Gut Pathogens

BRIEF REPORT Open Access

A fatal case of *Vibrio cholerae*-associated diarrhea and bacteremia in a 30-year-old carrier of beta-thalassemia

Mohammad Tarequl Islam[1†], Kazi Sumaita Nahar[1†], Nikhat Ara[2], Suma Mita Biswas[2], Waliullah[1], Jarin Tasnim[1], Mohammad Nazmus Sakib[1], Abdullah Al-Mamun[1], Alimul Islam[1], Anindita Bristi[1], Marzia Sultana[1], Dilruba Ahmed[1], Kimberley D. Seed[3], Andrew Camilli[4*], Tahmeed Ahmed[1] and Munirul Alam[1,5*]

Abstract

Bacterial infections leading to bacteremia and septicemic shock constitute an emerging public health concern globally, especially in areas where sanitation is poor and safe drinking water is scarce. Enteric pathogens such as *Vibrio cholerae* are responsible for many deaths caused by contaminated food and water in these areas. While cholera is the prominent clinical threat posed by *V. cholerae*, outcomes like bacteremia turning into sepsis and associated morbidity and mortality have been increasing globally in recent times. Here, we report an alarming case of fatal sepsis with a probable association of *V. cholerae* bacteremia in Bangladesh. In September 2023, a 30-year-old man with a pre-condition of beta-thalassemia presented to a tertiary care hospital with acute diarrhea, abdominal pain, nausea, and fever and died within 36 h of admission with acute cholecystitis, metabolic acidosis, acute kidney injury, pancytopenia, and refractory septic shock with multi-organ dysfunction syndrome. Blood culture detected *V. cholerae*, which was further characterized as hemolytic, carrying the hemolysin gene and genes for the virulence factor type-three secretion system. The isolate was confirmed as *V. cholerae* non-O1/O139 (NOVC), which differed in genetic properties from the few contemporary NOVC isolates associated with diarrheal cases in Bangladesh. To manage the diarrhea and septicemic condition, the patient was treated empirically with metronidazole and meropenem. However, antibiotic susceptibility testing showed the strain was susceptible to all the routinely prescribed drugs for *V. cholerae* infections. To the best of our knowledge, this investigation provides the first molecular description of a fatal case of *V. cholerae*-associated bacteremia in Bangladesh and underscores the need for comprehensive investigations on bacterial septicemia to prevent future casualties.

†Mohammad Tarequl Islam and Kazi Sumaita Nahar contributed equally to this work.

*Correspondence:
Andrew Camilli
andrew.camilli@tufts.edu
Munirul Alam
munirul@icddrb.org

[1]ICDDR,B (International Centre for Diarrheal Disease Research, Dhaka, Bangladesh
[2]Evercare Hospital, Dhaka, Bangladesh
[3]Department of Plant and Microbial Biology, University of California - Berkeley, Berkeley, CA, USA
[4]School of Medicine, Tufts University, Boston, USA
[5]Infectious Diseases Division International Centre for Diarrheal Disease Research, Shaheed Tajuddin Ahmed Sarani, Bangladesh 68, Mohakhali, Dhaka 1212, Bangladesh

Introduction

Septicemia is a life-threatening infection associated with organ injury resulting from a dysregulated host response to an infection [1, 2]. Due to underreporting, especially from low- and middle-income countries (LMICs), the true magnitude of the incidents is unknown, while the actual number of deaths is often masked because fatalities are attributed to the underlying conditions instead of sepsis. A recent global analysis extrapolated the incidence and mortality rate of sepsis to 48.9 million cases and 11 million fatalities each year [1], highlighting the gravity of the issue. Of the recorded incidences, diarrheal illness appears to be the most common disease that leads to septicemia [1].

Vibrio spp. are a diverse group of organisms, including notorious human pathogens like *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus*, responsible for diarrheal illnesses globally that can potentially cause bacteremia leading to sepsis. The bacteria can cause illnesses, categorized into two types: cholera, caused by toxigenic *V. cholerae*, and non-cholera infections or vibriosis. *V. cholerae* is primarily known for causing cholera and gastroenteritis but occasionally can cause infections in other body sites [3]. Alarmingly, the incidences of cholera and vibriosis are on the rise globally, potentially due to global climate change and rising temperatures [4]. This rise in cases of such diseases could dramatically escalate the threat posed by the bacteria to the burden of septicemia-associated illness and death.

V. cholerae is a gram-negative bacterium ubiquitously found in aquatic ecosystems worldwide and is the cause of the pandemic disease cholera [5]. There are more than 200 different serogroups of the bacteria, of which serogroups O1 and O139 have been found to consistently conserve the ability to produce cholera toxin, hence being associated with cholera [5]. The serogroup O1 has been associated with seven pandemics of cholera, affecting millions throughout the world. As a result, strains of *V. cholerae* O1 and O139 have been studied extensively. Strains belonging to other serogroups, collectively designated as non-O1/O139 V. cholerae (NOVC) [6, 7], typically do not produce cholera toxin but have been found to cause small outbreaks of cholera-like diseases and sporadic intestinal and extra-intestinal illnesses [8]. However, there have been growing reports of NOVC infections in recent years, a significant number of which progressed to severe and fatal infections [4, 9–12], underscoring the threat presented by these strains. Especially when the infection progresses to bacteremia and sepsis, *V. cholerae* infection can result in catastrophic outcomes [3, 10, 11, 13, 14]. In Bangladesh, there is a low incidence of reported gastroenteric infections caused by NOVC, and to the best of our knowledge, there have been no reports of bacteremia caused by *V. cholerae* to date. Thus, the true burden remains unknown, even though observations from similar settings [15, 16] suggest that these cases are hugely underreported. Here, we report a case of *V. cholerae*-associated diarrhea and bacteremia in a 30-year-old man who succumbed to death within 36 h of hospitalization and present a detailed phenotypic and genotypic characterization of the isolate for future reference in such unfortunate cases.

