The occurrence of potential pathogenic antibiotic-resistant bacteria in the marine environment in Ha Long Bay, Vietnam

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Abstract:

This study aimed to investigate the occurrence of potential pathogenic bacteria from surface water samples collected at 30 stations along Ha Long Bay, Vietnam. Among 116 bacterial isolates identified, the composition analysis indicated 24 distinct bacterial genera, with *Vibrio* (29.3%), *Pseudomonas* (23.3%), *Staphylococcus* (8.6%), and *Bacillus* (6.0%) being the most dominant. The taxonomic analysis revealed 61/116 (52.6%) isolates as potential pathogens (risk group 2) belonging to 21 bacterial species. Potential pathogens were detected in 28/30 (93.3%) sampling stations, with the most common pathogens including *Vibrio alginolyticus* (n=25), *Vibrio parahaemolyticus* (n=5), *Enterobacter cloacae* (n=3), *Pseudomonas mendocina* (n=3), *Aeromonas hydrophila* (n=2), *Enterobacter asburiae* (n=2), *Klebsiella oxytoca* (n=2), *Bacillus cereus* (n=2), and *Staphylococcus haemolyticus* (n=2). The selected pathogens were highly resistant to Amoxicillin (100%), Piperacillin (100%), Amoxicillin + Clavulanate (85.7%), Ticarcillin (71.4%), Cefoxitin (54.4%), Kanamycin (50%), and Fosfomycin (50%), and less resistant to Trimethoprim + sulfamethoxazole, Fusidic Acid, Tetracycline, Erythromycin, Pristinamycin, Dalacin, and Rifampicin (<25%), and susceptible to the remaining antibiotics. Notably, 12/23 (52.2%) isolates were resistant to at least three antibiotics and were multidrug-resistant bacteria. Our findings underline that the occurrence of pathogenic antibiotic-resistant bacteria in the marine environment of Ha Long Bay presents a potential threat to human health. Further genomic surveillance studies are necessary to manage and control the emergence and spread of pathogenic-resistant bacteria in this area.

Keywords: **antibiotic resistance, Ha Long Bay, marine environment, multidrug resistance, pathogenic bacteria.**

Classification numbers: **3.5, 5.3**

1. Introduction

Infectious diseases (IDs) and emerging infectious diseases (EIDs) affecting humans and wildlife are a significant and growing threat to health, the economy, resources, and biodiversity on a global scale [1, 2]. Each year, infectious diseases kill millions of people worldwide, resulting in an economic cost of $ billions in the United States alone [3]. For instance, it is estimated about 7.15 million waterborne illnesses occurrs in the United States annually resulting in 6,630 deaths and with the indirect healthcare costs of US $3.33 billion [4]. IDs and EIDs can also greatly influence ecosystem and wildlife health, contributing to population declines and sometimes extinction [5, 6]. Bacteria represent the pathogens more often (54.3%) involved in EID events in the last decades [7, 8]. IDs and EIDs are inherently difficult to study for various reasons, including the fact that their etiologic agents are poorly characterized. Therefore, identifying high-risk pathogens ranks among the greatest challenges facing modern science; critical to this effort is the need to predict geographic locations where disease outbreaks are likely to occur, identify the reservoir hosts from which pathogens will emerge, and predict host species at greatest risk of pathogen-mediated declines.

Waterborne infections are strongly associated with high morbidity and mortality globally. Previous studies [7, 8] have reported the occurrence of waterborne pathogens and antibiotic resistance in surface water, including coliforms, *Pseudomonas* sp., *Vibrio* sp., *Salmonella* sp., *Campylobacter* sp., *Acinetobacter* sp., *Aeromonas* sp., and new and re-emerging pathogens worldwide [9-14]. Unfortunately, most research on IDs and antimicrobial resistance has focused on inland systems with comparatively little effort directed towards marine habitats. However, marine environments can function as transmission foci for pathogens or antimicrobial resistance because seawater carries a large quantity of microorganisms, some harmful to humans, marine species, and ecosystems. Additionally, the aquatic life histories of many vectors and intermediate hosts can affect species interactions and trigger disease emergencies [9-12]. There is also evidence of microbial and antimicrobial resistance dispersal mechanisms between marine and terrestrial biomes. The direct transmission of human or marine livestock pathogens from marine habitats is therefore not negligible, but the major risk to public health and aquaculture production is assumed to be the development of a reservoir of resistance genes transferable to human or marine livestock pathogens and the emergence of pathogens with newly acquired antibiotic resistance. This risk increases in developing regions

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where the treatment of hospital and household sewage, important sources of pathogens, antibiotic-resistant genes, and antibiotics in the coastal environment, is limited (often non-existent) and where intensive aquaculture has developed. The risk of infection linked to seawater is therefore a major world health challenge [9-12].

