BIOCHEMICAL ASSAY FOR DETECTION OF PATHOGENIC AND PROBIOTIC BACTERIA AT SHRIMP AND PRAWN FROM WILD AND DIFFERENT CULTURE CONDITIONS IN BANGLADESH

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Abstract

We conducted biochemical assays on shrimp and prawn samples in Bangladesh, revealing the presence of both harmful and beneficial bacteria. Pathogens such as Escherichia coli, Klebsiella pneumonia, Vibrio cholera, Pseudomonas aeruginosa, Clostridium perfringens, Salmonella typhimurium, Shigella flexneri, Listeria monocytogenes, and Staphylococcus aureus were detected in these samples, with Escherichia coli being the most prevalent. Additionally, we identified three potential probiotic bacteria - Bacillus subtilis, Lactobacillus spp., and Enterobacter aerogenes. These probiotics can enhance immunity, support survival, and have minimal environmental impact. Shrimp and prawn farms in Bangladesh may be susceptible to harmful microorganisms due to contaminated water sources and subpar sanitation during post-harvest handling and processing. Our use of biochemical characterization proves a dependable method for distinguishing pathogenic and probiotic bacteria in shrimp and prawns, offering valuable insights for future disease research.

Keywords: **Biochemical tests; pathogenic bacteria; prawn; probiotic bacteria; shrimp**

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Introduction

The decline in grab fisheries has boosted aquaculture in Bangladesh, which is now the fourth largest sector globally (Voumik and Shah, 2014). Seawater shrimp and freshwater prawns are particularly vital for the country's fisheries industry and as a source of foreign exchange. Shrimp and prawn exports contribute significantly to Bangladesh's revenue, with 4.37% of GDP and 2.01% of export revenues attributed to this industry. In the 2012-2013 fiscal year, the United States exported 50,333 MT of frozen shrimp and prawns, valued at $788.03 (Zafar *et al*., 2015).

Bangladesh harvests 36 species of shrimp (Ali *et al*., 2016), including *Penaeus monodon* (marine shrimp) and *Macrobrachium rosenbergii* (freshwater prawn). Marine shrimp, locally known as Bagda, is farmed in saltwater ponds along the shore. Freshwater prawn, also called Golda, is raised in freshwater ponds along the coast and certain inland and northern regions. Shrimp and prawn exports are popular and contribute significantly to the country's GDP, with only three percent of output sold domestically. The majority are sent abroad (Hossain *et al*., 2013).

Generally, diseases in shrimp and prawn farming arise from inadequate pond management, lack of technical expertise, extension services, and credit facilities. Shrimp deaths are primarily caused by White spot syndrome virus (WSV), vibriosis, soft shell disease, muscle necrosis disease, black gill disease, fungal and protozoan infections (Afsharnasab *et al*., 2014). Prawns are susceptible to Metamorphosis Molt Mortality Syndrome, bacterial necrosis, larval mid-cycle sickness, and white prawn disease (Saurabh and Sahoo, 2008). Successful prevention of viral infection has been achieved through screening of spawners and fry at the hatchery.

The majority of this item is produced at coastal aquaculture facilities, providing thousands of job opportunities. The most common shrimp species caught in the country are Black Tiger Shrimp (Bagda), Brown Shrimp (Horina), Indian White Shrimp (Chaka), and Giant Freshwater Shrimp (Golda) (Rahman and Hossain, 2009). Farm-produced shrimp and prawns are primarily exported as frozen seafood to the United States, the EU, and several Asian nations. However, the cultivation and harvesting of shrimp and prawns have adverse environmental effects. Our shrimp products have been banned by several countries due to environmental damage and the presence of harmful bacteria (Martínez-Porchas *et al*., 2010).

Shrimp and prawn deterioration is caused by pathogenic and probiotic microorganisms (Luis-Villaseñor *et al*., 2012), and can be prevented by using probiotics, which increase the population of beneficial bacteria and outcompete any potentially hazardous pathogens.