Method and materials

The bacterium was isolated from the blood culture of the patient seeking treatment at The Evercare Hospital, Dhaka, Bangladesh. Identification of *V. cholerae* was performed according to standard culture and molecular methods as described previously [17]. Hemolytic property of the isolate was tested on 5% sheep blood agar plates with an overnight incubation at 37°C. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc.) as per the manufacturer's instructions. The serogroup of the *V. cholerae* isolate was confirmed by a slide agglutination test using *V. cholerae* O1 and O139-specific polyvalent antisera [17]. Molecular confirmation of serogroups, species-specific genes, and detection of virulence-associated genes namely *ompW*, *viuB*, *rfbO*1, *rfbO*139, *tcpA*, TTSS, *hlyA*, *ompU*, *rtxC* was done by Polymerase chain reaction (PCR) following methods described previously [17–20]. Using the Clinical and Laboratory Standards Institute guidelines [21], a standard disc diffusion test on Muller-Hinton agar (BD, USA) determined bacterial susceptibility to antimicrobials. Fourteen drugs of different drug classes, namely azithromycin (AZM), tetracycline (TE), ampicillin (AMP), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (C), erythromycin (E), imipenem (IPM), gentamicin (CN), aztreonam (ATM), amoxicillin-clavulanate (AMC), levofloxacin (LEV), ciprofloxacin (CIP), cefepime (FEP), ceftriaxone (CRO), meropenem (MEM), vancomycin (VA), colistin (CT), and rifampin (RD), were tested. The minimum inhibitory concentration (MIC) was determined by E-test (Biomeuriex). PCR was also performed to detect antimicrobial resistance-associated genes, namely *qnrVC*, *mphA*, *sxt*, *bla-NDM*, *bla-OXA*-48, using primers and procedures described elsewhere [22–24]. Nucleotide sequencing of *viuB*, *gyrA*, *parC*, and TTSS genes was performed using the Sanger di-deoxy termination method at the sequencing facility at the International Centre for Diarrheal Disease Research, Bangladesh (icddr, b). To amplify 293 bp of the *viuB* region from DNA extracted from water samples, a PCR reaction was performed in a total of 20 µL reaction mix using 1 µL each of 10 pmol forward and reverse primers

(viuB2f 5'-CCGTTAGACAATACCGAGCAC-3' and viuB5r 5'-TTAGGATCGCGCACTAACCAC3'), 2 µL of 10 mM dNTP mix (ThermoFisher), 1 µL of magnesium chloride (ThermoFisher), 0.2 of µL Taq DNA Polymerase (Invitrogen), 2 µL of 10× Buffer, and 2 µL of template DNA. The PCR reaction was performed using the following program: initial denaturation at 95 °C for 3 min; followed by 34 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 30 s and extension 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. Sequencing of the PCR product was performed in an Applied Biosystems 3500XL genetic analyzer using the BigDye Terminator v3.1 cycle sequencing ready reaction kit (PerkinElmer). The sequence files were processed and multiple sequence analysis was performed in Geneious (Geneious 2023.2) comparison using the existing *viuB* reference sequence types described in Kirchberger et al. [19].

Table 1 Summary of clinical laboratory tests results

Parameters	Value	Unit	Normal range
CRP (C Reactive Protein)	9.80	mg/dl	< 0.37
Procalcitonin	72.24	ng/ml	< 0.05
Hemoglobin	7.6	gm/dl	13.5–17.5
Hematocrit	23.30	%	40–52
WBC count	2.59	10^9/L	4–11
Neutrophils	45.1	%	40–80
Lymphocytes	45.2	%	20–40
Platelet count	100	10^9/L	150–400
Bilirubin	5.4	mg/dl	0.0–1.0
ALT (SGPT)	64	IU/L	< 50
AST (SGOT)	151	IU/L	15–45
Alkaline phosphatase	86	IU/L	40–115
Total Protein	6.8	g/dl	6.5–8.5
Albumin	2.1	g/dl	3.5-5
Globulin	4.7	g/dl	2–4
Urea	52	mg/dl	15–45
Creatinine	1.85	mg/dl	0.50–1.30
Sodium	132	m mol/L	135–145
Potassium	4.2	m mol/L	3.5-5.0
Chloride	98	m mol/L	98–108
Bicarbonates	12	m mol/L	24–32
Amylase	27	U/L	< 125
Lipase	65	U/L	73–393
NT proBNP	6303	pg/ml	< 125
Troponin I	19.2	ng/L	< 34.2
Prothrombin time (PT)	45.3	Seconds	9.8–12.1
International normalized ratio (INR)	4.31		
Activated Partial Thromboplastin Clotting Time (APTT)	48.7	Seconds	25.0-31.3
Fibrinogen	123.5	mg/dl	180–350
D-Dimer	2935	µg/L	< 500

mg/dl-milligram/decilitre, ng/ml-nanogram/decilitre, L-litre, IU/L-International Units/litre, g/dl-gram/decilitre, m mol/L-millimole/litre, U/L-Units/litre, pg/ml-picogram/millilitre, µg/L-microgram/millilitre

Case history and results

A 30-year-old nondiabetic, normotensive male patient presented to the emergency department of The Evercare Hospital on 8 September 2023 with severe upper abdominal pain since morning. He also experienced nausea, several episodes of loose stool, and a fever the previous night. The patient has been diagnosed with beta-thalassemia intermedia since the age of 4 years, had gross hepatosplenomegaly, and received his last blood transfusion more than a year ago (Table 1).

At the emergency department, a physical examination of the patient revealed mild disorientation (GCS was E4 V4 M6), hypotension, tachycardia, and tachypnoea with a normal body temperature. He had generalized abdominal tenderness with reduced urinary output. His blood picture showed pancytopenia with moderate anemia (hemoglobin 7.6 g/dl), mildly elevated liver enzymes (SGOT 151 IU/L, SGPT 64 U/L), raised serum urea (52 mg/dl) and creatinine (1.85 mg/dL) and a normal electrolyte level. His septic biomarkers were suggestive of sepsis (CRP 9.80 mg/dl, procalcitonin 72.24 ng/ml), and his coagulation profile was grossly deranged. His arterial blood gas analysis revealed metabolic acidosis with a pH of 7.2. An ultrasonogram of his abdomen showed features suggestive of acute intestinal obstruction or paralytic ileus, acute cholecystitis with thick sludge, moderate abdominopelvic ascites, and bilateral trace pleural effusion. He was moved to the intensive care unit as he had acute watery diarrhea with metabolic acidosis, septic shock, acute kidney injury, and pancytopenia. There, adequate hydration was ensured under central venous pressure (CVP) guidance, and he was started on intravenous sodium bicarbonate and the antimicrobials Inj. Meropenem (1 gm thrice a day) and metronidazole (500 mg four times a day) empirically.

At 7 p.m. the same day, the patient's condition became critical with a non-recordable pulse and blood pressure and subsequent cardiac arrest was evident. The patient was immediately taken into advanced cardiac life support with mechanical ventilation. He was also given one unit of packed red blood cell. At 11:45 p.m., his condition worsened again with non-recordable blood pressure, no pulse, and a non-detectable SPO2 with a fixed and dilated pupil. Upon medical examination, the patient was declared dead on 9 September 2023, with the multi-organ dysfunction syndrome as the main cause of death and acute cholecystitis, metabolic acidosis, acute kidney injury, beta thalassemia intermedia, pancytopenia, and refractory septic shock as secondary confounders.