Ha Long Bay is a spectacular scenic spot nominated as one of the seven world heritage sites by UNESCO in 1994. Located in Quang Ninh province in north-eastern Vietnam, Ha Long Bay covers approximately 65,650 ha with 1,133 islands and islets, featuring several creeks and arches, predominantly composed of limestone (https://whc.unesco.org/en/list/672/). However, the water environment in Ha Long Bay is polluted with oil, coliforms, heavy metals, chlorinated pesticides, organic matter, and plastics due to the rise of industrial, agricultural, domestic waste, and tourism activity. Intensive marine aquaculture is also one of the most important activities carried out in this area. In 2023, the total aquaculture area of Quang Ninh province reached 42,292 hectares with total aquaculture products reaching 175,324.6 tons. The seafood production value was 6,943 billion VND, accounting for nearly 50% of the value of the province's agriculture and rural sectors. All these activities could make Ha Long Bay a reservoir for the emergence and spread of potential pathogens in the marine environment to the human population. Unfortunately, little is known about its effects on microbial biodiversity, particularly the emergence and transmission of potential pathogens. In this context, this study aimed to investigate the occurrence of potential pathogenic antibiotic-resistant bacteria in marine water along Ha Long Bay, which are fundamental elements to reveal the water quality and risk to human health.

2. Materials and methods

2.1. Sample collection

Following the mission of the Centre for Marine Environmental Monitoring and Analysis, the Vietnam People's Navy, the quality of seawater in Ha Long Bay, Quang Ninh province, is assessed to evaluate potential risks to human health. This study was responsible for investigating potential pathogenic bacteria circulating in Ha Long Bay. The selected 30 sampling stations covered the entire coastal area within Ha Long Bay. The seawater samples were taken from 30 different stations (HL1-HL30) with geographical coordinates between 20°50'00.1'' N - 20°56'05.7'' N latitudes and 106°59'52.4'' E - 107°12'25.3'' E longitudes. At each sampling station, surface water was collected in autoclavable 500-ml polypropylene bottles with screw caps and kept cold at 4°C for microbial analysis.

2.2. Bacterial isolation

In this study, six culture media were selected for the isolation of potential pathogenic bacteria, including Tryptic Soy Agar (TSA, Sigma-Aldrich, Germany) for the isolation and cultivation of total cultivable bacteria, MacConkey Agar (MAC, Oxoid, UK) for the isolation and selection of Enterobacteriaceae species, Mannitol Salt Phenol Red Agar (MSPR, Sigma-Aldrich, Germany) for the isolation and selection of Staphylococcus, Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS, Merck, Germany) for the isolation and selection of *Vibrio* species, Salmonella-Shigella Agar (SS, Sigma-Aldrich, Germany) for the isolation and selection of Salmonella and Shigella species, and Klebsiella ChromoSelect Agar (KCS, Sigma-Aldrich, Germany) for *Klebsiella* species. For each sample, 1 ml of seawater was spread on a petri plate followed by incubation at 35°C for 18-24 hours. Bacterial colonies were primarily differentiated by morphological characteristics and then purified by sub-culturing using the streaking plate method. All purified isolates were maintained in TSB with 50% glycerol solution and stored at -80°C.