Due to shrimp and prawn's increasing significance as one of Bangladesh's key export goods, we made an effort to evaluate shrimp and prawns for pathogenic and probiotic microorganisms. The objectives of the study were to detect various pathogenic and probiotic bacteria through Biochemical tests and to understand the microbial status of exportable shrimp and prawn products in Bangladesh.

Materials and Methods

Sample Collection

Shrimp specimens (Figure 1(a)) were taken from markets in Bangladesh (Noakhali and Khulna), traditional gher (Shatkhira, Bagerhat, Patuakhali), and semi-intensive gher (Khulna). Prawn specimens (Figure 1(b)) were collected from rivers and canals in the wild, ponds, rice fields, and markets (Kachua and Fakirhat of Bagerhat, Chairman Ghat and Maijdee of Noakhali; Bangladesh) (Table 1). The samples were collected, aseptically transferred in a polythene bag to the lab for microbiological analysis, processed within three hours of collection, and stored at 4°C to 8°C.

Sample Processing

Each shrimp and prawn had 10 g of their heads, bodies, skin surfaces, gills, fins, guts, abdomens, and telsons removed aseptically and placed in sterile containers. After being homogenized, the material was diluted into 90 ml of sterile normal saline. After transferring 1 ml of the homogenized solution to 9 ml of normal saline, the dilution series was continued 10-fold up to 10^6 sequentially (Rahman *et al*., 2012).

Table 1. List of shrimp and prawn samples used during the present microbial study

Species	Collecting place	Location	No. Sample used
Penaeus monodon	a) Traditional gher	Shatkhira	5
	b) Traditional gher	Bagerhat	5
	c) Traditional gher	Patuakhali	5
	d) Semi-intensive pond	Khulna	3
	e) Local Market	Khulna	5
	f) Local Market	Maijdee, Noakhali	6
Macrobrachium rossenbergii	a) Meghna River	Chairman Ghat, Noakhali	3
	b) Prawn farm	Kachua, Bagerhat	4
	c) Canal	Suborno chor, Noakhali	5
	d) Rice Field	Fakirhat, Bagerhat	3
	e) Wholesale market	Chairmanghat, Noakhali	3
	f) Market	Maijdee, Noakhali	10

Isolation of Microorganisms from Shrimp and Prawn Samples

Standard microbiological procedures (Rahman *et al*., 2012) were used to analyze the samples in a completely sterile environment in the research laboratory of the Department of Biotechnology and Genetic Engineering at Noakhali Science and Technology University, Bangladesh.

Culture

Nutrient agar media (Mathivanan *et al*., 2021) was used to incubate the swabs at 37˚c in an aerobic setting (Hannan *et al*., 2019). Different morphological, physiological, and biochemical characteristics of the isolated bacterial colonies were used to confirm their identities.

***Escherichia coli, Pseudomonas* sp. *and Klebsiella* sp.**

A 0.1 ml suspension was placed over the surface of MacConkey (Rahman *et al*., 2012) and Nutrient agar medium and incubated at 37°C for 24 h to extract *Escherichia coli*, p*seudomonas* sp., and *Klebsiella* sp. from each 10^{-1} to 10^{-3} dilution tube. The plate was examined for typical colonies after incubation.

***Salmonella* sp., *Shigella-like organisms, and Vibrio* sp.**

In order to enrich for *Salmonella*, S*higella*-like organisms, and V*ibrio* spp., 1 ml of homogenized sample was added to 9 ml of selenite cystine broth and alkaline peptone water (10^{-1} dilution), and the mixture was incubated at 37°C for 6 h onto XLD and TCBS agar (Rahman *et al*., 2012).

***Clostridium* sp.**

To inactivate vegetative cells, we diluted each sample 1:8 in sterile saline and heated the mixture to 80°C for 15 min. Each tube of dilutions from 10^{-4} to 10^{-6} included 0.1 ml of suspension, which was poured onto agar plates containing *perfringens* (Rahman *et al*., 2012). The plates were placed in a candle jar and incubated at 37°C for 48 h.

***Listeria* sp.**

To identify *Listeria* from primary cultures, it was placed onto the medium for Listeria (Rahman *et al*., 2012) and incubated at 37ºC h. Colonies that looked olive green were identified and counted as possibly containing *Listeria* sp.