Laboratory investigations

Blood culture performed at the microbiology lab of Evercare Hospital revealed the growth of *V. cholerae*, and the isolate was sent to the *V. cholerae* reference laboratory at

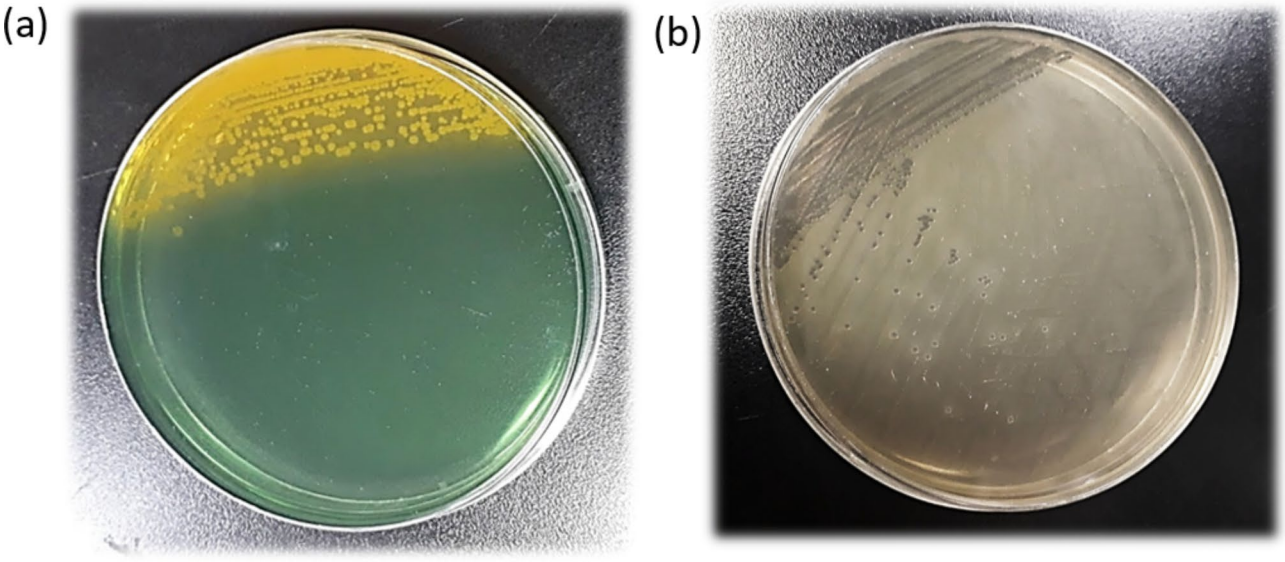

Fig. 1 The isolate showing colony morphology of a typical *V. cholerae* strain demonstrating yellow colonies on TCBS agar (**a**) and gelatinase production on TTGA (**b**)

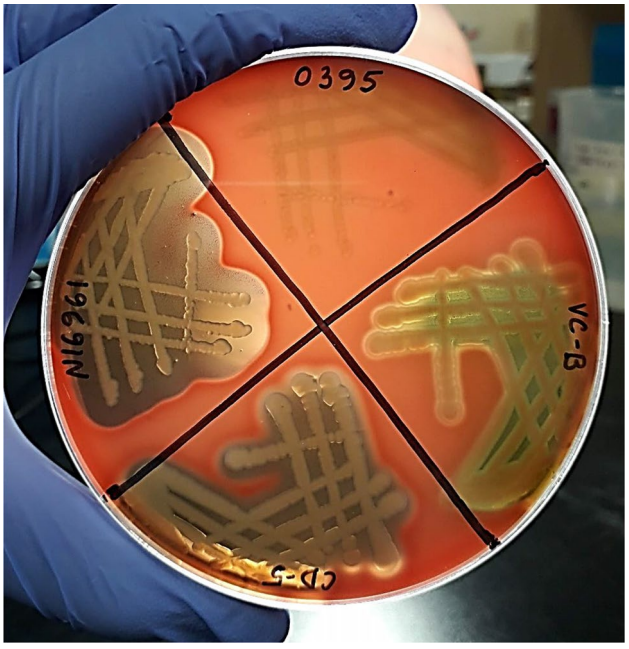

Fig. 2 The strain showing moderate hemolytic activity on 5% sheep blood agar compared to the reference strains. N16961 – *Vibrio cholerae* O1, ET biotype, O395 – *V. cholerae* O1, classical biotype, CD5 – *V. cholerae* non O1/O139, VC-B – SNVC 01; Non-hemolytic (-), Moderate Hemolytic Activity (+), Intermediate Hemolytic Activity (++), Strong Hemolytic Activity (+++)

the icddr, b for serotyping and further investigation. The isolate (SNVC-01) showed the colony morphology of a typical *V. cholerae* on thiosulfate citrate bile salt (TCBS) agar and taurocholate tellurite gelatine agar (TTGA) (Fig. 1). Reaction with polyvalent *V. cholerae* O1 and O139 antisera did not result in any agglutination of the strain; hence, it was typed as *V. cholerae* non-O1/O139 (NOVC).

When tested for hemolytic properties, the strain had moderate beta-hemolytic activity compared to the strong hemolytic activity by the reference *V. cholerae* O1 El Tor strain N16961 (Fig. 2). The hemolytic properties of the strain were compared with nine other clinical NOVC strains isolated from stool sample of diarrheal patients at icddr, b laboratory. Notably, these strains exhibited a wide range of hemolytic activity, from non-hemolytic to strongly hemolytic (Table 2).

PCR based investigation revealed that the strain carried *V. cholerae* species-specific genes, *ompW* and *viuB*, confirming the identity of the isolate. PCR for serogroup O1 and O139-antigen-specific genes, *rfbO*1 and *rfbO*139, gave a negative result typical of NOVC. Among the potential virulence genes tested, the isolate carried the hemolysin encoding gene *hlyA*, type-three secretion system (TTSS) associated genes *vcsC2*, *vopM*, and *vopG*, and the repeat-in toxin gene, *rtxC*. However, it was negative for the canonical virulence factors cholera toxin gene (*ctxA)*, toxin co-regulated pili (*tcpA*), and the outer membrane porin, *ompU*.

To assess the subtype properties of the isolate in the context of contemporary *V. cholerae* isolates, the marker gene *viuB* was sequenced, and the genotype was determined as *viuB*-56 following the criteria described by Kirchberger et al. BLAST search in the NCBI nucleotide database revealed three other *V. cholerae* strains, including one isolated from clinical sample, to possess genotype *viuB*-56 (Accession no. AP028804, CP078727, and CP078723). To assess additional genotypic information, nucleotide sequencing of the PCR amplicon of the genes

Table 2 Phenotypic and genotypic characteristics of *Vibrio cholerae* non O1/O139 isolated from the sepsis patient and dirrheal patients in Dhaka, Bangladesh