2.3. Bacterial identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

The bacterial isolates were rapidly identified using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) Biotyper system (Bruker, Germany). Briefly, a full loop of pure colonies was suspended in 300 µl of 70% ethanol, and the cells were harvested by centrifugation at 12,857 rcf (relative centrifugal force) for 5 minutes. Cell pellets were incubated with a mix containing 50 µl of 70% formic acid and 50 µl of acetonitrile for 5 minutes. The cell suspension was centrifuged at 12,857 rcf for 5 minutes, and then 1 µl of the clear supernatant was transferred onto a 96-spot MALDI target plate and air-dried at room temperature. Each spot was overlaid with 1 µl of MALDI matrix and dehydrated for 15 minutes, followed by processing on the MALDI-TOF Biotyper system. The protein spectra obtained were analysed by BioTyper 3.0 system software and compared against the CDC database for bacterial identification. A log (score) of MALDI Biotypes above 1.8 indicates a valid identification, while a score under 1.7 suggests poor performance and represents uncertain identification.

2.4. Bacterial identification by 16S rDNA gene sequencing

For isolates that were not identified using the MALDI-TOF MS system, they were further identified by 16S rDNA gene sequencing. A 500-bp DNA fragment of the 16S rDNA gene was amplified with the primers 16S-8F (Forward): 5'-GCTGGATAGGTTAAGGGCGG-3' and 16S-518R (Reverse): 5'-ATTACCGCGGCTGCTGG-3'. The thermal cycling parameters included a 1-minute denaturation at 95°C followed by 35 cycles of 15 seconds at 95°C, 15 seconds at 55°C, and 10 seconds at 72°C. PCR amplicons were examined by agarose gel electrophoresis. After that, the PCR amplicons were purified and sequenced at the First Base Company (Singapore). The obtained DNA sequences were processed using BioEdit software and then searched on the GenBank, National Center for Biotechnology Information (NCBI) database using the nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for bacterial identification based on sequence comparison.

2.5. Antibiotic susceptibility testing

The antibiotic susceptibility was determined for selected potential pathogenic bacteria on Mueller Hinton Agar (MHA, Sigma, Germany) using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), M100 version: performance standards for antimicrobial susceptibility (CLSI 2020). Specifically, *Aeromonas* species were tested for 6 antibiotics including fusidic acid (10 µg), Cefepime (30 µg), Ceftazidime (10 µg), Ciprofloxacin (5 µg), Trimethoprim + sulfamethoxazole (25 µg), and Levofloxacin (5 µg). *Vibrio* species were examined for 7 antibiotics including Cefotaxime (5 µg), Ciprofloxacin (5 µg), Trimethoprim + sulfamethoxazole (25 µg), Erythromycin (15 µg), Meropenem (10 µg), Piperacillin + Tazobactam (36 µg), and Tetracycline (30 µg). *Enterobacter* and *Klebsiella* (Enterobacteria family) were tested for 16 antibiotics including nalidixic acid (30 µg), amikacin (30 µg), amoxicillin + clavulanic acid (30 µg), amoxicillin (20 µg), Cefotaxime (5 µg), Cefoxitin (30 µg), Ceftazidime (10 µg), Ciprofloxacin (5 µg), Trimethoprim + sulfamethoxazole (25 µg), Ertapenem (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Piperacillin (30 µg), Ticarcillin (75 µg), and Tobramycin (10 µg). *Bacillus cereus* and *Staphylococcus haemolyticus* were tested for 14 antibiotics including Gentamicin (10 µg), Trimethoprim + Sulfamethoxazole (25 µg), Tobramycin (10 µg), Tetracycline (30 µg), Ofloxacin (5 µg), Fosfomycin (15 µg), Erythromycin (15 µg), Kanamycin (30 µg), Rifampicin (5 µg), fusidic acid (10 µg), Pristinamycin (15 µg), Cefoxitin (30 µg), Dalacin (10 µg), and Teicoplanin (30 µg). The sizes of the inhibition zones were interpreted to determine whether the microorganism was susceptible, intermediately resistant, or resistant to each antibiotic according to the CLSI guidelines. Multiple antibiotic-resistant phenotypes were reported for isolates that showed resistance to at least 3 antibiotics. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as quality controls for all tests.

