scientific reports

Check for updates

OPEN

Investigation and pathogenetic testing of *Shewanella* spp. positive diarrhea cases in Beijing, China

Ying Li[1,2], Keyi Yu[3], Guilan Zhou[4], Yuwei Liu[1,2], Aixia Yan[1,2], Mu He[1,2], Tao Peng[1,2] & Zhenzhou Huang[5]✉

The pathogenic profiles of seven *Shewanella* spp. positive cases identified during diarrhea surveillance in Beijing, China, in 2023 were characterised. Sentinel hospitals collected patient information and stool samples, while regional centres for disease control (CDC) performed cultures and real time PCR. Whole-genome sequencing (WGS), average nucleotide identity (ANI) analysis, phylogenetic analysis, virulence gene and resistance gene analysis of the *Shewanella* spp. isolates were conducted, as well as phenotypic resistance analysis. The detection rate in the stool samples collected from 354 diarrhea patients was 1.98% (7/354). The time of disease onset of six out of the seven patients ranged from July 17–22, 2023. The incubation period ranged from 8 to 12 h with 3–50 episodes/day. Three subjects reported having consumed potentially contaminated seafood. The seven isolated strains of *Shewanella* spp. (named as S1-S7) were closely related to *S. algae*, belonged to the algae clade, and were all novel ST (sequence typing) strains. A total of 125,738 SNPs (single nucleotide polymorphism) were identified in the core genomes of the seven *Shewanella* strains. Twenty-six virulence-related genes in five categories were identified, with chemotaxis and flagella-related genes being the most abundant (26.92%, 7/26), followed by secretion system- and serum resistance-related genes at 23.08% (6/26) and 15.38% (4/26), respectively. *Shewanella* spp. were detected in patients with diarrhea at a certain level. Seafood should be the key food category for monitoring and seafood markets should become a key monitoring site for *Shewanella* spp. The novel STs of the algae clade isolated from diarrhea patients in this study may potentially help in tracking circulating strains. Further in-depth investigations are required to precisely elucidate the correlation between *Shewanella* infections and human diarrhea and the pathogenic characteristics of this infection.

Keywords *Shewanella*, Epidemiologic investigation, Pathogen characterization, Whole-genome sequencing, Diarrhea

The genus *Shewanella* spp. belongs to the phylum *Pseudomonadota*, class *Gammaproteobacteria*, order *Alteromonadales*, family *Shewanellaceae*, and genus *Shewanella*[1]. To date, over 70 species of *Shewanella* have been identified[2]. Among them, those that cause disease in humans are *Shewanella algae*(*S. algae*), *S. putrefaciens*, and *S. xiamenensis*, while species such as *S. indica* and *S. chilikensis* have been identified as closely related to *S. algae* and belong to the algae clade[3]. *Shewanella* spp. produces tetrodotoxin (TTX) and belongs to the TTX-producing bacteria[4]. All *S. algae* are haemolytic. Khashe et al. confirmed that *S. algae* is more pathogenic than *S. putrefaciens* in mice and speculated that the haemolytic activity of *S. algae* may be an important virulence factor[5]. The isolation of *Shewanella* spp. from food poisoning cases or intestinal samples of diarrhea patients has been previously reported[6–9].

The incidence of *Shewanella* spp., an opportunistic pathogen newly included in China's *List of Pathogenic Microorganisms Transmitted From Human to Human*(2023 edition), in clinical infections has increased worldwide. The widespread transmission of *Shewanella* spp. poses a significant challenge to public health and clinical anti-infective treatment. Yu et al.[10] classified diseases of *Shewanella* infections into eight broad categories based on the site of infection, namely ear, nose, and throat (E.N.T) disorders, central nervous system (CNS) disorders,

