Infection and Drug Resistance

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ORIGINAL RESEARCH

Clonal Dynamics, Virulence Genes, Antimicrobial Resistance, and Early Diagnostic Indicators of Bloodstream Infections of *Vibrio vulnificus* in a Hospital in Eastern China from 2021 to 2024

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Objective: This study analyzes the characteristics and clonal dynamics of *Vibrio vulnificus* isolated from a Chinese hospital between 2021 and 2024.

Methods: We performed whole genome sequencing (WGS) on the six collected *Vibrio vulnificus* isolates. Antibiotic susceptibility testing was conducted using the bioMérieux automated antimicrobial susceptibility testing system. The clonal dynamics were studied through phylogenetic analysis, and the clinical diagnostic value indicators for bloodstream infections (BSI) were evaluated using the receiver operating characteristic (ROC) curve.

Results: The six *Vibrio vulnificus* strains collected in this study were phylogenetically closely related, including two of sequence type ST678 and one each of ST696, ST675, ST607, and ST367. All strains tested positive for the *vcgC* gene; five were positive for 16S rRNA type A, and one showed type AB. Virulence gene profiling revealed that the strains universally carried exotoxins (VvhA, RTX), adhesion factors (TadZ/CpaE, IlpA, VWA), and motility-associated genes (cheW/R, motA, flgC). Notably, one strain harbored a broader array of virulence factors, including effector protein secretion systems (ompA, vipA/tssB, sciN/tssJ), biofilm formation-related genes (mrkA/B/C), and siderophores (fepA/B, entE/F, iucA/B), which may significantly enhance its pathogenicity. According to CLSI guidelines for *Vibrio* spp. all isolates were resistant to ampicillin but susceptible to third-generation cephalosporins, carbapenems, tetracyclines, quinolones, and folate pathway inhibitors. ROC curve analysis showed that high-sensitivity C-reactive protein (HS-CRP), lactate (Lac), procalcitonin (PCT), and other indicators were of good clinical value for the early diagnosis of bloodstream infection caused by *Vibrio vulnificus*.

Conclusion: This study represents the inaugural whole-genome sequencing research conducted on *Vibrio vulnificus* isolates from eastern China. It comprehensively examines the current species, antibiotic resistance, virulence genes, and clinical characteristics of infected individuals, elucidating the pathogenic mechanisms and epidemiological features of *Vibrio vulnificus*. The findings offer essential references for clinical diagnosis and treatment.

Keywords: *Vibrio vulnificus*, prevalence, resistance, virulence

Introduction

Vibrio vulnificus is a Gram-negative, rod-shaped bacterium belonging to the Vibrionaceae family.[1] It is a deadly opportunistic human pathogen and is responsible for the majority of seafood-related fatalities worldwide.[2] The primary manifestation of *Vibrio vulnificus* infection is sepsis, which accounts for approximately 43% of cases. This type of infection can rapidly deteriorate and may be life-threatening. Wound infections make up 45% of cases, typically arising from exposure to contaminated seawater through open wounds or injuries caused by marine animals.[3,4] These infections

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can lead to skin and muscle necrosis, and in severe cases, amputation may be necessary. Gastroenteritis is rare, accounting for only 5% of cases.[3,5]

The prevalence shows obvious differences across various regions (Figure 1 and Supplementary Table 1) and different years (Figure 2), primarily influenced by climate, environment, public health measures, and aquaculture activities. In Southeast Asia, the prevalence of *Vibrio vulnificus* is relatively high, particularly in countries such as Thailand, Vietnam, and the Philippines, where it can reach 10% to 30%.[6] According to some academic studies[7] and public health reports, infections caused by *Vibrio vulnificus* are more prevalent in the summer and autumn, especially in coastal areas. In East

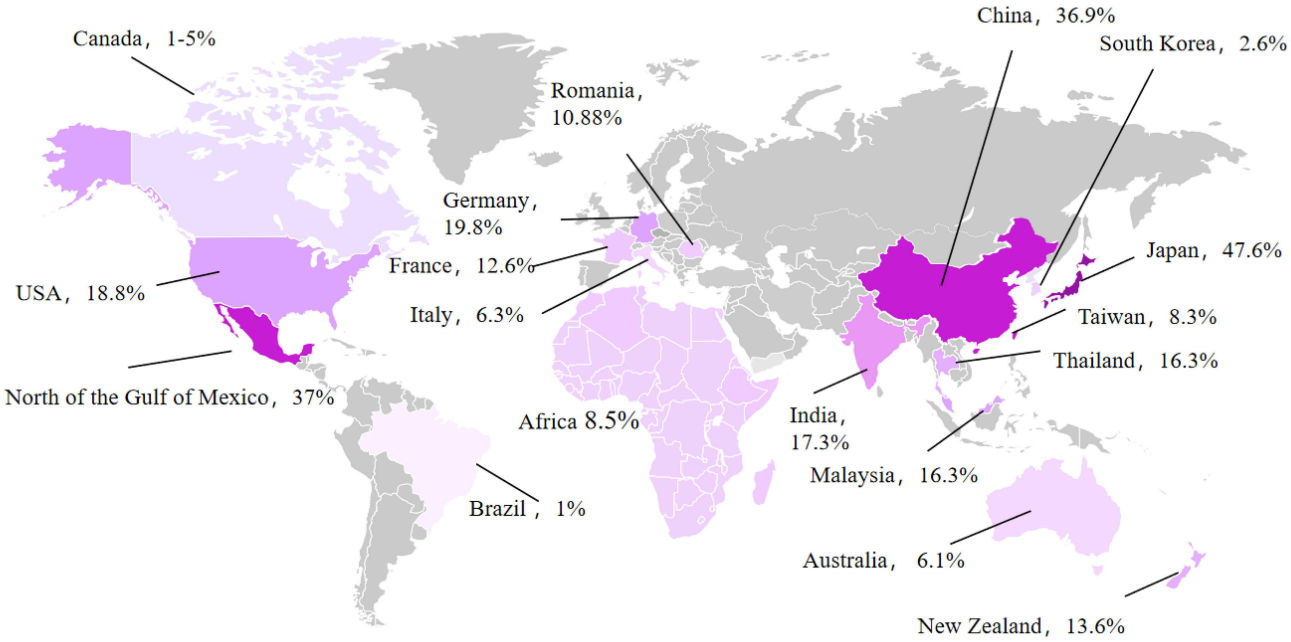

Figure 1 Global Distribution Patterns of *Vibrio vulnificus* Prevalence.
Note: Specific data are provided in Supplementary Table 2; color intensity indicates the prevalence of *Vibrio vulnificus* in each country.