Sl.	Strain ID	Serogroup	Year of Isolation	Resistance Profile	*Hemolytic properties	Genetic Profile												
						ompW	CTX-*tcpA*	*viuB*	*mphA*	*qnrVC*	SXT	T3SS-1	T3SS-2	T3SS-3	bla NDM	bla OXA-48	hlyA ET	ompU	rtxC
1	SNVC-01	VC Non O1/O139	2023	VA, CT	Beta (+)	+	-	viuB-56	+	-	-	+	+	+	-	-	+	-	+
2	CD - 5	VC Non O1/O139	2018	VA, CT	Beta (++)	+	-	viuB-10	+	+	+	-	-	-	-	-	+	+	-
3	CD - 8	VC Non O1/O139	2018	AMP, CRO, FEP, NA, AZM, E, VA, ATM	Alpha	+	-	viuB-67	+	-	-	+	-	+	-	-	+	+	-
4	CD - 26	VC Non O1/O139	2019	AMP, NA, AZM, E, SXT, TE, VA	Beta (++)	+	-	viuB-35	+	+	-	-	-	+	-	-	+	-	-
5	CD - 47	VC Non O1/O139	2023	CT, AMP, CXM, MEL, AZM, E, VA	Alpha	+	-	viuB-10	+	-	-	-	-	-	-	-	+	+	+
6	CM - 24	VC Non O1/O139	2019	VA, CT	Beta (++)	+	-	viuB-49	+	-	-	+	-	+	-	-	+	+	+
7	CM - 25	VC Non O1/O139	2019	VA, CT	Beta (++)	+	-	viuB-45	+	-	+	-	+	+	-	-	+	-	+
8	CM - 42	VC Non O1/O139	2023	CT, E, AZM, MEL, CXM, AMP, SXT, IPM, VA	Beta (++)	+	-	viuB-10	+	+	+	-	+	-	-	-	+	-	+
9	CM - 43	VC Non O1/O139	2023	AMP, CXM, MEL, AZM, E, CT, VA	Beta (+++)	+	-	viuB-10	+	+	-	-	+	-	-	-	+	-	+
10	CM - 44	VC Non O1/O139	2023	IPM, SXT, AMP, AMC, CXM, AZM, E, VA	Beta (++)	+	-	viuB-10	+	+	-	-	+	-	NDM-1	-	+	-	+
11	N16961 (Reference strain)	VC O1	1971	Sensitive to all drugs	Beta (+++)	+	+	viuB-73	+	+	+	-	-	-	-	-	+	+	+

*Moderate Hemolytic Activity (+), Intermediate Hemolytic Activity (++), Strong Hemolytic Activity (+++).

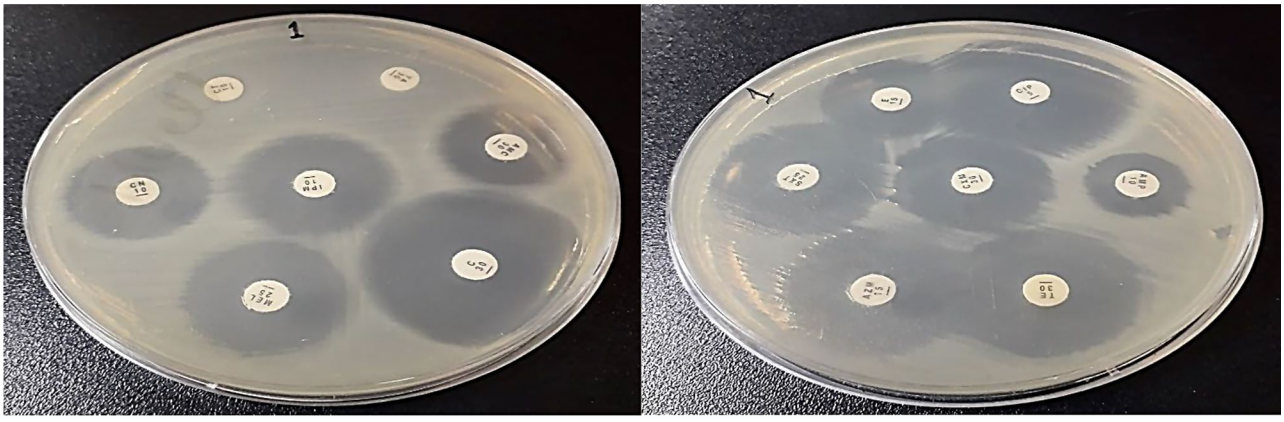

Fig. 3 Disc diffusion assay showing susceptibility of the strain against twelve different drugs, and resistant against colistin and vancomycin

gyrA and *parC* was done. Sequence analysis revealed that the isolate SNVC-01 possessed the wild-type allele of both *gyrA* and *parC*, whereas contemporary *V. cholerae* isolates worldwide are commonly known to have mutations 'Ser83 to Ile' and 'Ser85 to Leu' in *gyrA* and *parC*, respectively [25, 26].

In the icddr, b laboratory, the antimicrobial susceptibility of the isolate was determined against a panel of fourteen antibiotics and compared with that of contemporary NOVC isolates. The antimicrobial susceptibility test (AST) revealed that SNVC-01 was susceptible to twelve antibiotics of different drug classes, including the drugs (doxycycline, tetracycline, erythromycin, and azithromycin) commonly prescribed for cholera (Fig. 3). The strain was resistant to colistin and vancomycin, with no visible zone of inhibition. Minimum inhibitory concentration (MIC) was measured for colistin, and the breakpoint for inhibition was 64 µg/mL. In the molecular tests, antimicrobial resistance (AMR)-associated genes, *qnrVC*, *sxt*, *bla-NDM*, and *bla-OXA*-48 were not detected by PCR. However, the strain carried the macrolide resistance gene *mphA* despite showing susceptibility to the macrolide antibiotics azithromycin and erythromycin. Compared to the isolates from diarrheal patients, it was noted that although these *V. cholerae* strains share a similar AMR profile with SNVC-01, the strains differ significantly in other phenotypic and genetic features (Table 2).

Discussion

Presently available data on sepsis incidences in Bangladesh suggests that these cases are predominantly attributed to gram-negative bacteria [27–29]. However, none

of the studies detected *V. cholerae*, even though there have been recorded cases of bacteremia and septicemia caused by *V. cholerae* in several regions worldwide [6]. In a recent study on the ICU patients treated in icddr, b Dhaka Hospital, Sarmin et al. reported that 27% of the isolates from the stool of diarrheal patients diagnosed with severe sepsis were *Vibrio* spp [28]. The majority of the previous *V. cholerae* bacteremia case studies reported that the source of infection was either through the consumption of raw or undercooked seafood or by coming into direct contact with contaminated water while having an open wound [3, 6, 12, 13, 30–32]. While the exact causal relationship between enteric infections and sepsis is not completely understood, it has been shown that enteric pathogens can damage the gut mucosal layers and exacerbate intestinal infection by impacting the extraintestinal organs. Some enteric infections trigger a cascade of cellular events, ultimately causing cell death, increased gut permeability, and subsequent release of components from the host's intestinal lumen into the circulatory system and other organs. This leads to detrimental consequences such as sepsis. Whether or not the infection has spread beyond the gastrointestinal tract, bacterial endotoxins are known to enhance the severity of sepsis, most likely by amplifying the inflammatory processes [33]. A similar observation came up in several other studies, where translocated bacteria from the inflamed gut was thought to have resulted in severe sepsis [27].