[1]Shunyi District Center for Disease Control and Prevention, Beijing, China. [2]Workstation for Microbial Infectious Disease, Shunyi District Center for Disease Control and Prevention, Beijing, China. [3]Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, Zhejiang Province, China. [4]Nanjing Center for Disease Control and Prevention, Nanjing, Jiangsu Province, China. [5]Hangzhou Center for Disease Control and Prevention (Hangzhou Health Supervision Institution), Hangzhou, Zhejiang Province, China. ✉email: huangzhenzhou@hzcdc.com.cn

chest infections, cardiovascular diseases, bloodstream infections (bacteraemia and sepsis), intra-abdominal infections, osteoarthropathy, and skin and soft-tissue infections (SSTIs). In recent years, reports of sporadic cases of infections caused by *Shewanella* spp. have been on the rise due to improved clinical microbiological testing techniques[11]. Among patients with *Shewanella* infections, 43.59% had been exposed to the marine environment. The population infected with *Shewanella* spp. is mostly elderly and neonates, with a male to female ratio of 2.84:1[10]. This study was performed based on the investigation and pathogen characterization of seven *Shewanella* spp. positive cases detected in diarrhea surveillance in Beijing, China in 2023. It aims to establish a foundational basis for subsequent research regarding diarrhea caused by *Shewanella* spp.

Results

Epidemiological findings of *Shewanella* spp.-positive cases

A total of 354 stool samples from 354 diarrhea patients were collected, and the pathogen detection rates are as follows: *Shewanella* spp. 1.98% (7/354), *Salmonella* spp. 1.98% (7/354), *Shigella* spp. 0% (0/354), diarrheagenic *Escherichia coli* 8.19% (29/354), *Vibrio parahaemolyticus* 3.67% (13/354), *Campylobacter jejuni* 3.67% (13/354), *Campylobacter coli* 2.26% (8/354), *Yersinia enterocolitica* 1.41% (5/354), norovirus 29.94% (106/354), sapovirus 7.06% (25/354), rotavirus 0.03% (1/354), enteric adenovirus 8.47% (30/354), and astrovirus 8.19% (29/354). Seven strains of *Shewanella* spp. (named as S1-S7) were isolated from seven patients (named as P1-P7). The onset of P1 was July 1, 2023, while that of P2-P7 was July 17–22, 2023. No pathogens other than *Shewanella* spp. were detected in P1-P7.

Among the six patients with clustered onset times (P2-P7), there were five males and one female, and the six patients did not know each other. They were aged between 15 and 58 years old, and the incubation period ranged from 8 to 12 h. The frequency rates of clinical symptoms was 100% (6/6) for diarrhea, 50.00% (3/6) for nausea, 33.33% (2/6) for fever, 16.67% (1/6) for abdominal pain, 16.67% (1/6) for dehydration, 16.67% (1/6) for thirst, 16.67% (1/6) for vomiting, and 16.67% (1/6) for rectal tenesmus. The frequency of diarrhea episodes ranged from three to 50 times/day, and the faecal characterization was watery stools in 83.33% (5/6) patients and bloody purulent stools in 16.67% (1/6) patients, respectively. Suspected contaminated food consumed by P3 and P6 was purchased from the same market (Market X in Beijing). P4 and P5 both travelled to the tourist attraction Y in city B on the same day (the attraction is 240 km away from Beijing, and P4 and P5 did not know each other, were not in the same tour group, nor had any history of dining together) and dined at the attraction site. The suspected contaminated food product was seafood for both patients. The suspected contaminated food products that the six patients were exposed to were all purchased from the catering service establishment or retail market. The suspected contaminated food products consumed were seafood for three patients, cold drinks for two patients, and cold watermelon for one patient. P4, P5, and P7 all dined with another individual, who also contracted diarrhea (Table 1). The epidemiological data were unavailable for P1 as the call was disconnected.

Identification of isolated *Shewanella* spp.

Based on the average nucleotide identity (ANI) results, strains S1, S2, and S4 were identified as *S. indica*, strains S3, S6, and S7 were identified as *S. chilikensis*, and S5 was identified as *S. algae*(Fig. 1). The identification results of S1-S7 based on the three methods of matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS), fully automated bacterial biochemical identification instrument. Both MALDI-TOFMS and ANI results are shown in Table 2.

All seven *Shewanella* strains were of novel sequence typing (ST) types, with S1 being ST77, S2 and S4 being ST78, S3, S6, and S7 being ST79, and S5 being ST81, as also shown in Table 2. A total of 125,738 single nucleotide polymorphism (SNPs) were identified in the core genomes of the seven *Shewanella* strains, where the number of paired SNPs for S2-S4 and S3-S7 was 0, and the number of paired SNPs between S3-S7-S6 was 0–1, as shown in Fig. 2A. In the maximum likelihood phylogenetic tree constructed based on core genome single nucleotide polymorphism (cgSNP), S3, S6, and S7 were in the same genetic branch and were close to the reference strain *S. chilikensis* KCTC 22540^T. S5 was in an independent genetic branch and was close to the reference strain *S. algae* JCM 21037^T. S1 was in an independent genetic branch, and S2 and S4 were in the same genetic branch, which were all close to the reference strain *S. indica* KCTC 23171^T (Fig. 2B).