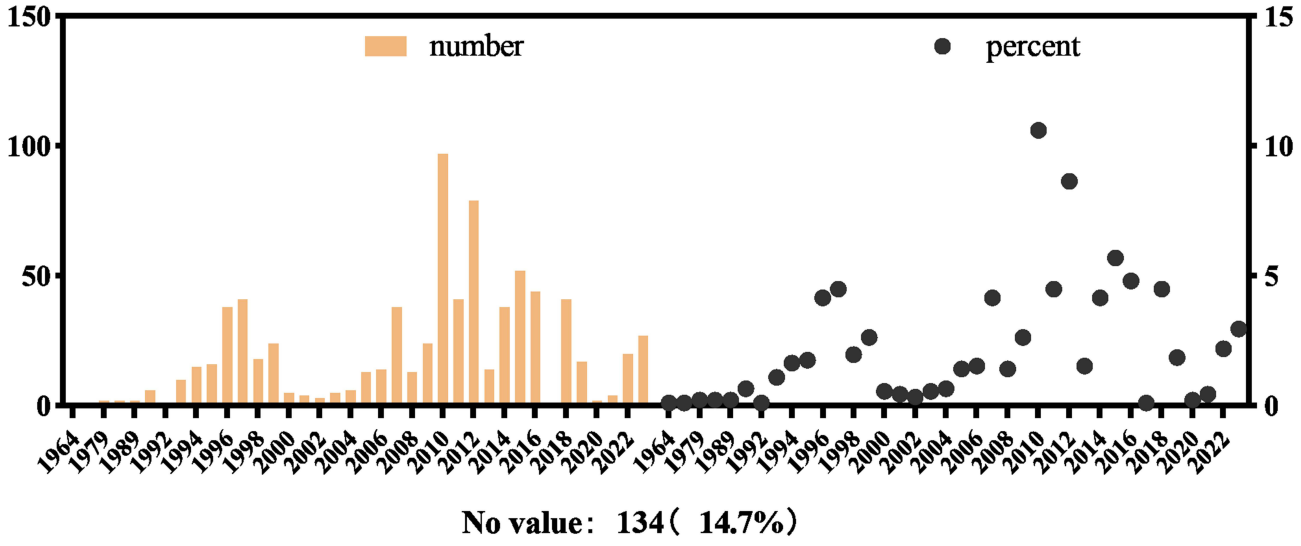

Figure 2 Epidemiological Situation of *Vibrio vulnificus* in Different Years.
Notes: Data are sourced from https://pubmlst.org/bigsdb?db=pubmlst_vvulnificus_isolates. The left bar chart illustrates the annual detection counts of *Vibrio vulnificus* isolated between 1964 and 2023, while the right scatter plot correspondingly displays the total annual prevalence of *Vibrio vulnificus*. "No value" indicates samples without a specific year of discovery.

Asia, Japan is a high-incidence area for infections, particularly during the summer when Japan's foodborne disease surveillance system regularly reports cases of *Vibrio vulnificus* infections. Monitoring data indicates that in certain years, the detection rate of *Vibrio vulnificus* in seafood can be as high as 20–30%, making Japan one of the countries with the highest prevalence currently.[8] A study[9] analyzing cases of *Vibrio vulnificus* infections in South Korea from 2001 to 2016 revealed that over 80% of oyster consumption-related infections occurred during the summer months, particularly in August and September. The data indicated that such incidents were extremely rare or completely absent during the cold season from December to March. This phenomenon suggests that low winter temperatures effectively inhibit the reproduction and spread of *Vibrio vulnificus*, thereby significantly reducing the risk associated with oyster consumption.[10] According to the latest report released by the Centers for Disease Control and Prevention (CDC) on February 1, 2024, approximately 150 to 200 cases of *Vibrio vulnificus* infections are reported annually in the United States, with a fatality rate of about 20%.[11] Additionally, a long-term surveillance study conducted in Maryland, which compared the periods from 2006 to 2012 and from 2013 to 2019, found that the average annual incidence rate of vibriosis (per 100,000 population) increased by 39%.[12] Although the CDC primarily focuses on epidemiology within the United States, there have also been reports of international travelers contracting *Vibrio vulnificus* in Mexico. Research indicates that the infection rate of *Vibrio vulnificus* in Mexico is relatively high, especially in the northern Gulf of Mexico, where the prevalence may reach 37%.[13] In Europe, the prevalence of *Vibrio vulnificus* is generally lower, with the incidence in seafood-related cases being about 6.1%, thanks to a robust public health system and effective water quality management that reduces the risk of infection.[14] In Australia, the risk of *Vibrio vulnificus* infection is low, with most cases associated with oysters, followed by clams and mussels. In coastal regions (such as Queensland and Sydney), the risk is relatively higher due to high seafood consumption and favorable water temperatures for *Vibrio vulnificus* growth.