***Staphylococcus* sp.**

A selective medium for the isolation of pathogenic *Staphylococci* is mannitol salt agar (Saito *et al*., 2011). It is particularly nutrient-dense due to the presence of beef extract and protease peptone.

Isolation of Probiotic Microorganisms from Shrimp and Prawn Samples

Samples were cleaned with distilled water to get rid of debris like dust and sediment. Under sterile circumstances, the fish's digestive systems were dissected. In addition, 10 ml of sterile distilled water was used to homogenize the GI tract, and then the specimen was centrifuged at 13,000 rpm for 10 min. The resulting supernatant was collected and serially diluted with sterile distilled water (Temmerman *et al*., 2003). Following a 24-hour incubation at room temperature, was placed on Nutrient Agar, LBS Agar, and MacConkey Agar respectively.

Probationary Identification of the Bacterial Isolates

A wide range of colony forms in a variety of culture mediums was extensively studied. The colonies were documented for their morphological properties such as color, form, elevation, surface roughness, opacity, etc.

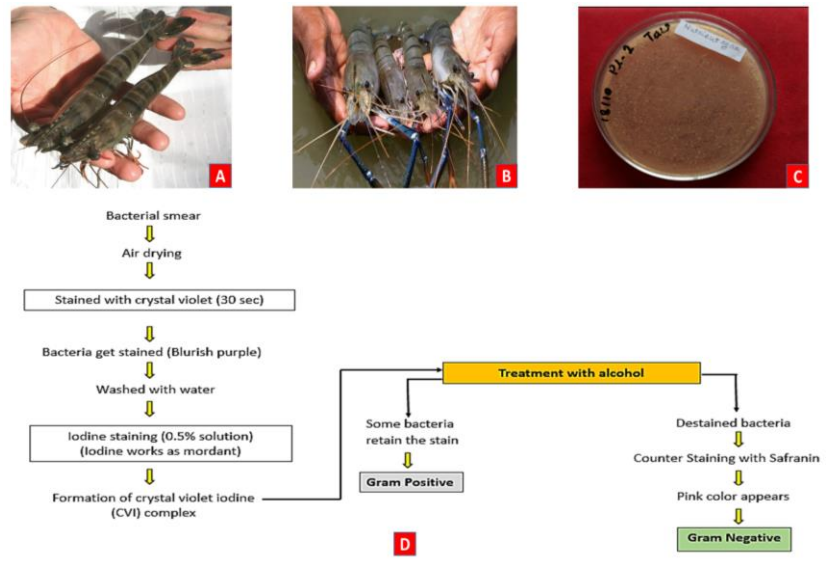

Figure 1. Samples of (A) Shrimp, (B) Prawn, (C) Bacterial growth on nutrient agar media, and (D) Gram staining procedure

Gram staining allowed for the analysis of cellular size and shape (Moyes *et al*., 2009) (Figure 1(d)). Finally, several biochemical tests, including those for motility, catalase, oxidase, OF (oxidation-fermentation), gelatin hydrolysis, indole production, methyl red, Voges-Proskauer, citrate utilization, carbohydrate fermentation, H_2S, nitrate reduction, and urease were carried out to provisionally identify the bacteria of interest, as outlined in the Manual of Methods for General Bacteriology.

Results

Colony Morphology

Bacteria growth was detected after incubating Nutrient agar at 37°C in an aerobic environment for 24 h (Figure 1(c)). The morphological features of the bacteria as they appeared on nutrient agar are shown in Table 2.

Subculture on Different Selective Media

Within the scope of this investigation, our objective was to cultivate a culture that is robust enough to survive protracted subculture. The outcomes are depicted in Figure 2.