3. Results and discussion

3.1. Bacterial isolation and purification

A total of 280 bacterial colonies were isolated from the six culture media according to their morphological characteristics (Fig. 1A). Most were isolated from TSA (n=94, 33.6%), followed by MAC (n=76, 27.1%), TCBS (n=47, 16.8%), and MRSP (n=43, 15.4%). Only a few bacterial colonies were recovered from KCS and SS media (5.7% and 1.4%, respectively). Overall, 14 out of 30 sampling spots yielded ≥10 bacterial colonies (Fig. 1B). Specifically, the number of bacterial isolates was highest in stations HL1 (n=13), HL2 (n=12), HL28 (n=12), HL6, HL21, HL23, HL29, and HL30 (each station, n=11), while fewer bacteria were isolated in stations HL15 (n=5), HL18, HL22, and HL25 (each station, n=7). The low number of bacteria isolated suggests that the culture media may not be suitable for the growth and survival of marine bacteria.

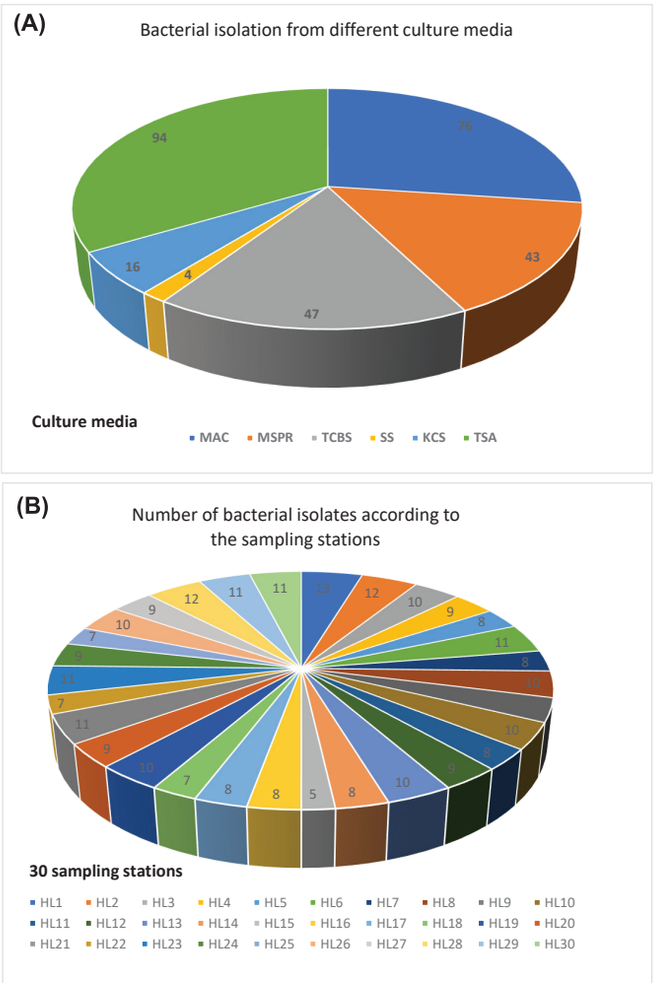

Fig. 1. Distribution of bacterial isolates according to the selected culture media (A) and sampling stations (B).

3.2. Identification of potential pathogenic bacteria

Among the 280 bacterial isolates, 85 could not be regrown; thus, 195 were subjected to taxonomic identification by MALDI-TOF MS. Since these bacterial isolates originated from the marine environment, the media used and culture conditions might not be suitable for their growth and survival. After removing duplicate isolates within each sampling spot, 116 distinct isolates were available for taxonomic analysis. The bacterial identification results showed high diversity in the marine water environment in Ha Long Bay. These isolates belonged to 24 different bacterial genera, with *Vibrio* being the most dominant (n=34, 29.3%), followed by *Pseudomonas* (n=27, 23.3%), *Staphylococcus* (n=10, 8.6%), and *Bacillus* (n=7, 6.0%). The remaining genera were less common, accounting for less than 3% of total isolates. *Vibrio* was widely distributed in Ha Long Bay, found in 26 out of 30 (86.7%) sampling stations, followed by *Pseudomonas* (19/30, 63.3%), *Staphylococcus* (8/30, 26.7%), and *Bacillus* (7/30, 23.3%).