Our understanding of NOVC strains linked to human diseases is still limited. With a few exceptions, most research on bacteremia and sepsis caused by *V. cholerae* primarily consists of case reports and lacks a thorough analysis of the causative strains. In this study, we performed extensive genetic screening of the potential virulence genes following the identification of the associated *V. cholerae* strain. While the strain lacked the cholera-toxin-encoding gene *ctx*, toxin coregulated pili (*tcpA*) and porin protein-encoding gene *ompU*, it carried virulence genes such as type three secretion system (TTSS), repeat in toxin (RTX) gene clusters, and the gene encoding hemolysin (*hlyA).* TTSS has been found to cause diarrheal symptoms comparable to full-blown cholera and, hence, can serve as a potent virulence factor for NOVC [34]. TTSS could be crucial for the clinical manifestation of the study isolate. Studies on animal models show that TTSS gene clusters play a critical role in the colonization of host epithelial cells, aid in initiating an infection, and are involved in the increased pathogenic potential of *V. cholerae* [35, 36]. While epidemic strains of *V. cholerae* use TCP as the primary colonization factor, NOVC strains that do not encode TCP instead employ TTSS. In addition to TTSS, *V. cholerae* may also rely on o*mpU* as an adherence factor and is recognized as an important virulence factor for its contribution to the colonization of host epithelial cells [37, 38]. We observed that the strain from the sepsis patient possesses essential translocation and effector protein-encoding genes of TTSS but does not have *ompU*. This indicates that TTSS may have facilitated gut colonization and led to diarrhea in the patient. RTX gene clusters have also been shown to be associated with cytotoxicity in mammalian cells [39]. Hemolysis of erythrocytes is a virulence trait widely distributed among pathogenic *Vibrio* spp. When tested for this property, SNVC-01 demonstrated moderate beta-hemolytic activity against sheep erythrocytes and was found to carry the *V. cholerae* El Tor hemolysin gene, *hlyA* ET. *hlyA* is strongly associated with invading pathogens and is involved in enhanced cytotoxicity and apoptosis during infection [11, 40]. This property of the strain would be important for future references, considering the potential association of thalassemia and hemolytic behaviour of the pathogen. Upon comparing the NOVC isolates from diarrheal patients, we found that irrespective of the phenotypic features, all the strains carried *hlyA*, while their hemolytic behaviour varied from no to strong hemolysis (Table 2). Other potential virulence factors might have contributed to the clinical outcome, and whole genome sequencing of the isolate will provide clues to mend the gaps in the understanding of the case. Recently, vibriobactin utilization protein subunit B (*viuB*) was shown to be a promising molecular marker to study the population structure and close relatives of *V. cholerae* as each allele of *viuB* roughly corresponds to a distinct lineage of *V. cholerae* [41]. When we compared the *viuB* sequence of SNVC-01 with the NCBI database, three isolates that were isolated from environmental and clinical sources shared 100% sequence identity. Considering the known diversity of the *viuB* genotype [19, 41] *viuB*-56 appears uncommon and found for the first time in Bangladesh.

Recent studies suggest that antimicrobial resistance (AMR) properties are not only associated with the disease's clinical outcome but also potentially serve as important evolutionary markers that possibly impact the survival and adaptability of the bacteria in environments like the human body [42]. Even though the isolate SNVC-01 was found sensitive to the commonly used antibiotics in preliminary screening, considering the gravity of the case, the context of the global rise of AMR and lack of information on the epidemiological patterns of NOVC, we expanded the phenotypic and molecular tests pertinent to AMR properties additionally including some non-conventional antibiotics for *V. cholerae*, like vancomycin and colistin as done in several studies [43–45]. When the AMR profile of the isolate was compared to that of contemporary NOVC clinical isolates in our collection, few strains (CD5, CM24, CM25) isolated from diarrheal patients showed similar AMR (Table-2) pattern. Resistance to antimicrobials is a challenge for the clinical

management of hospital-associated infections, including septicemia. The NOVC isolate SNVC-01 was susceptible to all the drugs that are commonly prescribed for vibriosis or bacteremia, in contrast to the contemporary multidrug resistant *V. cholerae* strains prevalent in Bangladesh (Table-2). The AMR gene profile of this strain was also strikingly different from the contemporary NOVC isolates associated with clinical cases in Bangladesh. Only *mphA*, encoding for macrolide resistance, was detected in the strain by PCR. Despite the strain's susceptibility to macrolide antibiotics, this gene indicates a potential functional role. Quinolone resistance in *V. cholerae* has been linked to mutations in the DNA gyrase gene *gyrA* and the topoisomerase gene *parC*. Over the years, the El Tor variants of *V. cholerae* acquired several mutations in these genes, thus serving as a marker to predict the strain type. It has recently been shown that mutations in the *gyrA* gene appear to be distinct among the lineages and could be an indicator of the current circulating lineage [26]. PCR amplicon sequencing of *gyrA* and *parC* revealed that SNVC-01 harbors the wild type genes and does not possess any mutations, in contrast to the contemporary O1 strains in Bangladesh [26].

Although the majority of infections caused by bacterial pathogens are treatable with available antibiotics [4] and the proportion of patients achieving complete recovery is significant, there are unfortunate incidences leading to mortality, many of which are associated with patients having pre-conditions like compromised immune systems and chronic illnesses [3, 10, 11, 13, 14] (J. Chen et al., 2020; Ding et al., 2022; Engel et al., 2016; Hwang et al., 2021; Phetsouvanh et al., 2008). Clinical manifestations of bacteremia and sepsis caused by *V. cholerae* can vary depending on the route of infection. The most common clinical presentations are diarrhea, abdominal discomfort, fever, and vomiting [6, 14, 30, 46]. Similarly, in the present case, the patient was suffering from abdominal pain, diarrhea, nausea, and fever. While the causal link between the severity of the disease and the pathogenic traits of the isolated bacteria is often obscure, it is widely acknowledged that host susceptibility is likely to play a significant role. Children, elderly people, individuals with liver diseases, and compromised immune systems are considered to be at higher risk of acquiring an infection with *V. cholerae* [14, 46]. In this context, knowing the comorbidity and plausible correlation with the disease progression is instrumental in better understanding a case. Eventhough, the patient in the present case was in rather stable condition before the infection, he had preconditions of hepatosplenomegaly and beta-thalassemia. Thalassemia is considered a risk factor for infection due to iron overload and immune abnormalities among these individuals [47]. There are several records of *V. cholerae* bacteremia or sepsis in individuals with thalassemia and liver disorders; nonetheless, these patients exhibited satisfactory recovery following treatment [10, 12, 30]. These cases indicate that thalassemia may not be the only causal factor for the increased probability of mortality. However, the presence of such co-morbidity can increase the host's vulnerability; hence, selecting a treatment regimen should be approached cautiously in such cases.