Gram Staining

Gram staining was used to classify bacteria into two broad categories defined by the components of their cell walls. Bacterial cells were dyed a distinctive color to indicate whether they belonged to the gram-positive (purple or violet) or gram-negative (reddish or pink) group. *Pseudomonas* sp., *Escherichia coli*, *Klebsiella* sp., and *Vibrio* sp. all appeared pink and rod-shaped under the microscope when cultured from shrimp, except *Vibrio* sp., which appeared comma-shaped. All of these pink or red bacteria belonged to a group known as gram-negative organisms. However, the gram-positive bacteria that resulted from a species of *Clostridium* had a blue-violet tint and was rod-shaped (Figure 3). Additionally, *Listeria* sp. and *Staphylococcus* sp. bacterial colonies from prawn cultures appeared tiny as violet-colored, rod-shaped follow-on gram-positive bacteria. Gram-negative bacteria produced from *Salmonella* sp. and *Shigella* sp. were pink in color and had short, plump, and rod shapes, respectively (Figure 3). Gram-positive bacteria *Bacillu*s sp. and *Lactobacillus* spp. were violet in color and rod-shaped. In contrast, *Enterobacter* sp. was gram-negative because it was obtained in a pink hue and had a rod form (Figure 3).

Table 2. Bacterial Morphological Characteristics on Nutrient agar

Name	Colony Color	Colony Shape
1) *E. coli*	Greyish-white	Smooth, Circular
2) *Vibrio* sp.	Pearly gray	Smooth, Flat
3) *Bacillus* sp.	White cream/Yellow	Rod-shaped
4) *Shigella* sp.	Colorless	Circular, Smooth surface
5) *Salmonella* sp.	Greyish-white	Circular
6) *Klebsheilla* sp.	Ash	Circular, Small
7) *Staphylococcus aureus*	Golden Yellow	Large, Circular, Smooth
8) Clostridium perfringens	Translucent, opaque, smooth colonies	Small rod, single or paired, violet in color
9) Listeria sp.	Very light growth & give bluish coloration	Reflect blue light
10) Pseudomonas sp.	Mucoid colonies with Blue-Green color	large, opaque, flat colonies with irregular margins and distinctively fruity odour colonies

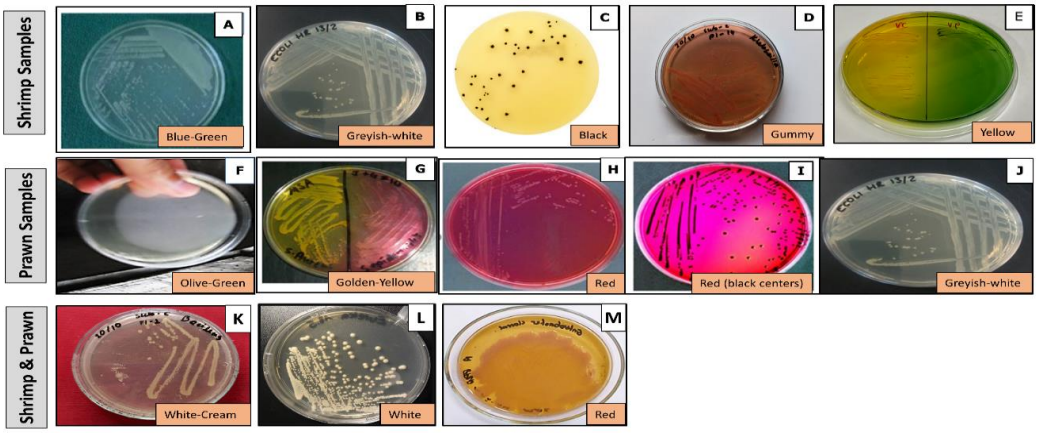

Figure 2. Growth of (A) *Pseudomonas* sp. [Nutrient agar], (B) *E. coli* [Nutrient agar], (C) *Clostridium* sp. [Perfringens agar], (D) *Klebsiella* sp. [MacConkey agar], (E) *Vibrio* sp. [TCBS agar], (F) *Listeria* sp. [Listeria isolation media], (G) *Staphylococcus* sp. [Mannitol Salt agar], (H) *Shigella* sp. [XLD agar], (I) Salmonella sp. [XLD agar], (J) E. coli [Nutrient agar], (K) Bacillus sp. [Nutrient agar], (L) *Lactobacillus* sp. [LBS agar], (M) *Enterobacter* sp. [MacConkey agar]; A-J: Pathogenic Bacteria, K-M: Probiotic Bacteria