The 116 isolates were further identified as 54 distinct bacterial species. The most common bacterial species were

Vibrio alginolyticus (n=25, 21.5%), followed by *Pseudomonas stutzeri* (n=10, 8.6%), *Pseudomonas* sp. (n=6, 5.2%), and *Vibrio parahaemolyticus* (n=5, 4.3%). Notably, 21/54 (38.9%) identified bacterial species were recognised as potential human pathogenic bacteria according to the classification of the risk group by ZKBS Germany (https://www.zkbs-online.de/ZKBS/DE/Datenbanken/Organismen) (Fig. 2). *Vibrio alginolyticus* was found in 25/30 (83.3%) sampling stations, and other potential pathogens were sporadically distributed in Ha Long Bay. Overall, potential pathogens were detected in 28/30 (93.3%) sampling stations, except for stations HL22 and HL26 (Fig. 2).

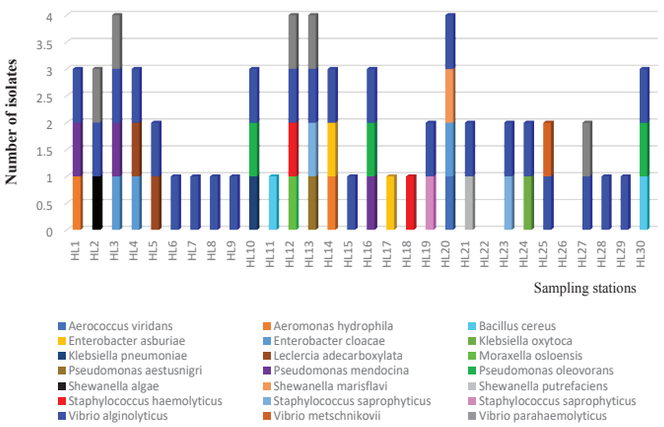

Fig. 2. Distribution of potential pathogenic bacteria according to the 30 sampling stations.

3.3. Antibiotic susceptibility testing

Among the gram-negative pathogenic bacteria isolated, *A. hydrophila* (n=2), *Enterobacter asburiae* (n=2), *Enterobacter cloacae* (n=3), *Klebsiella oxytoca* (n=1), *Klebsiella pneumoniae* (n=1), *Vibrio alginolyticus* (n=5), and *Vibrio parahaemolyticus* (n=5) were selected for evaluating susceptibility to selected antibiotics. A. hydrophila HL1 was sensitive to five antibiotics and was only intermediately resistant to ceftazidime. *A. hydrophila* HL14 was sensitive to three antibiotics but was resistant to Levofloxacin and intermediately resistant to Ciprofloxacin and Trimethoprim + Sulfamethoxazole. All seven enteric bacterial strains (Enterobacter asburiae, *Enterobacter cloacae*, Klebsiella oxytoca, *Klebsiella pneumoniae*) were resistant to 3-5 antibiotics. Specifically, they were resistant to Amoxicillin and Piperacillin (7/7, 100%), followed by Amoxicillin + Clavulanate (6/7, 85.7%), Cefoxitin and Ticarcillin (5/7, 71.4%). Nevertheless, all these strains were sensitive to antibiotics belonging to aminoglycosides, quinolones, and carbapenems. Finally, all 10 *Vibrio* isolates were sensitive to the seven antibiotics tested.

Regarding potential pathogenic gram-positive bacteria, *Bacillus cereus* (n=2) and *Staphylococcus haemolyticus* (n=2) were selected for phenotypic susceptibility testing towards 14 antibiotics. Specifically, *B. cereus* HL11 and *B. cereus* HL30 were intermediately resistant/resistant to nine antibiotics, belonging to at least five antibiotic groups. *S. haemolyticus* HL12 and *S. haemolyticus* HL18 were also intermediately resistant/resistant to five and four antibiotics, respectively, belonging to four antibiotic groups.

Table 1. Antibiotic-resistant profiles of the selected bacterial isolates.