Analysis of virulence genes of *Shewanella* spp.

A total of 26 virulence-related genes in five categories were identified in the genomes of seven *Shewanella* strains, among which chemotaxis- and flagella-related genes were the most abundant (26.92%, 7/26), followed by secretion system- and serum resistance-related genes at 23.08% (6/26) and 15.38% (4/26), respectively. Among them, multiple virulence genes related to the type VI secretion system (T6SS) and the effector delivery system were predicted in S5, including *hcp_1*, *hcp_2*, *vipB*, and *exeG*. Virulence genes related to the effector delivery system and motility were predicted in the other six strains. A total of three virulence gene profiles were obtained from the seven strains. The virulence gene profile of S5 was in a separate cluster, which differed significantly from the composition of the virulence gene profiles of the remaining six strains, as it contained *cheY*, *flgG*, *vipA/mglA*, and *katB* genes. The virulence gene profiles of S7, S3, and S6 clustered together (Cluster 1), while those of S1, S2, and S4 clustered together (Cluster 2). The *tviB*, *vasA*, and *vipB/mglB* genes were detected only in Cluster 1, while *xcpR* was detected only in Cluster 2 (Fig. 3).

Prediction of resistance genes and phenotypic resistance

A total of six classes of resistance genes were identified in the seven *Shewanella* strains, including genes resistant to aminoglycosides [*aph(3")-Ib*, *aph(6)-Id*], β-lactams (bla_{OXA-55}), quinolones (*qnrA1*, *qnrA2*), sulfonamides (*sul2*), chloramphenicol (*floR*), and tetracyclines [*tet(59)*]. All seven strains carried the gene resistant to β-lactam (bla_{OXA-55}). Moreover, all strains carried the quinolone resistance gene, with four strains (S3, S5, S6, and S7)

Patient no.	Mealtime	Time of onset	Incubation period	Gender	Occupation	Age	Clinical symptom	Suspected exposed food	Purchase location	City of purchase	Number of co-eaters	Whether diarrhea occurred among co-eaters
P2	17:00 Jul 16 2023	5:00 Jul 17 2023	12 h	Male	Cadres and staff	39	diarrhea (watery stools 10 times/day), nausea,	Herbal tea	Food service establishments	City A (180 km from Beijing)	1	/
P3	10:00 Jul 17 2023	20:00 Jul 17 2023	10 h	Male	Worker	36	diarrhea (watery stools 3 times/day), nausea	Icy water and drink	Retail market	Market X in Beijing	1	/
P4	12:00 Jul 21 2023	21:00 Jul 21 2023	9 h	Male	Student	15	diarrhea (watery stools 4 times/day), nausea, abdominal pain, dehydration, thirst, vomiting	Seafood	Food service establishments	Tourist attraction Y in city B (240 km from Beijing and 80 km from city A)	7	Yes
P5	12:00 Jul 21 2023	20:00 Jul 21 2023	8 h	Female	Domestic and non-working	58	diarrhea (watery stools 6 times/day), fever 39.0 °C	Seafood	Food service establishments	Tourist attraction Y in city B (240 km from Beijing and 80 km from city A)	20	Yes
P6	18:00 Jul 21 2023	5:00 Jul 22 2023	11 h	Male	Business services	37	diarrhea (bloody purulent stools 50 times/day), rectal tenesmus	Cold watermelon	Retail market	Market X in Beijing	1	/
P7	19:00 Jul 21 2023	7:00 Jul 22 2023	12 h	Male	Cadres and staff	56	diarrhea (watery stools 10 times/day), fever 38.0 °C	Seafood	Retail market	Market Z in Beijing	2	Yes

Table 1. Patient information, clinical symptoms, and distribution of suspected contaminated foods of six *Shewanella* spp.. Positive patients and a concentrated period of disease onset.