The risk of *Vibrio vulnificus* infections in Australia is relatively low, with most cases linked to oysters, followed by clams and mussels. The risk is higher in coastal areas such as Queensland and Sydney, where seafood consumption is significant and water temperatures are conducive to the growth of *Vibrio vulnificus*.[15] Additionally, eight pathogenic Vibrio species have been identified in African water bodies, with the highest detection rate being for *Vibrio cholerae* (59.5%), followed by *Vibrio parahaemolyticus* (10.4%), while *Vibrio vulnificus* ranked fourth at 8.5%. The presence of these pathogenic Vibrio species, especially in freshwater sources, clearly confirms the ongoing outbreaks in Africa.[16]

This study represents the first effort to integrate genomic data with clinical information and employ ROC curve analysis to assess the utility of conventional inflammatory markers, such as PCT, HS-CRP, and WBC, in the early diagnosis of *Vibrio vulnificus* bloodstream infections in eastern China. This research provides critical evidence for the establishment of relevant evidence-based standards within the country. Additionally, a comprehensive understanding of the pathogen's virulence factors and antimicrobial resistance characteristics not only facilitates the optimization of clinical treatment strategies but also provides a scientific basis for the development of effective infection prevention and control measures, ultimately contributing to the reduction of the global disease burden associated with this pathogen.

Materials and Methods

Bacterial Isolates

Between January 1, 2021, and December 31, 2024, a total of six strains of *Vibrio vulnificus* isolates were obtained from a tertiary hospital in Ningbo. Bacterial identification was performed using the VITEK®2 system (bioMérieux, France).

Antimicrobial Susceptibility Testing

Bacterial culture was performed on Mueller-Hinton (MH) agar plates, and then single colonies were picked and dissolved in 0.45% NaCl solution to adjust to a 0.5 McFarland suspension. This experiment utilized the VITEK®2 automated microbiological identification and susceptibility testing system from bioMérieux (France) to assess the antibiotic sensitivity of *Vibrio vulnificus*. Except for tigecycline, the antibiotic susceptibility results were interpreted according to the latest 2024 CLSI M100 34th edition guidelines and the CLSI M45 third edition for rare organisms. The former was interpreted using FDA standards for Enterobacteriaceae (susceptible≤2 mg/L; resistant≥8 mg/L). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains in this experiment.

WGS and Bioinformatics Analysis

Genomic DNA from the 6 isolates of *Vibrio vulnificus* was extracted using the AxyPrep Bacterial Genomic DNA Mini-Prep Kit (Axygen Scientific). DNA libraries were prepared using the NEB Next Ultra II DNA Library Prep Kit, followed by sequencing with the Illumina NovaSeq. Quality control of the raw sequence reads was performed using FastQC, and trimming was carried out with Fastp. The trimmed reads were then assembled using SPAdes. Multi-locus sequence typing (MLST) of the *Vibrio vulnificus* isolates was conducted using MLST (https://github.com/tseemann/mlst). Antibiotic resistance genes (ARGs) and virulence factors were identified using the CARD database (https://card.mcmaster.ca/download/) and the VFDB database (https://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi).

Phylogenetic Tree and Resistance Genes

Using Mega 5 software, the housekeeping gene sequences and reference sequences of six strains of *Vibrio vulnificus* were aligned and analyzed based on the Maximum Likelihood method, resulting in the construction of a bacterial phylogenetic tree derived from the alignment results. Subsequently, the WGS data of the six *Vibrio vulnificus* strains were analyzed using the VFDB database. The virulence gene information obtained was correlated with the strains in the phylogenetic tree to explore the relationship between virulence genes and strain evolution.

Clinical Characteristics of *Vibrio vulnificus* Infection

A retrospective analysis was conducted on the early clinical examination data of 6 hospitalized patients with BSI caused by *Vibrio vulnificus*, exploring the application value of clinical inflammatory diagnostic indicators, including procalcitonin (PCT), white blood cell count (WBC), neutrophil count (NE), red blood cell count (RBC), hemoglobin (Hb), high-sensitivity C-reactive protein (Hs-CRP), prothrombin time (PT), platelet count (PLT), lymphocyte count (LY), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), and D-dimer in the early clinical diagnosis of the disease. This provides an effective theoretical basis for guiding physicians in the timely and accurate implementation of evidence-based medical treatment for hospitalized patients. Data analysis was performed using SPSS 17.0, employing ROC curve analysis to evaluate the diagnostic value of different indicators for *Vibrio vulnificus* bloodstream infections; $P < 0.05$ was considered statistically significant.

Results

Species and Typing Identification of *Vibrio vulnificus*

The sequence analysis of the selected Vibrio isolates using 16S rRNA confirmed the species of all isolates (Figure 3). The virulence testing results indicated that 6 isolates of *Vibrio vulnificus* were positive for the vcgC gene, with 5 isolates showing positive results for the 16S rRNA A type and 1 isolate showing positive results for the AB type.

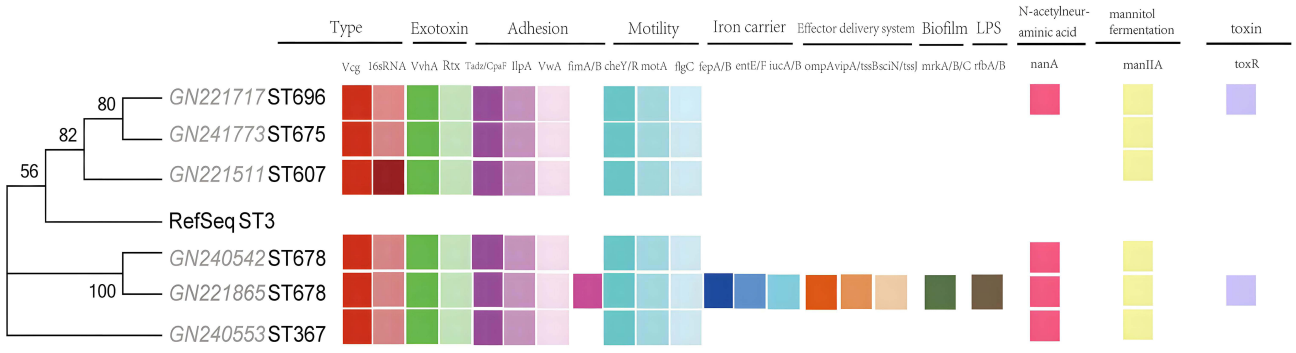

Figure 3 A comparison of the virulence factor spectra of six different genotypes of *Vibrio vulnificus*.