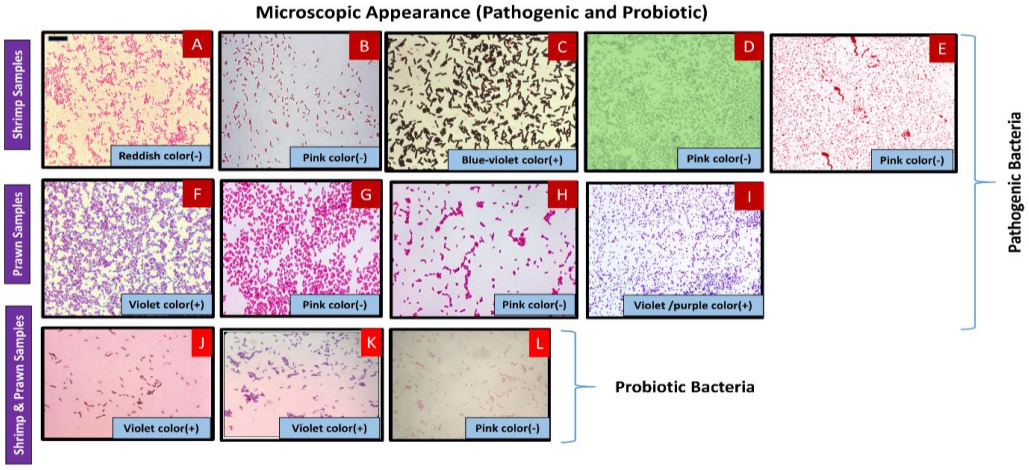

Figure 3. Gram staining result of (A) *Pseudomonas* sp., (B) *Escherichia coli*, (C) *Clostridium* sp., (D) *Klebsiella* sp., (E) *Vibrio* sp., (F) *Listeria* sp., (G) *Salmonella* sp., (H) *Shigella* sp., (I) *Staphylococcus* sp., (J) *Bacillus* sp., (K) *Lactobacillus* sp., (L) *Enterobacter* sp., Gram positive: '+', Gram negative, '-'.

Table 3. Biochemical tests confirmative of the presence of the specific pathogens

Colonies on media	Biochemical Tests of Pathogenic Bacteria (Shrimp)																		
							IMViC Tests				Carbohydrate Fermentation								
	Gram Staining	Motility	Catalase	Oxidase	OF	Gelatin Hydrolysis	Indole	MR	VP	Citrate Utilization	Glucose	Fructose	Lactose	Maltose	Sucrose	H_2S Production	Nitrate Reduction	Urease Production	Tentatively Identified Bacteria
Nutrient agar (Greyish-white)	-	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	*Escherichia coli*
MacConky (Gummy)	-	-	+	+	+	-	-	-	+	+	+	+	-	+	-	-	+	+	*Klebsiella pneumoniae*
TCBS (Small-Yellow)	-	+	+	+	+	+	+	+	-	+	+	+	V	+	+	-	+	-	*Vibrio cholera*
Nutrient (Blue-green)	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	+	-	*Pseudomonas aeruginosa*
Perfringens agar (Black)	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	*Clostridium perfringens*

N.B. V: Variable; +: Positive; -: Negative

Biochemical Tests

To put it another way, bio-oxidation processes are crucial for bacteria. By oxidizing organic compounds or fermenting sugars, these processes aid bacteria in producing energy. These responses were used to determine the identities of the bacteria, which are shown in Tables 3-5.

Discussions

Microbial Community of Shrimp and Prawn

There should not be too much time between harvesting and processing, since this might negatively affect the finished product's quality by causing the raw ingredients to lose some of their freshness. The quality of shrimp and prawns remains low during this time (Rahman *et al*., 2012). It's crucial to minimize the delay in shrimp/prawns getting from the docks to the processing facilities. Spoilage of food occurs when microorganisms develop due to improper handling and poor processing. In this investigation, ten different pathogenic bacteria were identified in shrimp and prawn samples.

