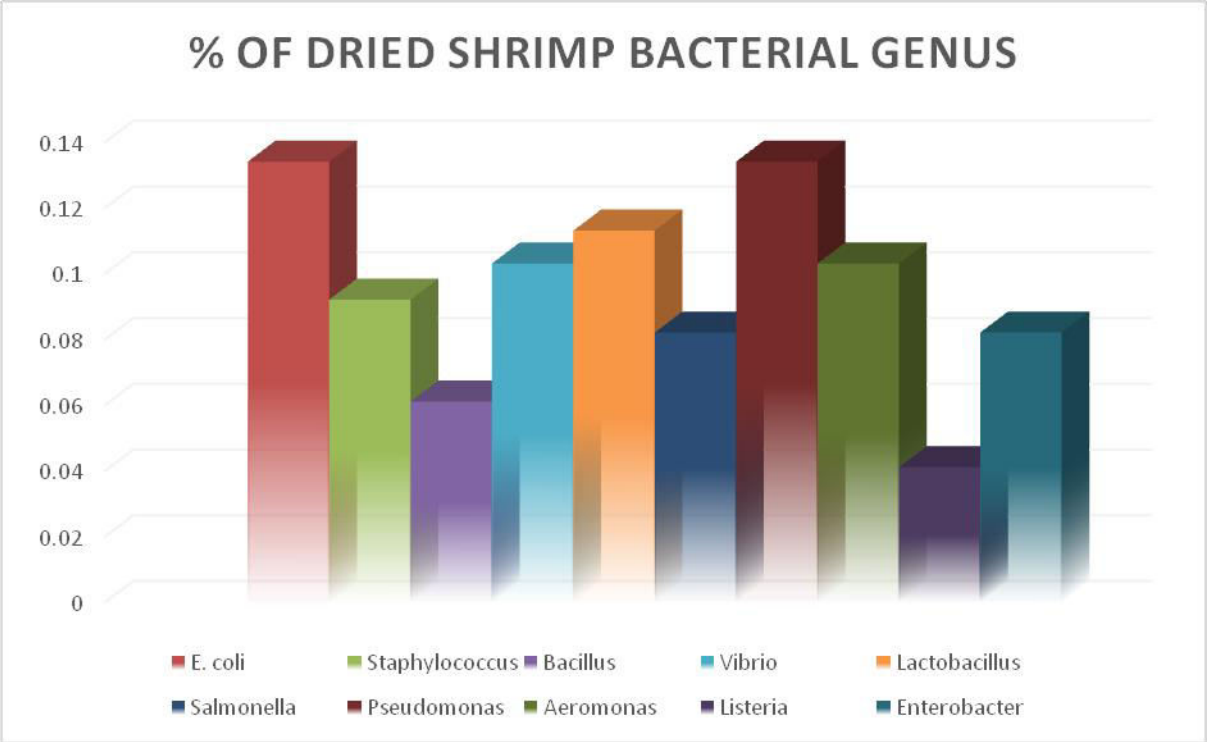

Fig. 3: Percentage of Dried shrimp bacterial genus.

contaminated with sewage or have been contaminated during the manufacturing stage. Based on the findings of the ICMSF (1986), it is expected that marine food items should not include *Salmonella* and *Vibrio cholera*. In the current investigation, the presence of *Salmonella* sp. was detected in the fresh shrimp products, rendering them unsuitable for human consumption. It is worth noting that the majority of shrimp undergo a cooking process before being consumed, which significantly reduces the potential health hazards to consumers. However, it is important to be cautious of cross-contamination in kitchen environments, as this could pose a risk to food safety (Huss, 1994; USDA, FSIS, 2008).

The resistance patterns shown by bacterial isolates obtained from fresh shrimp samples indicated the presence of several antibiotic resistances (Table 3). The isolates were 100% resistant to five antibiotics: AMP, CPD, DO, GEN. The antibiotics CZM, AMC, CN, LE, CF, NX, exhibited the lowest levels of resistance, with resistance rates of 85%. 97%, 93%, 81%, 75%, 76% respectively. The resistance pattern of individual isolates revealed that five isolates (*Shigella* sp, *Aeromonas* sp, *Listeria* sp, *Bacillus* spp and *Pseudomonas* sp) exhibited complete resistance (100%) to all antibiotics examined in the present study. The resistance profiles of bacterial isolates obtained from frozen shrimp revealed that all isolates exhibited resistance to several antibiotics (Table 4). All isolates showed complete resistance to antibiotics, namely AMP, CF, NX, GEN. Nevertheless, it is worth noting that only *Bacillus* spp, *Aeromonas* sp, *Streptococcus* sp, *Flavobacterium* sp strains obtained from frozen prawns showed 100% resistance to all eleven (11) drugs that were subjected to testing of resistance. Dried shrimp revealed all isolates exhibited resistance to five antibiotics namely AMP, CXM, OF, CF, DO. The antibiotics AMC, CPD, GEN, LE, NX, CN exhibited the lowest levels of resistance, with resistance rates of 85%. 91%, 98%, 71%, 95%, 68%, respectively (Table 5). Previous studies have shown that *Escherichia coli* is the primary carrier of antibiotic resistance in faecal flora, whereas resistance in other genera is