Antimicrobial Resistance of *Vibrio vulnificus*

All tested *Vibrio vulnificus* isolates (n = 6) were found to be susceptible to 13 out of the 14 antibiotics tested, including the drug classes recommended by the Centers for Disease Control and Prevention (CDC) for the treatment of *Vibrio vulnificus* infections: β-lactams, tetracyclines, fluoroquinolones, and folate pathway inhibitors (Table 1). According to the CLSI recommendations, 100% of the *Vibrio vulnificus* isolates exhibited resistance to ampicillin, and no isolates showed resistance to third-generation cephalosporins, penicillins, tetracyclines, fluoroquinolones, or folate pathway inhibitors. Furthermore, whole-genome sequencing of the bacteria did not detect any associated resistance genes.

Detection of Virulence Genes

In the detection of virulence genes, the presence of the nanA gene from the sialic acid degradation metabolic cluster, enzyme IIA (manIIA) from the mannitol fermentation operon, the virulence toxin (toxR) of *Vibrio vulnificus*, and the Vibrio-specific hemolysin (VVH) was examined. Among these, only 4 isolates tested positive for the nanA gene, and 2 isolates were positive for the toxR gene, while the remaining 6 isolates carried both the manIIA and VVH genes.

Phylogenetic Tree and Virulence

The 6 isolates of *Vibrio vulnificus* collected in this study showed a close phylogenetic relationship, with 2 isolates classified as ST678 and the others as ST696, ST675, ST607, and ST367. All isolates carried exotoxins (VvhA, RTX), adherence factors (TadZ/CpaE, IlpA, VWA), and motility factors (cheW/R, motA, flgC). However, the GN221865 isolate carried a more diverse array of virulence factors, including the effector delivery system (ompA, vipA/tssB, sciN/tssJ), biofilm formation factors (mrkA/B/C), iron transporters (fepA/B, entE/F, iucA/B), as well as lipopolysaccharides (LPS, rfbA/B) involved in self-immune regulation. These virulence factors enhance the pathogenicity of the bacteria toward the host (Figure 3).

Table 1 Antimicrobial Susceptibility Results of Six *Vibrio vulnificus* Strains Based on CLSI Recommended Drugs

Categories of Antibiotics	Antimicrobial Agent	Breakpoints Susceptibility, Resistance	K-B/mm	MIC_{50}	MIC_{95}	Resistance Rates (%)
β-lactams	Imipenem	≤1 S, ≥4 R	–	≤0.25	≤0.25	0
	Meropenem	≤1 S, ≥4 R	–	≤0.25	≤0.25	0
	Ceftazidime	≤4 S, ≥16 R	–	1	2	0
	Cefepime	≤2 S, ≥16 R	–	0.25	1	0
	Ampicillin	≥17 S, ≤13 R	14.5	14.5	15	100
	Cefuroxime	≥18 S, ≤14 R	20.7	21	21	0
	Piperacillin/Tazobactam	≤1 S, ≥4 R	–	≤4	4	0
	Amoxicillin/Clavulanic Acid	≤8 S, ≥32 R	–	≤8	≤8	0
Quinolones	Amikacin	≤16 S, ≥64 R	–	8	16	0
	Ciprofloxacin	≤1 S, ≥4 R	–	≤0.25	≤0.25	0
	Levofloxacin	≤2 S, ≥8 R	–	≤0.12	≤0.12	0
Tetracyclines	Doxycycline	≤4 S, ≥16 R	–	≤0.5	≤0.5	0
	Tigecycline	≤2 S, ≥8R	–	≤0.5	≤0.5	0
Sulfonamides	Trimethoprim/ Sulfamethoxazole	≤2-38 S, ≥4-76 R	–	≤20	≤20	0

Notes: Except for tigecycline, the antibiotic susceptibility results were interpreted according to the latest 2024 CLSI M100 34th edition guidelines and the CLSI M45 third edition for rare organisms. The former was interpreted using FDA standards for Enterobacteriaceae (susceptible ≤ 2 mg/L; resistant ≥ 8 mg/L). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains in this experiment.

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Clinical Features of *Vibrio vulnificus* Infection

During the 3-year study period, 6 patients were diagnosed with laboratory-confirmed *Vibrio vulnificus* infections, and the isolates were frozen in the hospital. The average age of the patients was 59 years (ranging from 40 to 72 years), with 5 males and 1 female. All patients were from southeastern China and sought medical attention during the summer months of June to August. The 6 isolates were obtained from blood (n=5) or pus specimens (n=1). Among the patients, 4 exhibited bilateral lower limb edema, 2 reported having consumed seafood, and 2 presented with fever and diarrhea without any obvious triggers. The lesions were primarily located in the distal extremities, with the most common clinical symptoms being fever, pain, erythema, and local swelling, followed by vomiting and diarrhea. Three patients died from septicemia, two-thirds of people had a history of cirrhosis, while the other three were discharged, resulting in an overall mortality rate of 50% (Table 2). Upon admission, a comprehensive workup including a blood routine test, coagulation profile, blood gas analysis, PCT, and blood cultures was performed. Before the results of blood cultures became available, ROC curve analysis indicated that conventional laboratory parameters, specifically PCT, WBC, NE, RBC, Hb, HS-CRP, PT, TT, D-Dimer, and Lac, had significant clinical diagnostic value in the early detection of *Vibrio vulnificus* bloodstream infections, and detailed information is provided in the Supplementary Materials. The diagnostic efficiency, ranked from highest to lowest, was as follows: PCT/HS-CRP/Lac/PT/D-Dimer > RBC > Hb > TT > NE > WBC (Table 3 and Figure 4).