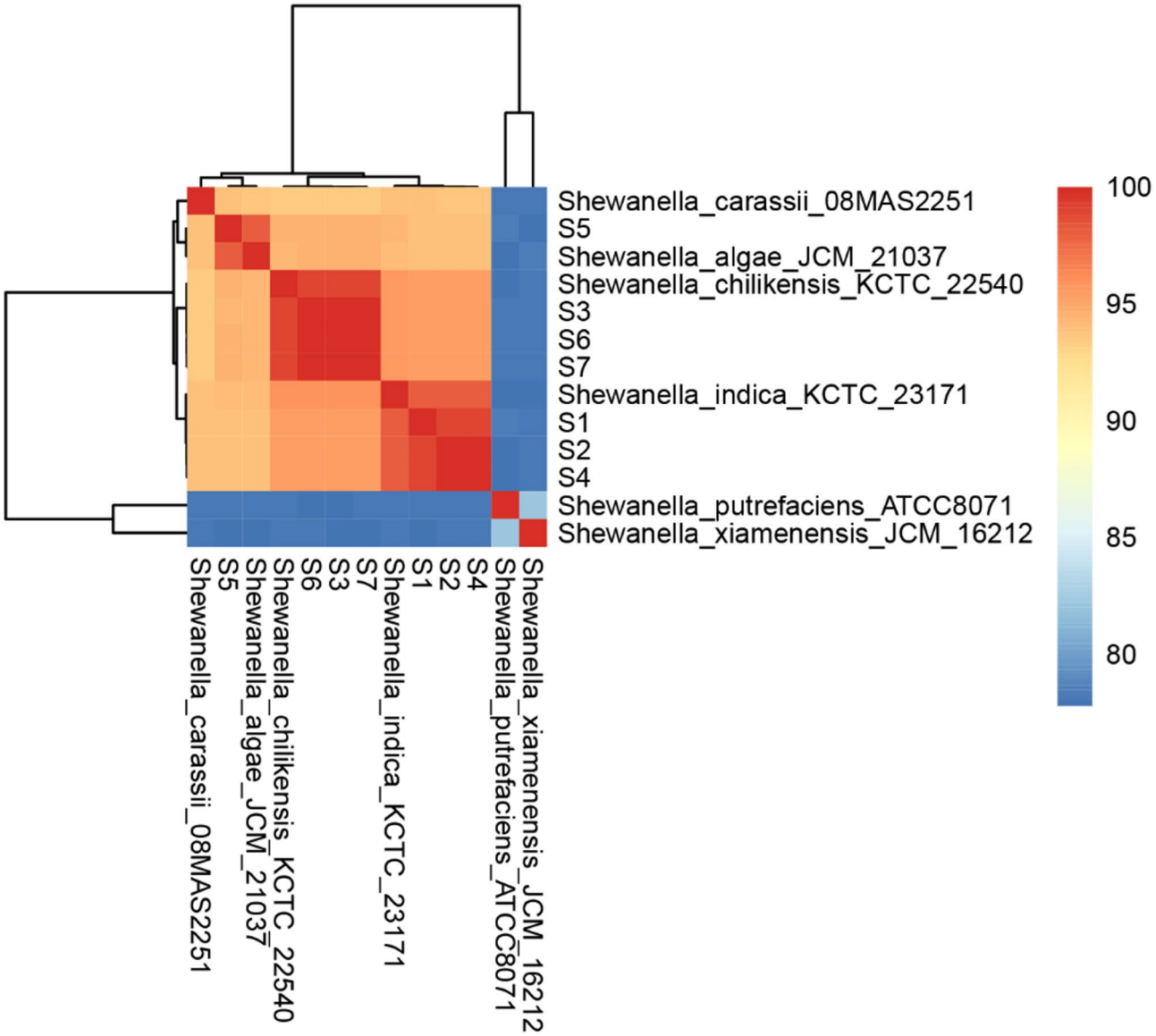

Fig. 1. Heatmap of ANI analysis of the seven strains of *Shewanella* spp.. Six type strains (*S. algae* JCM 21037^T, *S. carassii* 08MAS2251^T, *S. chilikensis* KCTC 22540^T, *S. indica* KCTC 23171^T, *S. putrefaciens* ATCC 8071^T, *S. xiamenensis* JCM 16212^T) were used as reference genomes. The color from blue to red represents the value of ANI from <80 to 100.