No.	Bacterial species	Isolates sensitive to all antibiotics	Isolates intermediately resistant or resistant to ≤2 antibiotics	Isolates intermediately resistant or resistant to multiple antibiotics (n ≥3)	Total tested isolates
1	*Aeromonas hydrophila*	0	1	1	**2**
2	*Enterobacter asburiae*	0	0	2	**2**
3	*Enterobacter cloacae*	0	0	3	**3**
4	*Klebsiella oxytoca*	0	0	1	**1**
5	*Klebsiella pneumoniae*	0	0	1	**1**
6	*Vibrio alginolyticus*	5	0	0	**5**
7	*Vibrio parahaemolyticus*	5	0	0	**5**
8	*Bacillus cereus*	0	0	2	**2**
9	*Staphylococcus haemolyticus*	0	0	2	**2**
	Total	10	1	12	23

The antibiotic-resistant profiles showed that 12/23 (52.2%) bacterial isolates tested were multidrug-resistant phenotypes (Table 1). These multidrug-resistant bacteria included *A. hydrophila, E. asburiae*, *E. cloacae*, *K. oxytoca*, *K. pneumoniae*, *B. cereus*, and *S. haemolyticus*. Resistance to at least 18 out of 29 antibiotics tested was detected (Fig. 3). The selected pathogens were completely resistant to Amoxicillin and Piperacillin (100%). A high proportion of bacterial isolates were resistant to Amoxicillin + Clavulanate (85.7%), Ticarcillin (71.4%), Cefoxitin (54.4%), Kanamycin (50%), and Fosfomycin (50%). Resistance to Trimethoprim + sulfamethoxazole, Fusidic Acid, Tetracycline, Erythromycin, Pristinamycin, Dalacin, and Rifampicin was also detected at a low proportion (<25%). These bacterial isolates were still susceptible to 10 antibiotics including Amikacin, Tobramycin, Gentamicin, Piperacillin + Tazobactam, Cefotaxime, Imipenem, Meropenem, Ertapenem, Nalidixic Acid, and Ofloxacin (Fig. 3).

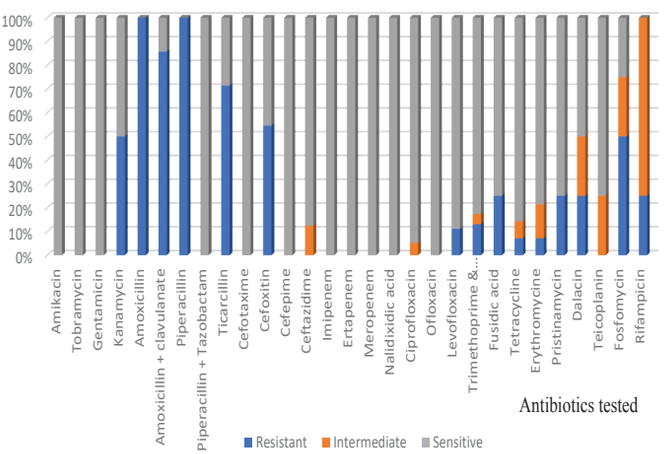

Fig. 3. Proportion of antibiotic-resistant bacteria in the marine water in Ha Long Bay.

4. Discussion

Pathogenic bacteria in marine environments can present a major threat to human health, with bathing and consumption of seafood being the major transmission routes to humans [12, 13]. The primary sources of microbial contamination in marine environments and transmission mechanisms are the faecal-oral route, involving sewage, runoff, river discharge, groundwater seepage, and sewage sludge disposal [14, 15]. The survival and growth of potential pathogenic bacteria in marine environments are greatly influenced by human activities and environmental factors, mainly triggered by man-made eutrophication. Unfortunately, the health threat posed by pathogenic bacteria in marine environments is not yet well understood [16-18]. Therefore, the present study investigated the distribution and antibiotic resistance of potential pathogenic bacteria in the marine environment of Ha Long Bay, Vietnam.