Table 2 Clinical Characteristics and Prognosis Analysis of Patients with *Vibrio vulnificus* Infection

Sample ID	Isolation Time	Sex	Age	Specimen Type	Infection Route	Immunosuppressed	Raw Seafood	Final Condition
GN221717	2022.07.24	Male	56	Blood	Small scattered purpura on the left lower leg	History of cirrhosis	No	Death
GN221511	2022.07.12	Male	54	Blood	Rash and swelling in the lower extremities	None	Yes	Recovery
GN221865	2022.08.01	Male	67	Blood	Bilateral lower extremity swelling and pain with multiple blisters	History of cirrhosis for more than 10 years	Yes	Death
GN240773	2024.08.30	Male	70	Blood	Fever with nausea and vomiting	Cirrhosis	No	Recovery
GN240542	2024.06.19	Female	40	Pus specimens	Fever with diarrhea	Diabetes	No	Recovery
GN240553	2024.06.22	Male	72	Blood	Erythroderma	None	No	Death

Table 3 Clinical Diagnostic Value of Different Inflammatory Indicators for Early Septicemia of *Vibrio vulnificus*

Inflammatory Indicators	AUC	Optimal Cutoff Value	Sensitivity	Specificity	Significance	95% Confidence Interval	
						Lower Limit	Upper Limit
HS-CRP	1	5.64	1	1	0.004	1	1
Lac	1	2.15	1	1	0.004	1	1
PCT	1	0.34	1	1	0.004	1	1
D-Dimer	1	591	1	1	0.004	1	1
PT	1	13.15	1	1	0.004	1	1
TT	0.89	15.35	1	0.67	0025	0.697	1
NE	0.88	3.85	0.83	0.5	0.031	0.666	1
APTT	0.82	31.8	0.83	0.5	0.066	0.566	1
WBC	0.86	5.475	0.83	0.67	0.037	0.604	1
FIB	0.32	2.2	0.5	0.83	0.298	0	0.641
PH	0.33	7.37	0.33	0.83	0.337	0	0.711
PLT	0.19	170.5	0.17	0.83	0.078	0	0.494
LY	0.17	1.15	0.17	0.83	0.055	0	0.465
RBC	0.03	3.92	0.17	0.83	0.006	0	0.111
Hb	0.06	121.5	0.17	0.83	0.010	0	0.186

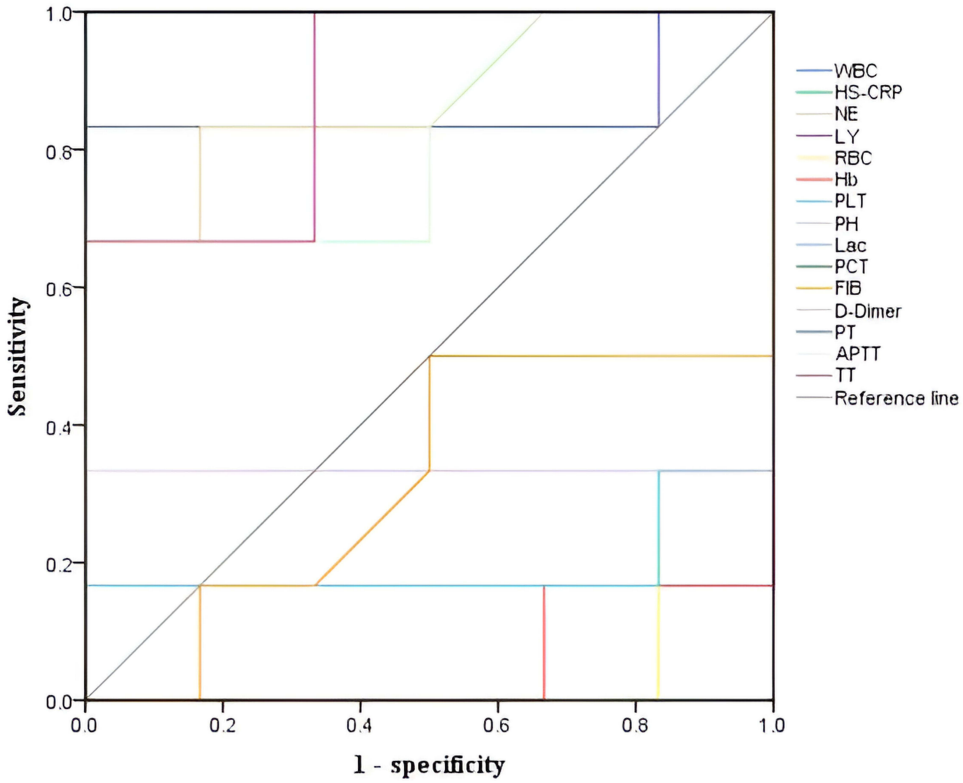

Figure 4 ROC curve analysis of *Vibrio vulnificus* bacteremia routine detection indicators.