carrying *qnrA1* and three strains (S1, S2, and S4) carrying *qnrA2*. In addition, one genetic branch (including strains S3, S6, and S7) also carried genes resistant to aminoglycosides [*aph(3")-Ib*, *aph(6)-Id*], sulfonamides (*sul2*), and tetracyclines [*tet(59)*]. However, S3 and S6 carried a chloramphenicol resistance gene (*floR*), whereas S7 did not, as shown in Fig. 4. Based on the phenotypic resistance test, three resistance profiles were detected for the seven *Shewanella* strains, among which S1, S2, and S4 were sensitive to all tested antibiotics, S3 and S6 were resistant to tetracycline (TET) + streptomycin (STR) + florfenicol (FLO), S7 were resistant to TET + STR, and S5 was resistant to ampicillin (AMP) + Colistin (CT), as shown in Fig. 4.

Discussion

In this study, *Shewanella* spp. were detected as a target microorganism together with other common diarrhea-causing pathogens during a 1-year diarrhea surveillance program. The detection rate of *Shewanella* spp. was 1.98% (7/354) among total diarrhea patients. In the samples that were positive for *Shewanella* spp., no other diarrhea-causing pathogens were detected, indicating that these were cases of single infection by *Shewanella*. Among them, six cases were reported on July 17–22, 2023, demonstrating a cluster of the time of disease onset. Based on the ANI analysis results, among the seven isolated *Shewanella* strains, there were three strains of *S. chilikensis*, three strains of *S. indica*, and one strain of *S. algae*(Fig. 1). They were all members of the algae

Strain No.	Result of fully automatic bacterial biochemical identification	MALDI-TOFMS result	Result of ANI analyses (ANI value %)	Housekeeping gene								ST
				16S_rRNA	*gyrB*	*mdh*	*recA*	*adk*	*rpoS*	*guaA*	*atpB*	
S1	*S. algae*	*S. putrefaciens*	*S. indica*(98.05%)	15	51	16	21	14	**47**	**88**	**22**	**77**
S2	*S. algae*	*S. putrefaciens*	*S. indica*(98.00%)	**23**	59	**50**	21	14	41	**90**	4	**78**
S3	*S. algae*	*S. putrefaciens*	*S. chilikensis*(99.01%)	**14**	54	**49**	33	3	4	**89**	**5**	**79**
S4	*S. algae*	*S. putrefaciens*	*S. indica*(98.05%)	**23**	59	**50**	21	14	41	**90**	**4**	**78**
S5	*S. algae*	*S. putrefaciens*	*S. algae*(98.03%)	**24**	63	25	**52**	1	11	**91**	**1**	**81**
S6	*S. algae*	*S. putrefaciens*	*S. chilikensis*(98.99%)	**14**	54	**49**	33	3	4	**89**	**5**	**79**
S7	*S. algae*	*S. putrefaciens*	*S. chilikensis*(98.99%)	**14**	54	**49**	33	3	4	**89**	**5**	**79**

Table 2. Identification and multilocus sequence typing analysis of seven *Shewanella* spp. strains. Bold text indicates new housekeeping gene number and ST number.

A

	S1	S2	S3	S4	S5	S6	S7	*S.algae* _JCM_21037	*S.chilikensis* _KCTC_22540	*S.indica* _KCTC_23171
S1	0	18639	93782	18639	125738	93783	93782	124121	94425	40551
S2	18639	0	94425	0	125228	94426	94425	123527	95194	40471
S3	93782	94425	0	94420	114307	1	0	113743	18535	88933
S4	18639	0	94420	0	125223	94421	94420	123522	95189	40471
S5	125738	125228	114307	125223	0	114308	114307	34235	114513	118237
S6	93783	94426	1	94421	114308	0	1	113744	18536	88934
S7	93782	94425	0	94420	114307	1	0	113743	18535	88933
S.algae _JCM_21037	124121	123527	113743	123522	34235	113744	113743	0	113750	116633
S.chilikensis _KCTC_22540	94425	95194	18535	95189	114513	18536	18535	113750	0	90330
S.indica _KCTC_23171	40551	40471	88933	40471	118237	88934	88933	116633	90330	0