There remain substantial challenges towards understanding septic associated deaths as simultaneous derangement of multiple pathways likely mediate mortality rather than a single mediator [48]. The patient in this case displayed splenomegaly which can contribute to pancytopenia and predispose the patient to septicemia [49]. Additionally, pre-existing liver dysfunction can be a contributor to the progression of infection to sepsis, multiple organ dysfunction and sepsis-induced death [50]. There are reports on hemochromatosis (iron overload) inducing gut leakage, systematic inflammation and sepsis in patients with thalassemia [51]. Adding to the confounding factors, the potential consequences of drug toxicity, which can significantly affect a patient's condition, especially in a severely diseased state when drug metabolism could be hindered cannot be ruled out. Considering the patient's medical history of being diagnosed with hepatosplenomegaly, it is plausible that the patient could have experienced impaired drug metabolism. However, it is challenging to attribute the adverse outcome of a patient to antibiotics since multiple factors operate in unison within the human body. For example, it is shown that carbapenems, which rely heavily on renal routes of elimination, may have a negative impact on renal function. In adverse cases, they were also associated with neutropenia and leucopenia [52]. Given the high occurrence of acute renal failure and associated death in ICU patients [53] and the possible side effects of such drugs, it is advisable to evaluate all relevant factors including pre-existing comorbidities, local drug resistance trend, ecology of the potential pathogen associated while developing policies for empirical drug usage in such cases. Hence, it is imperative to consider the potential of antibiotic-induced toxicity in sepsis, necessitating extensive research in the field.

Concluding remarks

V. cholerae has gained significance as an emerging etiology of septicemia worldwide. Yet ours was the first case of *V. cholerae* septicemia from Bangladesh, where cholera is endemic. The study's major limitation may be the inability to compare and analyse the case due to a lack of data, and the measurements were made only on the day of his admission at the hospital. Nevertheless, this report bears importance as a reference case for forming comprehensive studies in the future on the burden, source, clinical, microbiological and immunological aspects of

bacteremia and sepsis in Bangladesh. This would require a nationwide surveillance program involving hospitals of different categories that are representative of the burden. The detailed collection of metadata, and timely microbiological and molecular analysis should be included in the policy for managing such cases. Furthermore, there is a pressing need to implement a data-driven antimicrobial usage policy rather than empirical usage to give the best chance to our current clinical settings to save lives.

Acknowledgements
icddr, b gratefully acknowledges the unrestricted support from Governments of Bangladesh and Canada.

Author contributions
M.T.I. designed and wrote the manuscript; K.S.N. performed laboratory tests, wrote the manuscript and prepared the figures; N.A. coordinated clinical aspects and wrote the clinical case report section; S.M.B. curated clinical data and performed preliminary identification of the isolate; W.U. performed laboratory tests; J. T. performed laboratory tests; M.N.S. performed laboratory tests; A.A. performed laboratory tests; A.I. performed laboratory tests; M.S. coordinated the laboratory part at icddr, b, D.A. coordinated between the clinic and laboratories; K.D. S. did critical review of the manuscript; A.C. did critical review of the manuscript; T.A. did critical review of the manuscript and M.A. designed, guided and did critical review of the manuscript.

Funding
This research was supported by icddr, b and National Institutes of Health grants AI055058.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

Received: 15 July 2024 / Accepted: 7 October 2024
Published online: 19 December 2024