Table 3: Antibiotic sensitivity results of fresh shrimp sample

S. No.	Fresh shrimp Bacterial Genus	Antibiotic Disc Names											
		AMP (25 µg)	CXM (30 µg)	AMC (30 µg)	CPD (10 µg)	CN (30 µg)	DO (30 µg)	LE (5 µg)	GEN (10 µg)	CF (5 µg)	NX (10 µg)	OF (5 µg)	%
1.	*Vibrio* sp.	R	R	S	R	S	R	R	R	R	R	R	85%
2.	*Salmonella* sp.	R	R	S	R	R	R	S	R	R	R	R	97%
3.	*Shigella* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
4.	*Aeromonas* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
5.	*E .coli*	R	R	R	R	R	R	S	R	R	R	R	93%
6.	*Staphylococcus* sp.	R	S	S	R	R	R	R	R	S	S	R	81%
7.	*Klebsiella* sp.	R	S	R	R	R	R	R	R	R	S	S	75%
8.	*Listeria* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
9.	*Bacillus* spp.	R	R	R	R	R	R	R	R	R	R	R	100%
10.	*Pseudomonas* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
11.	Unknown	R	R	R	R	S	R	R	R	S	R	S	76%

Table 4: Antibiotic sensitivity results of frozen shrimp sample

S. No.	Frozen shrimp Bacterial Genus	Antibiotic Disc Names											
		AMP (25 µg)	CXM (30 µg)	AMC (30 µg)	CPD (10 µg)	CN (30 µg)	DO (30 µg)	LE (5 µg)	GEN (10 µg)	CF (5 µg)	NX (10 µg)	OF (5 µg)	%
1.	*Pseudomonas* sp.	R	R	R	S	S	R	S	R	R	R	R	66%
2.	*Bacillus* spp.	R	R	R	R	R	R	R	R	R	R	R	100%
3.	*Micrococcus* sp.	R	S	R	R	R	S	R	R	R	R	R	85%
4.	*Enterobacter* sp	R	R	R	S	R	R	R	R	R	R	S	87.5%
5.	*Aeromonas* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
6.	*Proteus* sp.	R	R	S	R	R	S	R	R	R	R	R	95%
7.	*Streptococcus* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
8.	*Flavobacterum* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
9.	*Lactobacillus* sp.	R	R	R	S	S	R	S	R	R	R	R	75%
10.	*Acetobacter* sp.	R	R	R	R	R	R	R	R	R	R	S	92.7%
11.	Unknown	R	R	R	R	R	R	R	R	R	R	R	100%

Table 5: Antibiotic sensitivity results of fresh dried sample

S. No.	Dried shrimp Bacterial Genus	Antibiotic Disc Names											
		AMP (25µg)	CXM (30µg)	AMC (30µg)	CPD (10µg)	CN (30µg)	DO (30µg)	LE (5µg)	GEN (10µg)	CF (5µg)	NX (10µg)	OF (5µg)	%
1.	*E. coli*	R	R	R	S	R	R	R	R	R	S	R	85%
2.	*Staphylococcus* sp.	R	R	R	R	S	R	R	R	R	R	R	91%
3.	*Bacillus* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
4.	*Vibrio* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
5.	*Lactobacillus* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
6.	*Salmonella* sp.	R	R	R	R	S	R	R	R	R	R	R	98%
7.	*Pseudomonas* sp.	R	R	S	R	R	R	R	S	R	R	R	71%
8.	*Aeromonas* sp.	R	R	S	R	R	R	R	S	R	R	R	95%
9.	*Listeria* sp.	R	R	R	R	R	R	R	R	R	R	R	100%
10.	*Enterobacter* sp.	R	R	R	S	S	R	R	R	R	S	R	69%
11.	Unknown	R	R	S	R	R	R	S	S	R	S	R	88%

rare, particularly in the absence of antimicrobial selection (Guardaasi *et al*., 1998; Osterblad *et al*., 2000).