B

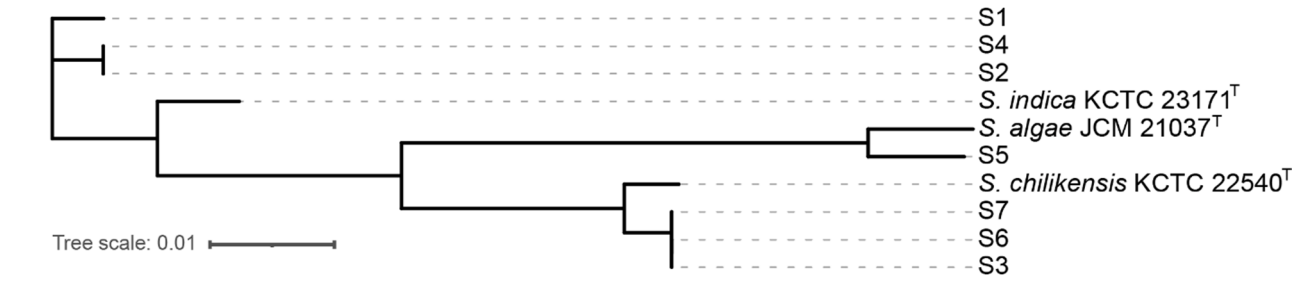

Fig. 2. SNP difference matrix of seven *Shewanella* strains **(A)** and the phylogenetic tree based on cgSNP **(B)**. Three type strains (*S. algae* JCM 21037^T, *S. chilikensis* KCTC 22540^T, *S. indica* KCTC 23171^T) were used as reference genomes. The SNP difference of the same strain is 0, highlighted in blue in matrix **(A)**. The SNP difference of the same clonal group is 0 or 1, include same clonal group of *S. indica*(S2 and S4), and same clonal group of *S. chilikensis*(S3, S6 and S7), were highlighted in color orange in matrix **(A)**.

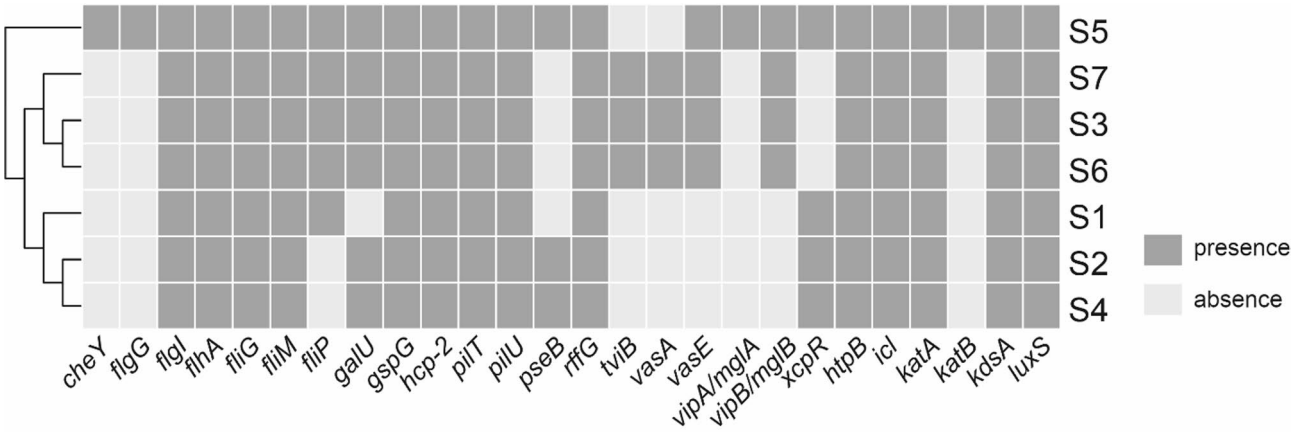

Fig. 3. Distribution of virulence genes of seven *Shewanella* strains. Colors grey and blue represent the absence and presence of virulence genes, respectively.