References

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S, Fleischmann-Struzek C, Machado FR, Reinhart KK, Rowan K, Seymour CW, Watson RS, West TE, Marinho F, Hay SI, Lozano R, Lopez AD, Angus DC, Murray CJL, Naghavi M. Global, regional, and national sepsis incidence and mortality. 1990–2017: analysis for the global burden of Disease Study. Lancet. 2020;395(10219):200–11. https://doi.org/10.1016/S0140-6736(19)32989-7.
2. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers. 2016;2:16045. https://doi.org/10.1038/nrdp.2016.45.
3. Hwang S, Kim Y, Jung H, Chang HH, Kim SJ, Park HK, et al. A fatal case of bacteremia caused by vibrio cholerae non-O1/O139. Infect Chemother. 2021;53(1):384–90. https://doi.org/10.3947/ic.2020.0301.
4. Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MK, Qadri F. Martinez- Urtaza1 J. Vibrio spp. infections. Nat Rev Dis Primers. 2018;4(1):8. https://doi.org/10.1038/s41572-018-0005-8.
5. Monir MM, Islam MT, Mazumder R, Mondal D, Nahar KS, Sultana M, Morita M, Ohnishi M, Huq A, Watanabe H, Qadri F, Rahman M, Thomson N, Seed K, Colwell RR, Ahmed T, Alam M. Genomic attributes of Vibrio cholerae O1 responsible for 2022 massive cholera outbreak in Bangladesh. Nat Commun. 2023;14(1):1154. https://doi.org/10.1038/s41467-023-36687-7.
6. Deshayes S, Daurel C, Cattoir V, Parienti JJ, Quilici ML, de La Blanchardière A. Non-O1, non-O139 Vibrio cholerae bacteraemia: case report and literature review. Springerplus. 2015;4:575. https://doi.org/10.1186/s40064-015-1346-3.
7. Feghali R, Adib SM. Two cases of Vibrio cholerae non-O1/non-O139 septicaemia with favourable outcome in Lebanon. East Mediterr Health J. 2011;17(8):722-4. www.public-health.gov.lb
8. Kaper JB, Morris JG, Levine MM, Cholera. Clin Microbiol Rev. 1995;8(1):48–86. https://doi.org/10.1128/CMR.8.1.48.
9. Chang-Chien CH. Bacteraemic necrotizing fasciitis with compartment syndrome caused by non-O1 Vibrio cholerae. J Plast Reconstr Aesthet Surg. 2006;59(12):1381–4. https://doi.org/10.1016/j.bjps.2006.06.002.
10. Chen J, Huang J, Huang M, Chen Z, Chen A, Yang J, Zhang T, Min X. Two cases of septic shock with different outcomes caused by non-O1/non-O139 Vibrio cholerae isolates. J Int Med Res. 2020;48(6):300060520933459. https://doi.org/10.1177/0300060520933459.
11. Ding Y, Hao J, Zeng Z, Jinbo, Liu. Identification and genomic analysis of a Vibrio cholerae strain isolated from a patient with bloodstream infection. Heliyon. 2022;8(11):e11572. https://doi.org/10.1016/j.heliyon.2022.e11572.
12. Faisal US, Zainulabid UA, Kori N, Periyasamy P, Khaithir TMN. Vibrio Cholera O1 Ogawa Bacteremia with Extraintestinal Manifestation. Intl J Infect Dis. 2023;134:S7. https://doi.org/10.1016/j.ijid.2023.05.034.
13. Phetsouvanh R, Nakatsu M, Arakawa E, Davong V, Vongsouvath M, Lattana O, Moore CE, Nakamura S, Newton PN. Fatal bacteremia due to immotile Vibrio cholerae serogroup O21 in Vientiane, Laos - a case report. Ann Clin Microbiol Antimicrob. 2008;7:10. https://doi.org/10.1186/1476-0711-7-10.
14. Engel MF, Muijsken MA, Mooi-Kokenberg E, Kuijper EJ, van Westerloo DJ. Vibrio cholerae non-O1 bacteraemia: description of three cases in the Netherlands and a literature review. Euro Surveill. 2016;21(15). https://doi.org/10.2807/1560-7917.ES.2016.21.15.30197.
15. Chowdhury G, Joshi S, Bhattacharya S, Sekar U, Birajdar B, Bhattacharyya A, Shinoda S, Ramamurthy T. Extraintestinal infections caused by non-toxigenic Vibrio cholerae non-O1/non-O139. Front Microbiol. 2016;7:144. https://doi.org/10.3389/fmicb.2016.00144.
16. Sachu A, Johnson D, Thomas S, Mathew R. Non O1/ O139 Vibrio Cholerae Septicemia in a patient with Hepatocellular Carcinoma. Ethiop J Health Sci. 2021;31(6):1303–6. https://doi.org/10.4314/ejhs.v31i6.27.
17. Jubyda FT, Nahar KS, Barman I, Johura FT, Islam MT, Sultana M, Ullah W, Tasnim J, Biswas SR, Monir MM, George CM, Camilli A, Ahmed N, Ross AG, Clemens JD, Alam M. Vibrio cholerae O1 associated with recent endemic cholera shows temporal changes in serotype, genotype, and drug-resistance patterns in Bangladesh. Gut Pathog. 2023;15(1):17. https://doi.org/10.1186/s13099-023-00537-0.
18. Singh DV, Matte MH, Matte GR, Jiang S, Sabeena F, Shukla BN, Sanyal SC, Huq A, Colwell RR. Molecular analysis of Vibrio cholerae O1, O139, non-O1, and non-O139 strains: clonal relationships between clinical and environmental isolates. Appl Environ Microbiol. 2001;67(2):910–21. https://doi.org/10.1128/AEM.67.2.910-921.2001.
19. Kirchberger PC, Orata FD, Nasreen T, Kauffman KM, Tarr CL, Case RJ, Polz MF, Boucher YF. Culture-independent tracking of Vibrio cholerae lineages reveals complex spatiotemporal dynamics in a natural population. Environ Microbiol. 2020;22(10):4244–56. https://doi.org/10.1111/1462-2920.14921.
20. Morita M, Yamamoto S, Hiyoshi H, Kodama T, Okura M, Arakawa E, Alam M, Ohnishi M, Izumiya H, Watanabe H. Horizontal gene transfer of a genetic island encoding a type III secretion system distributed in Vibrio cholerae. Microbiol Immunol. 2013;57(5):334–9. https://doi.org/10.1111/1348-0421.12039.
21. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated. Or Fastidious Bacteria [Internet].2016;35(17): Available from: www.clsi.org.
22. Thungapathra M, Sinha KK, Chaudhuri SR, Garg P, Ramamurthy T, Nair GB, Ghosh A. Occurrence of antibiotic resistance gene cassettes aac(6')-Ib, dfrA5, dfrA12, and ereA2 in class I integrons in non-O1, non-O139 Vibrio cholerae strains in India. Antimicrob Agents Chemother. 2002;46(9):2948–55. https://doi.org/10.1128/AAC.46.9.2948-2955.2002.
23. Baranwal S, Dey K, Ramamurthy T, Nair GB, Kundu M. Role of active efflux in association with target gene mutations in fluoroquinolone resistance in clinical isolates of Vibrio cholerae. Antimicrob Agents Chemother. 2002;46(8):2676–8. https://doi.org/10.1128/AAC.46.8.2676-2678.2002.
24. Rashed SM, Hasan NA, Alam M, Sadique A, Sultana M, Hoq MM, Sack RB, Colwell RR, Huq A. *Vibrio cholerae* O1reducedesusceptibilitybiliciprofloxacinoxaciazithromycinoisolatedolated frural Rural Coastal Area of Bangladesh. Front Microbiol. 2017;8:252. https://doi.org/10.3389/fmicb.2017.00252.
25. Hasan NA, Choi SY, Eppinger M, Clark PW, Chen A, Alam M, Haley BJ, Taviani E, Hine E, Su Q, Tallon LJ, Prosper JB, Furth K, Hoq MM, Li H, Fraser-Liggett CM, Cravioto A, Huq A, Ravel J, Cebula TA, Colwell RR. Genomic