Escherichia coli

The detection of *Escherichia coli* (*E. coli*) on the external abdominal area of shrimp and prawns, isolated using Nutrient agar medium. The *E. coli* colonies on the agar appeared bluish-gray. Identification was performed using Gram's staining and biochemical assays. While most *E. coli* strains are non-pathogenic, some can cause illnesses like diarrhea, urinary tract infections, respiratory ailments, or pneumonia. Poor sanitation can lead to its presence in shrimp and prawn farms. The research aimed to find *E. coli* isolates of public health significance in shrimp and prawns from wholesale and local markets in Noakhali Chairman Ghat (Maijdee) and Khulna, Bangladesh, to assess their microbiological quality.

Table 4. Biochemical tests confirmative of the presence of the specific pathogens

Colonies on media	Biochemical Tests of Pathogenic Bacteria (Prawn)																		
							IMViC Tests				Carbohydrate Fermentation								
	Gram Staining	Motility	Catalase	Oxidase	OF	Gelatin Hydrolysis	Indole	MR	VP	Citrate Utilization	Glucose	Fructose	Lactose	Maltose	Sucrose	H_2S Production	Nitrate Reduction	Urease Production	Tentatively Identified Bacteria
Nutrient agar (Greyish White)	-	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	*Escherichia coli*
XLD agar (Red colonies with black centers)	-	+	+	-	-	-	-	+	-	-	+	+	-	+	-	+	+	-	*Salmonella typhimurium*
XLD agar (Red colonies)	-	-	+	-		-	V	+	-	-	+	+	-	V	-	-	+	-	*Shigella flexneri*
Listeria isolation (Olive green)	+	+	+	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	*Listeria monocytogenes*
Mannitol Salt Agar (Golden Yellow)	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	*Staphylococcus aureus*

N.B. V: Variable; +: Positive; -: Negative

Table 5. Biochemical tests confirmative of the presence of the specific bacteria

Colonies on media	Biochemical Tests of Probiotic Bacteria (Shrimp and Prawn)																		
							IMViC Tests				Carbohydrate Fermentation								
	Gram Staining	Motility	Catalase	Oxidase	OF	Gelatin Hydrolysis	Indole	MR	VP	Citrate Utilization	Glucose	Fructose	Lactose	Maltose	Sucrose	H_2S Production	Nitrate Reduction	Urease Production	Tentatively Identified Bacteria
Nutrient agar (White cream)	+	+	+	V	-	+	-	-	+	+	+	+	V	+	+	-	+	-	*Bacillus subtilis*
LBS Agar (White Colonies)	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	*Lactobacillus* spp.
MacConkey (Red colonies)	-	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	+	*Enterobacter aerogenes*

N.B. V: Variable; +: Positive; -: Negative

Klebsiella pneumonia

The presence of *Klebsiella pneumoniae* (*K. pneumoniae*) bacteria in the telson of a shrimp sample. These bacteria, commonly found in human and animal feces and intestines, were isolated using MacConkey agar plates, appearing as a sticky mass. Identification was achieved through Gram's staining and a biochemical assay. While generally harmless in the intestines, *K. pneumoniae* can cause life-threatening infections if it spreads to other parts of the body. The extensive use of antibiotics in shrimp farming has contributed to the spread of these bacteria, posing a greater risk, especially when someone is already ill. *K. pneumoniae* is known for its versatility as a pathogen, capable of infecting various organs and systems (Schmiedecke *et al*., 2019).

Vibrio cholerae

Head and gill samples from shrimp commonly contained *Vibrio cholerae*. The agar medium TCBS was used for the isolation process. Colonies of *Vibrio cholerae* on TCBS agar appeared small and yellow. Bacterial identification was achieved through the use of Gram staining and biochemical testing. Pathology is brought on by *V. cholerae* strains that produce toxins. *V. cholerae* is a gram-negative, comma-shaped bacteria that has a single polar flagellum and moves around very quickly. The prevalence of *Vibrio* infections in shrimp and prawns is likely to increase as a result of water pollution in the shrimp growing system.