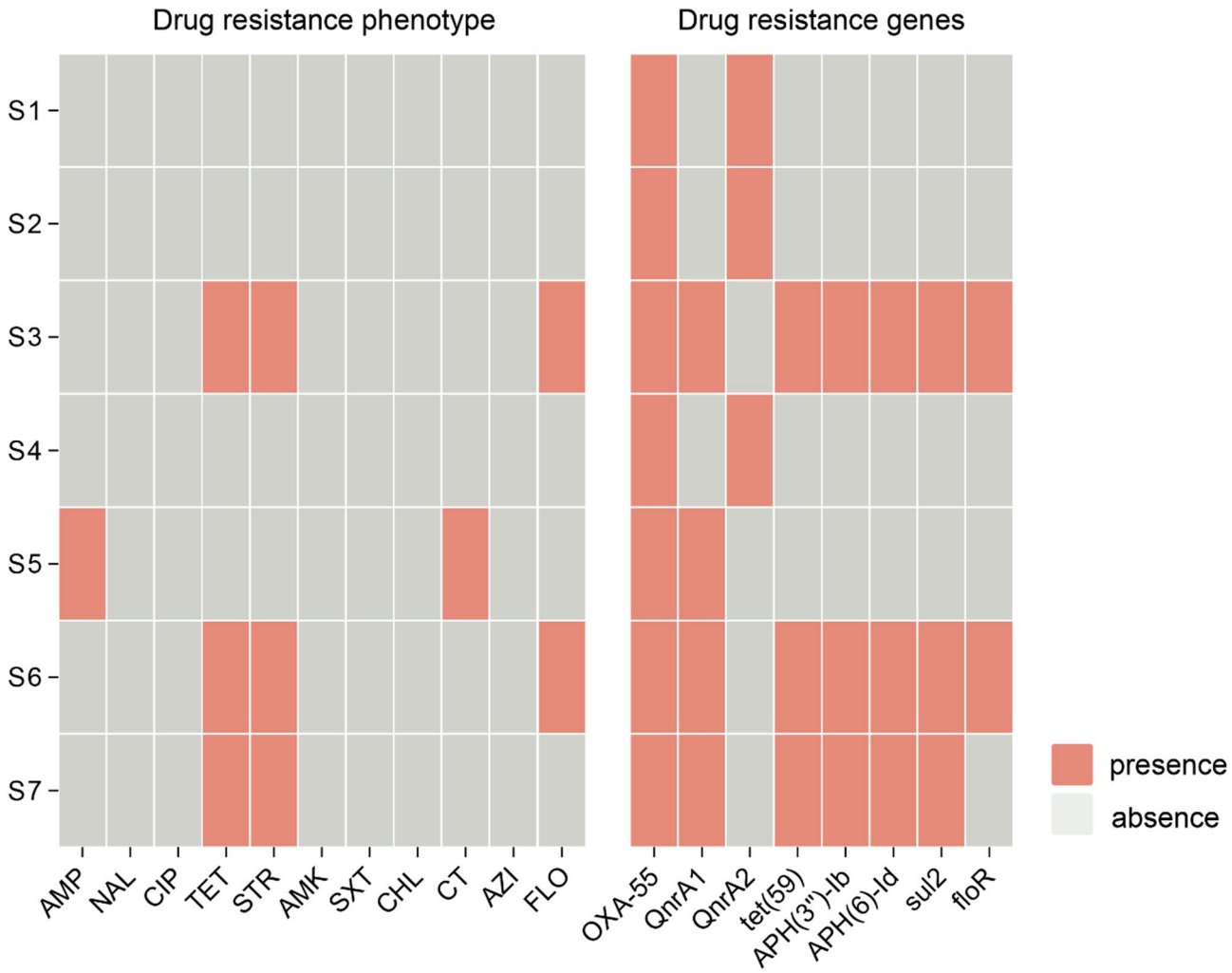

Fig. 4. Heatmap of phenotypic resistance and resistance genes of seven *Shewanella* spp. strains. Color grey represents the absence of phenotypic resistance and resistance genes, and color orange represents the presence of phenotypic resistance and resistance genes. AMP = ampicillin, TET = tetracycline, CT = colistin, CIP = ciprofloxacin, AZI = azithromycin, CHL = chloramphenicol, NAL = nalidixic acid, STR = streptomycin, SXT = sulfamethoxazole tablets, AMK = amikacin, FLO = florfenicol.

clade[3], all of which were new STs (Table 2). *S. indica* isolated from P2 and P4 were strains of the same clonal group, and *S. chilikensis* isolated from P3, P6, and P7 were strains from another group of clones (Fig. 2).