diversity of 2010 Haitian cholera outbreak strains. Proc Natl Acad Sci U S A. 2012;109(29):E2010–7. https://doi.org/10.1073/pnas.1207359109.
26. Monir MM, Hossain T, Morita M, Ohnishi M, Johura FT, Sultana M, Monira S, Ahmed T, Thomson N, Watanabe H, Huq A, Colwell RR, Seed K, Alam M. Genomic characteristics of recently recognized Vibrio cholerae El Tor lineages Associated with Cholera in Bangladesh, 1991 to 2017. Microbiol Spectr. 2022;10(2):e0039122. https://doi.org/10.1128/spectrum.00391-22.
27. Sarmin M, Afroze F, Sharifuzzaman, Alam T, Shaly NJ, Ahmed T, Shaly NJ, Ahmed T, Chisti MJ, et al. Predictor of death in Diarrheal Children under 5 years of Age having severe Sepsis in an urban critical care Ward in Bangladesh. Glob Pediatr Health. 2019;6:2333794X19862716. https://doi.org/10.1177/2333794X19862716.
28. Sarmin M, Begum M, Islam F, Afroze F, Shahrin L, Sharifuzzaman T, Shahid ASMSB, Ahmed T, Chisti MJ, et al. Factors associated with severe sepsis in diarrheal adults and their outcome at an urban hospital, Bangladesh: a retrospective analysis. PLoS ONE. 2021;16(9):e0257596. https://doi.org/10.1371/journal.pone.0257596.
29. Sands K, Carvalho MJ, Portal E, Thomson K, Dyer C, Akpulu C, Andrews R, Ferreira A, Gillespie D, Hender T, Hood K, Mathias J, Milton R, Nieto M, Taiyari K, Chan GJ, Bekele D, Solomon S, Basu S, Chattopadhyay P, Mukherjee S, Iregbu K, Modibbo F, Uwaezuoke S, Zahra R, Shirazi H, Muhammad A, Mazarati JB, Rucogoza A, Gaju L, Mehtar S, Bulabula ANH, Whitelaw A. BARNARDS Group; Walsh TR. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. Nat Microbiol. 2021;6(4):512–23. https://doi.org/10.1038/s41564-021-00870-7.
30. Deris ZZ, Leow VM, Hassan W, Lah N, Lee SY, Hawa SH, Asma HS, Ravichandran M. Non-O1, non-O139 Vibrio cholerae bacteraemia in splenectomised thalassaemic patient from Malaysia. Trop Biomed. 2009;26(3):320-5. https://www.msptm.org/files/320_-_325_Deris_Z_Z.pdf
31. Azeem A, Nogles T, Cherazard R. Septicemia secondary to Vibrio cholerae (non-O1/non-O139) in wound. BMJ Case Rep. 2019;12(10). https://doi.org/10.1136/bcr-2019-231901.
32. Piersimoni C, Morbiducci V, Scalise G. Non-O1 Vibrio cholerae gastroenteritis and bacteraemia. Lancet. 1991;337(8744):791–2. https://doi.org/10.1016/0140-6736(91)91409-n.
33. Rogers AP, Mileto SJ, Lyras D. Impact of enteric bacterial infections at and beyond the epithelial barrier. Nat Rev Microbiol. 2023;21(4):260–74. https://doi.org/10.1038/s41579-022-00794-x.
34. Chaand M, Miller KA, Sofia MK, Schlesener C, Weaver JWA, Sood V, Dziejman M. Type 3 Secretion System Island Encoded proteins required for colonization by Non-O1/non-O139 Serogroup *Vibrio cholerae*. Infect Immun. 2015;83(7):2862–69. https://doi.org/10.1128/IAI.03020-14.
35. Tang J, Li S, Zhang M, Li F, Tang Y, Yang F. Whole genome analysis of a Non-O1, Non-O139 Vibrio cholerae detected from human blood in China. Infect Drug Resist. 2023;16:5453–61. https://doi.org/10.2147/IDR.S420095.
36. Alam A, Miller KA, Chaand M, Butler JS, Dziejman M. Identification of Vibrio cholerae type III secretion system effector proteins. Infect Immun. 2011;79(4):1728–40. https://doi.org/10.1128/IAI.01194-10.
37. Sperandio V, Giro´n JA, Giro´n G, Silveira WD, Kaper JB. Infect Immun. 1995;63(11):4433–8. https://doi.org/10.1128/iai.63.11.4433-4438.1995.
38. Potapova A, Garvey W, Dahl P, Guo S, Chang Y, Schwechheimer C, Trebino MA, Floyd KA, Phinney BS, Liu J, Malvankar NS, Yildiz FH. Outer membrane vesicles and the outer membrane protein OmpU govern *Vibrio cholerae* biofilm matrix assembly. mBio. 2024;15(2):e0330423. https://doi.org/10.1128/mbio.03304-23.
39. Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, Calderwood SB, Fraser C, Mekalanos JJ. Identification of a vibrio cholerae RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. Proc Natl Acad Sci U S A. 1999;96(3):1071–6. https://doi.org/10.1073/pnas.96.3.1071.
40. Li Z, Fan Y, Li Z, Li X, Sun H, Li J, Lu X, Liang W, Kan B. Nonhemolysis of epidemic El Tor biotype strains of Vibrio cholerae is related to multiple functional deficiencies of hemolysin A. Gut Pathog. 2019;11:38. https://doi.org/10.1186/s13099-019-0316-7.
41. Islam MT, Nasreen T, Kirchberger PC, Liang KYH, Orata FD, Johura FT, Hussain NAS, Im MS, Tarr CL, Alam M, Boucher YF. Population Analysis of Vibrio cholerae in aquatic reservoirs reveals a Novel Sister species (*Vibrio paracholerae* sp. nov.) with a History of Association with humans. Appl Environ Microbiol. 2021;87(17):e0042221. https://doi.org/10.1128/AEM.00422-21.
42. Lassalle F, Al-Shalali S, Al-Hakimi M, Njamkepo E, Bashir IM, Dorman MJ, Rauzier J, Blackwell GA, Taylor-Brown A, Beale MA, Cazares A, Al-Somainy AA, Al-Mahbashi A, Almoayed K, Aldawla M, Al-Harazi A, Quilici ML, Weill FX, Dhabaan G, Thomson NR. Genomic epidemiology reveals multidrug resistant plasmid spread between Vibrio cholerae lineages in Yemen. Nat Microbiol. 2023;8(10):1787–98. https://doi.org/10.1038/s41564-023-01472-1.
43. Kumar PA, Patterson J, Karpagam P. Short Communication Multiple Antibiotic Resistance Profiles of Vibrio cholerae non-O1 and non-O139. Jpn. J. Infect. Dis. 2009; 62(3):230-2. https://www.jstage.jst.go.jp › 62_JJID.2009.230 › _pdf.
44. Abioye OE, Nontongana N, Osunla CA, Okoh AI. Antibiotic resistance and virulence genes profiling of Vibrio cholerae and Vibrio mimicus isolates from some seafood collected at the aquatic environment and wet markets in Eastern Cape Province, South Africa. PLoS ONE. 2023;18(8):e0290356. https://doi.org/10.1371/journal.pone.0290356.
45. Dua P, Karmakar A, Ghosh C. Virulence gene profiles, biofilm formation, and antimicrobial resistance of Vibrio cholerae non-O1/non-O139 bacteria isolated from West Bengal, India. Heliyon. 2018;4(12):e01040. https://doi.org/10.1016/j.heliyon.2018.e01040.
46. Chen YT, Tang HJ, Chao CM, Lai CC. Clinical manifestations of non-O1 Vibrio cholerae infections. PLoS ONE. 2015;10(1):e0116904. https://doi.org/10.1371/journal.pone.0116904.
47. Vento S, Cainelli F, Cesario F. Infections and thalassaemia. Lancet Infect Dis. 2006;6(4):226–33. https://doi.org/10.1016/S1473-3099(06)70437-6.
48. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, Remick DG. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. Physiol Rev. 2013;93(3):1247–88. https://doi.org/10.1152/physrev.00037.2012.
49. Sharma R, Nalepa G. Evaluation and management of chronic Pancytopenia. Pediatr Rev. 2016;37(3):101–11. https://doi.org/10.1542/pir.2014-0087. quiz 112-3.
50. Yan J, Li S, Li S. The role of the liver in sepsis. Int Rev Immunol. 2014;33(6):498–510. https://doi.org/10.3109/08830185.2014.889129.
51. Visitchanakun P, Saisorn W, Wongphoom J, Chatthanathon P, Somboonna N, Svasti S, Fucharoen S, Leelahavanichkul A. Gut leakage enhances sepsis susceptibility in iron-overloaded-thalassemia mice through macrophage hyperinflammatory responses. Am J Physiol Gastrointest Liver Physiol. 2020;318(5):G966–79. https://doi.org/10.1152/ajpgi.00337.2019.
52. Alván G, Nord CE. Adverse effects of monobactams and carbapenems. Drug Saf. 1995;12(5):305–13. https://doi.org/10.2165/00002018-199512050-00003.
53. Granowitz EV, Brown RB. Antibiotic Adverse Reactions and Drug Interactions. Crit Care Clin. 2008;24(2):421 – 42, xi. https://doi.org/10.1016/j.ccc.2007.12.011

Publisher's note