The findings of this research indicate that other genera have shown the potential to acquire multiple antibiotic resistance (MAR) capabilities, exceeding those of *Escherichia coli*. The range of resistance discovered, when linked to *Escherichia coli*, suggests that the MAR genes responsible for the unique characteristic identified in the research may also be inherent in some bacterial taxa. The prevalence of Gram-negative bacteria was more extensive compared to Gram-positive bacteria. This discovery aligns with the findings reported by Jeljaszewicz *et al.* (2000). In research conducted by Jeyasekaran *et al*. (2006), a variety of resilient human infections were identified and isolated. These pathogens included *Escherichia coli*, *Enterococcus* sp., *Salmonella* sp., *Shigella flexneri*, *Staphylococcus* sp., and *Vibrio* sp. The potential health danger posed by antibiotic-resistant bacteria in animal-derived food products has been acknowledged by the United States Department of Agriculture's Food Safety and Inspection Service (USDA, FSIS, 2008) as well as by Courvalin and Weber (2005). This concern arises due to the ability of bacteria to transmit resistance traits among themselves, leading to the possibility of antibiotic-resistant infections that are unresponsive to treatment with antibiotics (Lery, 2002). Previous studies conducted in Nigeria by Adebayo *et al*. (2012) have identified the presence of multi-drug-resistant (MDR) organisms in seafood. Additionally, Akubuenyi *et al*. (2011) have reported the isolation of MDR organisms from wastewater samples. Therefore, the extensive commercialization of this medication might potentially serve as a means for the rapid proliferation of antibiotic-resistant bacteria.

In extreme instances, typhoid fever has been shown to result in the development of septicemia and ulcers (Banwart, 1989; USDA/FSIS, 2008). According to Frazier and Westthoff (1978), infection with *Shigella* sp. leads to the development of bacillary dysentery, characterised by symptoms such as cramping, diarrhoea accompanied by the presence of blood and mucus. *Vibrio parahaemolyticus* is responsible for

inducing symptoms such as diarrhoea and fever, but *V. cholerae* is recognised for its ability to produce cholera, characterised by manifestations including rice water stool, vomiting, and fast dehydration (NAS, 1964; Nester *et al*.,1998). According to Safe Food and Public Health (2008*), Staphylococcus aureus* has been associated with cases of food poisoning resulting in symptoms of vomiting and diarrhoea. Similarly, *Bacillus cereus* has been identified as a causative agent of gastroenteritis.

Staphylococcus aureus is known to make an enterotoxin that causes staphylococcal enterotoxicosis or staphylococcal intoxication (Frazier and Westthoff, 1978; Evenson *et al*., 1988). According to Ekpo *et al*. (2010) and Elliott and Michener (2003), the lysis of *B. cereus* inside the digestive tract results in the production of an exoenterotoxin, which is what causes *Bacillus cereus* gastroenteritis. The presence and composition of indicator bacteria on prawns show variability depending on the contact environment and preservation technique used (Tobor, 1984; USDA, FSIS, 2008).

Conclusion

Shrimp often exhibit contamination by fecal-derived infections, which may result from water bodies in their cultivation environment being polluted with fecal matter or during the stages of harvesting and processing. However, the presence of these bacteria in fish, prawns, or other aquatic food sources may also be attributed to cross-contamination. All the genera identified in this investigation exhibited multiple antibiotic resistances (MAR), with the greatest resistance level seen against the most often used antibiotics. Gram-positive bacteria often exhibit greater susceptibility to antibiotics compared to Gram-negative bacteria. The potential health implications for the general population might be significant if prawns with a high concentration of multidrug-resistant bacterial infections were consumed. The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) established microbiological requirements that, when adhered to, may successfully reduce the transmission of food-borne illnesses via prawns. It is essential to establish and implement regulations to discourage shrimp harvesting in water bodies contaminated with fecal matter. This measure is crucial in mitigating the potential transmission of food-borne illnesses resulting from eating shrimp products that have been contaminated. To minimize the occurrence of cross-contamination in shellfish items offered in retail markets, it is essential to adhere to environmental sanitation practices, ensure adequate handling techniques, and maintain personal hygiene standards. Furthermore, it is important to maintain a consistent temperature in the refrigerator during frozen storage and sun drying in order to inhibit the proliferation of possible contaminants, including psychrophilic bacteria. The appropriate use of prescription antibiotics for managing food-borne bacterial illnesses is recommended to consider the presence of multiple antibiotic resistance (MAR) in pathogenic bacteria.

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