Among the 116 identified isolates, 21 bacterial species were potential pathogens to humans and animals. Among them, *Vibrio* (29.3%), *Pseudomonas* (23.3%), *Staphylococcus* (8.6%), and *Bacillus* (6.0%) were the most dominant. It is worth noting that these pathogens were found in 93.3% of the studied areas. In nature, *Vibrio* and *Aeromonas* species typically exist at low abundance in marine environments but represent a reservoir from which epidemics can arise [19, 20]. These pathogens are often found in marine environments with high-intensity aquaculture activities and are considered indicators of water quality in aquaculture. Particularly, *Vibrio* strains and their associated infections are on the rise globally due to increasing sea surface temperatures, representing an emergent threat to human and animal health, and causing significant economic losses in the aquaculture industry worldwide [20, 21]. In the present study, *Vibrio* species were found in 27/30 sampling stations in Ha Long Bay. The absence of *Vibrio* in the remaining 3 stations could be explained by the loss of *Vibrio* strains during the isolation and culture steps. Among marine *Vibrio* species, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* can cause serious seafood poisoning, wound and ear infections, with fatal diseases including necrotising soft-tissue infections, bacteraemia, septic shock, and multiple organ failures. These two pathogens are considered the major causative agents for seafood poisoning outbreaks in Asia [22-24]. Additionally, *Aeromonas hydrophila*, a well-known opportunistic aqueous pathogen, is now recognised as an emerging foodborne pathogen capable of causing human gastroenteritis, with its main reservoir being the aquatic environment. Foodborne outbreaks associated with *Aeromonas hydrophila* have been reported worldwide [25, 26]. This pathogen can be transmitted via the faecal-oral route through direct consumption of contaminated water or food. Notably, this pathogen can survive at very low temperatures, meaning frozen seafood contaminated with *Aeromonas hydrophila* still poses a potential risk of food poisoning. Furthermore, *Bacillus cereus*, a common food-poisoning pathogen, along with other human pathogenic species of Enterobacteriaceae (*Enterobacter asburiae*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*), were also detected in Ha Long Bay. Our findings emphasise the possible harm to human health that eating raw seafood poses, as it can lead to foodborne infections and microbiological food poisoning.

Recently, antibiotic-resistant bacteria have become a severe issue that endangers people's lives globally and places a heavy financial strain on the health system [27]. In this study, resistance to amoxicillin and piperacillin was the most commonly detected (100%), followed by amoxicillin + clavulanate (85.7%), ticarcillin (71.4%), cefoxitin (54.4%), kanamycin (50%), and fosfomycin (50-85.7%). Resistance to trimethoprim + sulfamethoxazole, fusidic acid, tetracycline, erythromycin, pristinamycin, dalacin, and rifampicin was less commonly detected (<25%). It has been reported that beta-lactams, tetracycline, erythromycin, and quinolones are commonly used in fisheries for the prevention and treatment of bacterial infections [28]. Therefore, the presence of *Aeromonas*, *Enterobacter*, *Klebsiella*, *Bacillus*, and *Staphylococcus* isolates resistant to at least one of these antibiotics is consistent with previous studies [11, 29, 30]. For *Vibrio* species, previous studies have reported that the prevalence of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* isolates resistant to antibiotics is often relatively low. Nevertheless, the high prevalence of antibiotic-resistant genes in marine environments [30, 31], combined with the high incidence of bacterial infections in aquaculture and the overuse of antibiotics and horizontal gene transfer mechanisms, would promote the selection and transmission of antibiotic-resistant bacteria in this area. Finally, we found that 52.3% of the selected bacterial isolates were multidrug-resistant phenotypes. These pathogens exhibited resistance to 3 to 9 antibiotics. The presence of multi-resistant bacteria in these samples highlights the potential risks to the health of marine organisms and humans. Additionally, this coastal area also receives discharges from urban canals and rivers, meaning human pathogens and AMR on land can be transported to the sea. Therefore, it is necessary to conduct monitoring and genomic surveillance studies to rapidly respond to the emergence of new pathogens in this important marine environment.

5. Conclusions

The wide distribution of potential human bacterial pathogens and the occurrence of multidrug-resistant strains in the surface water environment of Ha Long Bay pose a threat to the environment and community health. Thus, further studies should focus on monitoring the distribution, prevalence, and transmission pathways of antibiotic-resistant genes in this area to provide insight into the risk of the emergence and spread of multidrug-resistant bacteria. This knowledge can contribute to developing appropriate management and policy measures to reduce the human health risks associated with bathing and consuming seafood in Quang Ninh province.

CRediT author statement

Nguyen Quang Huy: Methodology, Experiment, Data analysis, Writing, Editing; Pham Quynh Trang: Experiment, Data analysis; Nguyen Thi Loi: Sample Collection, Data analysis, Writing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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