Discussion

Genotyping of *Vibrio vulnificus*

In recent years, researchers have conducted extensive studies on the virulence and ecological characteristics of *Vibrio vulnificus* (Supplementary Table 2), continuously advancing their knowledge in this field. Based on biochemical characteristics and ecological environments, *Vibrio vulnificus* is currently classified into three biotypes: Biotype I is the earliest isolated subtype, possessing various LPS types, and is the primary pathogenic strain in clinical settings, with a mortality rate exceeding 50% in cases of primary sepsis, and is widely distributed globally. Biotype II, characterized by serogroup E LPS, primarily infects marine invertebrates such as eels.[17] Biotype III was first isolated during an outbreak in Israel in 1996–1997, displaying a biochemical phenotype distinct from Biotype I and Biotype II strains,[18] mainly related to the farming and processing of tilapia, with a lower mortality rate compared to Biotype I.[19]

Research has demonstrated that Biotype I strains can be further categorized into two lineages, vcgC and vcgE, based on virulence-related genes.[20] The vcgC-type strains[21] are more frequently isolated from clinical samples and are linked to systemic infections with elevated mortality rates. All *Vibrio vulnificus* isolates collected in this study are clinical (C-type) strains exhibiting strong pathogenicity, with a mortality rate of 50%. A study by Nilsson et in 2003 indicated that there are two 16S rRNA genotypes (A and B) for *Vibrio vulnificus*. Further research has found that Type B exists only in Biotype I, while Type A may be related to the potential virulence of *Vibrio vulnificus*.[22] Sanjuan et al[23] categorized *Vibrio vulnificus* into three lineages based on the diversity in housekeeping genes and virulence gene sequences. Subsequent studies further categorized eighty strains of *Vibrio vulnificus* into five lineages, with most clinical and environmental isolates belonging to lineages L1 and L2, while fish pathogens primarily fell into L3. These results indicate that the existing typing methods each have their focus and have not yet formed a unified standard. Currently, pulsed-field gel electrophoresis (PFGE) is widely recognized as the "gold standard" for typing *Vibrio vulnificus* due to its high resolution, clear patterns, and good reproducibility.[24] Furthermore, multi-locus variable number tandem repeat analysis (MLVA)[25] has also contributed to revealing the diversity of *Vibrio vulnificus* and its epidemiological connections.

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In this clinical sample, all detected strains of *Vibrio vulnificus* were identified as 16S rRNA Type A, which contradicts findings from previous studies on clinical isolates. While 16S rRNA Type B is associated with high mortality rates, this does not imply that 16S rRNA Type A is of low virulence or avirulent. Virulence may be influenced by various factors, including the expression of other genes, environmental conditions, and the immune status of the host. In this study, the prevalence of clinical *Vibrio vulnificus* strains exhibiting the 16S rRNA A/vcg C genotype met expectations.

Virulence Gene Analysis

To confirm the potential toxic threat to humans, we examined the status of virulent and non-virulent strains of *Vibrio vulnificus* in the aquatic environment based on combinations of virulence and avirulence genes. The results showed that all six isolated strains of *Vibrio vulnificus* carried various combinations of virulence genes. VvhA (which encodes *Vibrio vulnificus* hemolysin) and manIIA (which encodes enzyme IIA of the mannitol fermentation operon) are the primary virulence genes identified, followed by two other toxin genes: nanA (which encodes N-acetylneuraminic acid; 75%) and toxR (which encodes *Vibrio vulnificus* toxin; 33.3%).

Different strains carried exotoxins (VvhA, RTX) and also possessed adhesion factors (TadZ/CpaE,[26] IlpA,[27] VWA[28]) associated with bacterial adhesion and biofilm formation. These factors play an important role in the attachment process of bacteria, helping them adhere to host cells, while motility-related factors (cheW/R,[29] motA,[30] flgC[31]) regulate the bacteria's adaptation to the environment, nutrient searching, and avoidance of harmful substances.

However, the GN221865 strain possesses a more diverse array of virulence factors, and the presence of these genes enhances the pathogenicity of the bacteria, thereby increasing their competitiveness in the host environment. The effector delivery system, outer membrane protein ompA, and vipA/tssB and sciN/tssJ,[32,33] which are involved in the bacterial type VI secretion system (T6SS), enable the direct transfer of effector factors (such as toxins) into host cells or other bacteria, enhancing the pathogenicity of the bacteria. The genes mrkA, mrkB, and mrkC are associated with biofilm formation.[34] The proteins encoded by these genes play crucial roles in bacterial adhesion and biofilm maintenance. The formation of biofilms enables bacteria to persist in diverse environments, thereby enhancing their resistance to antibiotics and host immune responses.

Additionally, GN221865 also contains virulence factors related to iron acquisition, including fepA/B, entE/F, and iucA/B. Among these, the proteins encoded by the fepA and fepB genes play a critical role in the synthesis and function of iron transporters.[34] Iron is essential for bacterial growth and metabolism; thus, the effective acquisition of iron sources is vital for bacterial survival and pathogenicity. Specifically, fepA[35,36] is an outer membrane iron transporter (a receptor for the iron-tyrosine complex) primarily responsible for binding and transporting the iron-tyrosine complex, while fepB is an inner membrane protein associated with fepA, participating in the transport of iron from the outer membrane into the cell, assisting in iron absorption, and ensuring that bacteria can effectively utilize iron sources from the external environment. Iron-regulated anemia induced by the host serves as a significant defense mechanism against *Vibrio vulnificus* infection.[37] Serum iron is an essential nutrient for growth; higher serum iron concentrations correlate with increased virulence of *Vibrio vulnificus*. The hemolysin produced by *Vibrio vulnificus* can lead to elevated levels of free hemoglobin and nutritional iron in the bloodstream. In such an environment, *Vibrio vulnificus* proliferates rapidly, resulting in a dramatic increase in iron consumption by the infected host. The synergistic effects of these two virulence factors ultimately lead to increased mortality in individuals infected with GN221865. This phenomenon also elucidates why individuals with liver diseases, hemochromatosis, or Mediterranean anemia exhibit heightened susceptibility to the pathogenic effects of *Vibrio vulnificus*.[34,35]

Additionally, the enzymes encoded by the entE/F and iucA/B genes are related to the synthesis of iron transporters[38,39] and are responsible for producing tyrosine-derived iron transporters. These genes assist *Vibrio vulnificus* in acquiring iron sources, thereby enhancing its survival capability and pathogenic potential. Furthermore, the LPS (rfbA/B) involved in autoimmune regulation is related to the synthesis of polysaccharides in *Vibrio vulnificus*.[40,41] Specifically, rfbA and rfbB genes play important roles in the synthesis of O-antigen polysaccharides LPS, which are crucial for the bacteria's pathogenicity, immune evasion, and biofilm formation.