Pseudomonas aeruginosa

Pseudomonas aeruginosa, a gram-negative encapsulated bacterium, was detected in shrimp's abdominal cavity, posing potential health risks to various organisms, including humans. Isolation was performed using Nutrient agar, revealing distinct blue-green colonies. Identification through Gram's staining showed rod-shaped bacteria. Improper cleaning, decomposition, or installation likely introduced these bacteria to the shrimp colony.

Pseudomonas aeruginosa is a multidrug-resistant bacterium with significant medical implications due to its

widespread prevalence, advanced antibiotic resistance mechanisms, and association with severe conditions like ventilator-associated pneumonia and septic shock.

Research by Chau *et al*., (2011) suggests that *Pseudomonas* sp. exhibits antibacterial properties against pathogens and adapts well to various physicochemical conditions in shrimp rearing systems. It also produces extracellular enzymes and is safe for shrimp larvae and juveniles, making it a potential candidate for controlling shrimp illnesses and a probiotic for shrimp cultivation (Chau *et al*., 2014).

Clostridium perfringens

The spore-forming gram-positive bacteria *Clostridium perfringens* (*C. perfringens*) is widely distributed in the environment and has been isolated from the digestive tracts, tissues, and telson of shrimp and prawns. *Perfringens* agar was used during the isolation procedure. Black colonies were seen as typical of *Clostridium perfringens* when cultured on *Perfringens* agar. The bacteria were identified using Gram's staining and a biochemical assay. It is usual to find *C. perfringens* on uncooked chicken and pork. It thrives in oxygen-deplete environments and may reproduce fast when circumstances are right. In the gut, *C. perfringens* creates a toxin that is harmful to humans. The prevalence of these pathogens in the shrimp production system is mostly attributable to the use of unhealthy feed.

Salmonella typhimurium

Salmonella typhimurium, a harmful gram-negative bacterium, was detected in shrimp and seafood. Isolation on XLD agar revealed red colonies with black cores. Identification was done using Gram's staining and biochemical analysis.

This bacterium, often transmitted among birds through droppings, can lead to acute salmonellosis, causing diarrheal illness, chronic health problems, and significant costs, as noted by Archer and Kvenberg (Heinitz *et al*., 2000).

To prevent *Salmonella* contamination, fishery product processors are required by law to adopt Hazard Analysis and Critical Control Point (HACCP) systems. These systems encompass key sanitation practices, ensuring water and food contact surface safety, cross-contamination prevention, protection from adulterants, safe toxic substance use, employee health monitoring, and pest control. Compliance with these regulations is crucial for food safety.

Shigella flexneri

A *Shigella* species, *Shigella flexneri*, was isolated from the prawn's skin's outer layer. The bacterium *Shigella* is a rod-shaped, gram-negative, non-motile, spore-less bacterium. For the separation, we utilized XLD agar media. Colonies of *Salmonella typhimurium* on XLD agar will stain a distinctive red. Gram's staining and a biochemical experiment helped to positively identify the bacteria. Bacillary dysentery, caused by the extremely dangerous *Shigella* bacteria, is a terrible illness. *S. flexneri* is primarily found in developing countries. While *Shigella*-related deaths in Bangladesh are extremely uncommon (0.01%), it is nevertheless a serious health concern (Kotloff *et al*., 1999).

Listeria monocytogenes

The gram-positive pathogenic bacteria *Listeria monocytogenes* is often detected in prawn gill and gut samples, where it is consumed by humans and transferred to other people. *Listeria* isolation agar was used in the process of isolating the bacteria. *L. monocytogenes*, when cultured on *Listeria* isolation agar, produces olive-green colonies. The bacteria were identified using Gram's staining and a biochemical assay. Human listeriosis, which is caused by this bacterium, is a devastating illness that has a high fatality rate and may cause meningitis, encephalitis, septicemia, and even miscarriage. According to Nelson *et al*. (2004), ready-to-eat food intake has been linked to listeriosis outbreaks (Abdollahzadeh *et al*., 2016). As a result, the occurrence of *L. monocytogenes* pathogenic serotypes is a major issue of concern for the seafood sector and public health organizations.

