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# Bacteria with an Antimicrobial Resistance in Shrimp and Shrimp Farms

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## Abstract

This study's goal was to look into the antibiotic resistance of pathogenic bacteria, mainly Escherichia coli, Salmonella, and Vibrio species, in the shrimp farming facilities of Bagerhat (Bangladesh). Both Penaeus monodon and *Macrobrachium rosenbergii* farms provided sediment samples, and the *Macrobrachium rosenbergii* facility provided shrimp samples. Five Enterobacterales (*Proteus penneri, Proteus alimentorum, Morganella morganii, Enterobacter hormaechei subsp. xiangfangensis, and Plesiomonas shigelloides*) were discovered, but not the previously listed bacteria. The presence of Enterobacter *hormaechei subsp. xiangfangensis* in a shrimp farm has never been before reported. Nine antibiotics were chosen for testing for antibiotic resistance: ampicillin, gentamicin, chloramphenicol, oxytetracycline, nitrofurantoin, levofloxacin, ciprofloxacin, azithromycin, and co-trimoxazole. The majority (88.9%) had at least one resistant strain. 78.0% of isolates from various sources were 29.3% of the isolates were found to be multidrug resistant and to be resistant to at least one antibiotic. The results of this experiment highlight that antimicrobial-resistant bacteria may be an issue for shrimp farms in Bagerhat despite the small number of samples examined—just nine in total. The health of consumers and the quality of shrimp may suffer as a result.

## Introduction

The majority of shrimp production is derived through aquaculture, making shrimp one of the most widely traded seafood commodities globally. Antimicrobial resistance (AMR) as a result of the treatment of disease in shrimp cultivation is one of the most significant issues facing the shrimp business. When microbial organisms become resistant to the antimicrobials that would typically kill them, AMR occurs. AMR is one of the most significant issues affecting human health, according to the World Health Organization. Since antimicrobial medications are typically used in preventative and therapeutic doses, some shrimp farms constitute a significant source of AMR. Exporting these treated shrimp throughout the globe could spread AMR microbes. In addition, there is an increase in the overuse and abuse of antibiotics. the possibility of antibiotic residues or AMR bacteria in seafood as well as the likelihood of the antimicrobial or AMR bacteria spreading into the environment [1].

As a result, shrimp farm sediment and shrimp hatcheries have been reported to contain antibiotic residues and AMR bacteria, which have caused widespread shrimp production losses. AMR is anticipated to grow as a result of rising global temperatures, which is another worry raised by climate change. The majority of shrimp production takes place in Asia (87% in 2017), where AMR has been found in shrimp farm effluents and the local environment. For instance, a number of factors have led to the identification of the Southeast Asian region as having a high risk of AMR development and dissemination. The manufacturing of shrimp frequently entails the direct interaction between workers and the pond's water and sediment. Additionally, along the supply chain, workers at the market and the processors come into contact with the shrimps directly, which aids in the spread of resistant bacteria [2].

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## Description

The samples were all gathered from shrimp farms in Bagerhat, Khulna, close to Bangladesh's coast, in the Ganges River delta, in the spring of 2018. Samples of silt from farms raising Penaeus monodon and *Macrobrachium rosenbergii*, as well as samples of shrimp from the *M. rosenbergii* facility, were obtained (MR shrimp). The P. monodon farm has 10 ponds totaling 3.25 ha, with pond sizes ranging from 0.2 to 0.4 ha. Three distinct ponds, each measuring about 0.2 hectares, were chosen at random for sample collection. The *M. rosenbergii* farm has four ponds totaling 1.5 ha, with pond sizes varying from 0.2 to 0.3 ha. For the purpose of collecting sediment and shrimp, three ponds were chosen at random. Each chosen pond contains, from ponds with water levels below the typical water line, sediment (top 6 cm, 500 mg) was aseptically collected in glass jars, and the sediment was then homogenised. For MR shrimp, each shrimp was put into a sterile bag (one shrimp was collected by pond). Nine samples in all were kept at 20°C following collection [3].

Three distinct samples of MR shrimp, MR sediment, and PM sediment were taken from each pond (n = 3), and a total of 27 samples were examined. Samples were thawed at 2 to 5°C in order to carry out the isolation technique. The shrimp's head and shell were cut off, and the bacteria were isolated only from the flesh. A blender was used to grind the flesh (Cuisinart food processor) [4]. Each replication was divided into three sub-samples for a total of 27 sub-samples, of which 1.0 g of sediment and 25 g of shrimp tissue were taken into consideration. The standard petrifilm method (3MTM, Maplewood, MN, USA, PetrifilmTM *E. coli*) was used for the *E. coli* isolation. The sample was combined aseptically with 225 mL of sterile phosphate-buffered saline, and solutions up to 104 dilutions were made. The centre of the 3M petrifilm was filled with a 1 mL diluted sample, and the lid was then secured [5].

The gestation the blue gas-forming colonies were counted as an indication of *E. coli* over the 24-hour period at 35°C. The standard Bacteriological Analytical Manual was used for the Salmonella isolation. The samples were homogenised aseptically with 225 mL of sterile lactose broth (Himedia, Mumbai, India), and then they were incubated for 24 hours at 35°C. Following that, 10 mL of Rappaport-Vasiliadis (RV) broth and 0.1 mL of the pre-enriched sample were combined and incubated at 42°C for 24 hours [6]. A 3 mm loop of RV broth was incubated for 24 hours at 35°C before being streaked on xylose lysine deoxycholate (XLD) agar (Himedia, Mumbai, India). On nutrient agar (NA), the susceptible colonies (pink colonies with or without black cores) were streaked to identify one susceptible colony. Colony incubation took place for 24 hours at 35°C [7].

Both lysine iron agar (Himedia-M377, Mumbai, India) and triple sugar iron agar (Himedia-M021, Mumbai, India) were utilised for the confirmation test. For samples of shrimp and sediment, the Bacteriological Analytical Manual was used for the Vibrio isolation. 225 mL of alkaline peptone water and 25 g of sample were combined aseptically and incubated at 35°C for 24 hours. For streaking, TCBS agar plates from Himedia in Mumbai, India, were utilised. They were incubated at 35°C for 24 hours. The isolates underwent standard sub-culture on NA plates and a 24-hour incubation period at 37°C [8]. The red colonies from the petrifilm (seen as being bacteria other than *E. coli*, Salmonella spp., and Vibrio spp.) were used to identify other bacteria using the criteria of total coliform, the oxidase positive yellow colonies from XLD agar and the oxidase negative yellow and green colonies from TCBS agar were chosen. Prior to molecular identification, such isolates were routinely sub-cultured on NA [9,10].

## Conclusion

The two Bagherhat shrimp farms under study had five multidrug-resistant Enterobacterales: *Proteus penneri, Proteus alimentorum, Morganella morganii, Enterobacter hormaechei subsp. xiangfangensis, and Plesiomonas shigelloides*. This is the first instance of Enterobacter hormaechei subsp. xiangfangensis being found in shrimp harvested from Bangladeshi shrimp farms. Additionally, 88.9% of the time, at least one isolate was resistant to one of the nine antimicrobials that were evaluated. Despite the small number of samples examined, the results show that antibiotic resistance bacteria may be a concern in Bagerhat shrimp farms, which may have an adverse effect on shrimp quality and consumer health.

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