Antimicrobial Resistance Analysis

Since the discovery of penicillin in the 1920s, hundreds of antibiotics have been synthesized and applied in clinical settings, animal treatment, or growth promotion.[42] However, it is widely recognized that the overuse of antibiotics is associated with the emergence of antibiotic resistance. The CDC in the United States recommends the doxycycline, cephalosporins (such as cefotaxime), and fluoroquinolones (such as levofloxacin and ciprofloxacin) for the treatment of *Vibrio vulnificus* infections. Additionally, trimethoprim-sulfamethoxazole, in combination with aminoglycosides, tetracyclines, or ciprofloxacin, may also be employed.[43] According to CLSI 2024, the recommended antibiotics for treating Vibrio species include penicillins (such as ampicillin), cephalosporins, carbapenems (including imipenem and meropenem), aminoglycosides (such as amikacin and gentamicin), fluoroquinolones, and trimethoprim-sulfamethoxazole.

In our study of *Vibrio vulnificus* collected from the Ningbo area, we found that it exhibited resistance solely to ampicillin, while remaining susceptible to other antibiotics, which resulted in favorable treatment outcomes. Additionally, we analyzed the antibiotic resistance of *Vibrio vulnificus* strains from various geographical locations, including estuaries, coastal areas, and aquaculture sites (Supplementary Figure 1). The *Vibrio vulnificus* isolates obtained from tilapia and Asian sea bass collected along the western coast of the Malay Peninsula demonstrated significant resistance to a range of antimicrobials, exhibiting a complete resistance rate of 100% to ampicillin and a multidrug resistance (MDR) rate of 8.3%.[44] Vibrio species are commonly found in coastal and estuarine waters. In the United States, studies from the Savannah River[45] reported unexpectedly high frequencies of multidrug resistance among bacteria from various sources, particularly in summer, with a significant proportion of isolates (17.3%) exhibiting resistance to eight or more antimicrobial agents. Among the entire environmental collection (151 strains), 68 strains (45%) were resistant to three or more structural classes of antibiotics. In the Chesapeake Bay in the United States,[46] no strains collected from 2019 to 2022 exhibited resistance to any antibiotics, while strains from 2009 to 2012 showed relatively low levels of resistance to Amphetamine (16%), Cefotaxime (6%), Sulfamethoxazole/Trimethoprim (4%), Ceftazidime (2%), and Aminoglycosides (2%). On the coast of Maryland,[47] *Vibrio vulnificus* exhibited the highest resistance to cephalosporins (one of the recommended antibiotics for treating Vibrio infections), followed by cefoperazone, cefotaxime, ceftriaxone, cefazolin, ceftazidime, amikacin, and meropenem.

Pan et al tested the sensitivity of *Vibrio vulnificus* strains isolated from retail shrimp in Hangzhou, China, to 21 antibiotics and found a high frequency of resistance to aminoglycosides and some cephalosporins, such as gentamicin (93.94%), tobramycin (100%), cephazolin (10%), streptomycin (45.45%), aztreonam (24.24%), and cefepime (3.03%).[25] In Guangdong Province, Yuan et al[48] reported the detection of *Vibrio vulnificus* in freshwater fish in Shenzhen, with a detection rate as high as 38.6% in some freshwater fish, which is even higher than the detection rate in seafood. The antibiotic resistance of *Vibrio vulnificus* strains from aquaculture farms was found to be higher than that of strains from ponds, and antibiotic-resistant bacteria are widely present in shrimp and ponds in India.[49] Mohney et al[50] found that the minimum inhibitory concentration (MIC) range for tetracycline of *Vibrio* species isolated from shrimp in the United States was 0.0–30.0 mg/L, while the MIC range for bacteria isolated from shrimp farms in Thailand was 0.39–100 mg/L, with an average MIC of 37.5 mg/L, nearly three times that of the United States. Numerous studies have confirmed that the misuse of antibiotics in aquaculture is particularly severe, especially in Asia. Statistical data indicates that major aquaculture countries, including China, India, Iran, Thailand, and Malaysia, consume over a hundred tons of antibiotics annually.[51] This phenomenon has directly contributed to a significant increase in AMR within aquatic ecosystems and food animals, with resistance rates typically ranging from 10% to 50%.[52]

The Antibiotic Resistance Mechanisms of *Vibrio vulnificus*

For decades, *Vibrio* species exhibiting resistance to multiple antibacterial drugs have posed a significant health concern. The mechanisms underlying antibiotic resistance in *Vibrio* include biofilm formation, drug inactivation, target protection, reduced permeability to antibiotics, and active efflux of antimicrobials (Supplementary Figure 2). Within *Vibrio* cells, the cellular targets of antimicrobial drugs are located inside the bacterial cell, and the growth-inhibiting effects of these intracellular drugs can be diluted by various solute efflux systems. Extended-spectrum beta-lactamase (ESBL) genes have been detected in *Vibrio vulnificus* strains isolated from imported seafood,[53] reporting the presence of ESBL genes such as

bla$_{\text{CTX-M}}$ (87.5%), *bla*$_{\text{TEM}}$ (40.6%), and *bla*$_{\text{SHV}}$ (21.8%) among Vibrio isolates. Recent studies have also documented the presence of *Vibrio cholerae* and *Vibrio parahaemolyticus* strains harboring the *bla*$_{\text{NDM}}$ gene in seafood and environmental samples.[54]