Staphylococcus aureus

A genus of bacteria, including Staphylococcus aureus, was isolated and identified from the prawn sample in the present investigation. There, a selective agar medium was used for isolation (Mannitol Salt Agar). Golden yellow, rounded, and convex were the *S. aureus* colony features seen on Mannitol Salt agar. The cultural features of *Staphylococcus aureus* reported by Konuku *et al*., (2012) were similar to those observed in the research (Konuku *et al*., 2012). The bacteria were identified using Gram's staining and biochemical analysis. *Staphylococcus aureus* was cocci-shaped and clustered like grapes in Gram's staining. *S. aureus* exhibited similar staining properties, according to Islam *et al*. (2019) and Ballah *et al*. (2022). *S. aureus* was found catalase positive while *E. coli* produced gas and acid from the fermentation of dextrose, lactose, maltose, and sucrose. The results of the biochemical test coincided with the findings of the earlier researcher.

Probiotics in Shrimp and Prawn Samples

Bacillus subtilis, Lactobacillus* spp., *and Enterobacter aerogenes

The gut of shrimp and prawn samples contained the majority of these three probiotic bacteria. Bacteria were isolated by cultivating them in a variety of mediums, including Nutrient agar, LBS agar, and MacConkey agar. The colony characteristics of *Bacillus subtilis, Lactobacillus* spp., *and Enterobacter aerogenes* exhibited White-cream colonies, White colonies, and Red colonies correspondingly. To identify those bacteria, a Gram stain, as well as a biochemical test, were performed. Choudhary *et al.* (2005) determined *Bacillus, Carnobacterium, Enterococcus, Lactococcus, and Lactobacillus* as probiotics in aquaculture (Hossain *et al*., 2017). Bergey's handbook of systematic bacteriology outlines the morphological and physiological tests that Holt (1994) used to characterize *Lactobacillus* (Seifu *et al*., 2012). In order to maximize output, probiotics are increasingly being used in modern shrimp and prawn farming procedures (Adel *et al*., 2017).

Future Thrust and Recommendation

In addition to the recommendations mentioned earlier, the integration of advanced technologies such as Polymerase Chain Reaction (PCR) (Salihah *et al*., 2016) and Molecular Sequencing (Fernandes *et al*., 2021) will be pivotal for advancing the safety and quality assurance of seafood in Bangladesh. PCR allows for the rapid and accurate detection of specific DNA sequences, making it invaluable for identifying and monitoring pathogens in shrimp and prawn samples. Molecular Sequencing technology, on the other hand, offers a comprehensive analysis of genetic material, enabling us to gain deeper insights into the microbial composition of seafood. The utilization of these cutting-edge techniques will not only enhance our ability to detect and combat pathogens but also shed light on the genetic diversity of probiotics, further elevating the potential for improved seafood quality. The integration of PCR and Molecular Sequencing technology holds promise for establishing Bangladesh as a reliable source of shrimp and prawn exports and bolstering consumer confidence in the global market.

Concluding remarks

In our investigation, we found *E. coli* and ten pathogenic bacteria in both shrimp and prawn samples, even in healthy individuals, emphasizing the need for thorough cooking. A significant discovery was the presence of three probiotics in these samples, offering a potential antibiotic alternative. Bangladesh, a major shrimp and prawn producer, faces challenges from poor sanitation and bacterial infections, threatening seafood production. To address this, it's vital to maintain clean shrimp ponds, avoid antibiotic use in feed, and prevent pollution. Adhering to HACCP principles during processing and handling can minimize contamination risks and disease outbreaks in the industry.

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Author Contributions

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. Muhammed Amanat, Muhammad Tawhid, Sapna Tandon: Wrote paper draft, Data collection and analysis. Mohammed Mafizul Islam, Muhammed Amanat: Corrected the paper draft and Visualization. Mohammed Mafizul Islam: Supervised the Experiment and Validation

Ethical Approval

This research has been approved by Noakhali Science and Technology University Ethical Committee (NSTU/SCI/EC/2021)

Declaration of Statement

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available within the manuscript and supplementary file.

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