The genome of *Vibrio vulnificus* contains 11 putative RND (Resistance-Nodulation-Division) efflux pump encoding genes, three of which (the VexAB and VexCD of *Vibrio cholerae* and the homologous AcrAB of *Escherichia coli*) have been characterized through gene deletion studies.[55,56] Although the specific mechanisms by which RND efflux systems regulate the expression of virulence factors have not been fully elucidated, there is evidence suggesting that the effects of efflux pump inhibitors on virulence genes may involve various mechanisms, rather than merely inhibiting the efflux pumps. In *Vibrio cholerae*, cholera toxin (CT) and toxin co-regulated pilus (TCP) are two important virulence factors.[57] Studies have proposed that efflux pump inhibitors can reduce the expression of tcpPH and toxT genes, thereby affecting the production of CT and TCP, indicating a complex interaction between virulence factors and RND efflux pumps.[58] Additionally, the outer membrane protein TolC, as part of the tripartite efflux system, is involved in the secretion of the RtxA1 toxin in *Vibrio vulnificus*.[59,60] Although there is limited information on the adhesion and biofilm formation of *Vibrio vulnificus*, reports have indicated that the eel pathogen *Vibrio* E serotype can form biofilms between the epidermal cells of eels.[61] Furthermore, a small-molecule inhibitor called BrpT (Bacterioferritin-associated protein T) has been identified to combat the biofilm formation of *Vibrio vulnificus*.[62]

Research Findings and Limitations

This study investigates the genetic diversity and pathogenic mechanisms of *Vibrio vulnificus*, emphasizing the critical role of bacterial virulence genes in the infection process. Additionally, it elucidates the influence of environmental and host factors on the pathogenicity of this bacterium. While the statistical power and generalizability of the study's conclusions may be limited due to the small sample size, the findings offer significant scientific insights into the pathogenic mechanisms of *Vibrio vulnificus* and its potential threats to human health. Through comprehensive testing of six strains of *Vibrio vulnificus*, the study reveals that all strains possess multiple virulence genes, including those that encode cytolysins and other toxins. Furthermore, strain GN221865 exhibited a wider array of virulence factors, involving effector delivery systems and genes related to iron acquisition. The presence of these genes enhances the bacterial pathogenicity and increases its competitiveness in the host environment.

The results of this study indicate that the *Vibrio vulnificus* strains isolated from the Ningbo region exhibited 100% resistance to ampicillin but were susceptible to other recommended antibiotics such as doxycycline, cephalosporins, and fluoroquinolones, suggesting good treatment outcomes. This finding contrasts with studies from other regions, such as Malaysia and the United States, where isolates showed high rates of multidrug resistance. There are significant differences in the prevalence of antibiotic resistance in different geographical environments. In the United States, the frequency of multidrug resistance among *Vibrio* species is higher, especially in the summer, suggesting that climatic and environmental factors may influence the evolution of bacterial resistance. *Vibrio* species possess various resistance mechanisms, among which the RND efflux pump plays a critical role in antibiotic resistance and may have complex effects on the expression of virulence factors. In the early diagnosis of *Vibrio vulnificus* infections, traditional laboratory indicators (such as PCT, WBC, RBC, and Lac) showed good prognostic value. This indicates that timely identification of infections before blood culture results are available is crucial for improving patient survival rates.

Research Conclusions and Prospects

In summary, the high prevalence and mortality rate of *Vibrio vulnificus* pose a significant threat to public health, necessitating increased attention. Future research should further investigate how different virulence factors synergistically enhance the pathogenicity of *Vibrio vulnificus*, particularly under varying host and environmental conditions, through multicenter and large-sample studies. Additionally, in-depth research into the mechanisms of antibiotic resistance in *Vibrio vulnificus* is crucial for understanding the transmission pathways of resistance genes and the epidemiological trends of resistance, which are essential for formulating effective public health strategies and clinical treatment protocols.

Publication Agreement

All participants provided written informed consent for publication of their clinical details.

Abbreviations

WGS, whole genome sequencing; ROC curves, Receiver Operating Characteristic curves; CLSI, Clinical and Laboratory Standards Institute; MLVA, Multi-Locus Variable Number Tandem Repeat Analysis; MLST, Multi-locus sequence typing; CDC, The Centers for Disease Control and Prevention; VvhA, Vibrio-specific hemolysin A; toxR, virulence toxin; PFGE, Pulsed-Field Gel Electrophoresis; T6SS, type VI secretion system; LPS, lipopolysaccharides; MDR, multidrug resistance; ARGs, Antibiotic resistance genes; manIIA, mannitol-specific enzyme IIA; RtxA1, multifunctional autoprocessing repeats-in-toxin A1; nanA, N-acetylneuraminic acid lyase; toxR, toxin regulator; vcgC, virulence-correlated gene C; vcgE, virulence-correlated gene E; MIC, minimum inhibitory concentration; ESBL, Extended-spectrum beta-lactamase; RND, Resistance-Nodulation-Division; CT, cholera toxin; TCP, toxin co-regulated pilus; PCT, procalcitonin; WBC, white blood cell count; NE, neutrophil count; RBC, red blood cell count; Hb, hemoglobin; HS-CRP, high-sensitivity C-reactive protein; PT, prothrombin time; TT, thrombin time; D-D, D-dimer; Lac, Lactate; PLT, Platelet; LY, Lymphocyte; APTT, Activated partial thromboplastin time; FIB, Fibrinogen; TolCBrpT, Bacterioferritin-associated protein T.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of The First Hospital of Ningbo University (approval number: 087RS). Informed consent was waived for all participants due to the retrospective nature of the study. The study was conducted by the principles of the Declaration of Helsinki.

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Disclosure

The authors declare that they have no competing interests.

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