Epidemiological investigations showed that the suspicious food described by P3 and P6 was purchased from the same market in Beijing, with an interval of four days between purchases (Table 1). P4 and P5 did not know each other previously but had travelled to the same scenic spot on the same day (the scenic spot was 240 km away from Beijing) and ate seafood at the scenic spot (Table 1). Moreover, diarrhea was seen in seven and 20 of the companies who dined together with P4 and P5, respectively (Table 1). The presence of six cases unknown to each other that did not originate from the same foodborne disease outbreak, combined with the fact that the *Shewanella* spp. isolates were all members of the algae clade and that there were two identical clones suggested that the novel ST of the algae clade isolated in this study may be a potentially circulating strain with a certain level of pathogenicity. We hypothesize that even a single food contaminated with *Shewanella* may harbor a mixture of multiple *Shewanella* species (or polyclonal strains), exhibiting microbial diversity. When humans consume foods contaminated with *Shewanella* exhibiting such diversity, different cases may carry distinct dominant species (or clones) in their stools. These findings also suggest the emergence and dispersed outbreak of the algae clade in Beijing. More attention should be paid to diarrhea surveillance and regular surveillance should be enhanced to improve the early warning of diarrhea. This study enriched the genomic database of *Shewanella* spp. and provided basic information for epidemiological studies related to human diarrhea caused by *Shewanella* spp..

In this study, food suspected of causing infection described by six patients (P2-P7) included seafood, beverages, and cold watermelon (Table 1). Given the presence of an incubation period intervening between the exposure to contaminated food and the manifestation of illness, and considering the highly subjective nature of the patients accounts regarding the suspected contaminated food, the actual contaminated food responsible for inducing diarrhea might deviate from the information provided by the patients. In previous epidemiological

studies on the algae clade, seafood should be a priority food category, and seafood related vending establishments should be identified as priority monitoring sites.

Among the six cases (P2-P7), the incubation period ranged from 8 to 12 h, and watery diarrhea was the most important clinical symptom (Table 1). In particular, P5, from whom *S. algae* was isolated, showed bloody purulent stool, with diarrhea episodes up to 50 times/day and the clinical symptom of rectal tenesmus, a common symptom in bacillary dysentery (Table 1). A previous study has also reported on the isolation of *S. algae* from bloody purulent stool, with the patient diagnosed with bacillary dysentery[8]. Based on the number of diarrhea episodes and faeces traits, P5 was found have more severe symptoms among the six patients mentioned above, which was in line with reports that *S. algae* may be correlated with high human pathogenicity[5]. The whole genome prediction of S5 (*S. algae*) showed 24 virulence genes in five categories, higher than the other six strains (Fig. 3).

Virulence genes related to motility were detected in the majority of *Shewanella* strains in this work. By tracking bacterial surface communities at single-cell resolution, Lee et al. also found that appendages such as pili and flagella of *Shewanella* had a significant impact on the growth rate diversity, and cell size homeostasis during its surface colonization process[12]. Biofilm formation plays a crucial role in the survival and colonization of *Shewanella*[13]. It can affect the intestinal colonization of *Shewanella* by regulating the methylation levels of genes related to lactate and iron homeostasis in the shrimp intestine[14].

The chemokine-related virulence gene *cheY*, flagellum-related virulence gene *flgG*, effector delivery system-related virulence genes *vipA/mglA*, and enzyme-related virulence gene KatB were only predicted in S5 (Fig. 3). The CheY protein expressed by the chemokine-related virulence gene *cheY* is a key component of the two-component system (TCS), which senses environmental changes and regulates the corresponding cellular responses, critical for the pathogenicity of *Helicobacter pylori*[15]. When *H. pylori* is exposed to certain chemicals, the CheA protein is activated and transfers phosphate groups to CheY. Phosphorylated CheY then further interacts with other proteins to enable the bacteria to move towards or away from specific chemical stimuli, helping the bacteria to find a suitable site for colonisation. In *Escherichia coli*, *Salmonella*, and other Gram-negative bacteria, the CheY protein may assist the bacteria in sensing nutrient concentration gradients in their surroundings, as well as the presence of harmful compounds, thereby efficiently seeking favourable conditions for survival and avoiding unfavourable ones[16,17]. Therefore, we hypothesised that S5 had a higher capacity in environmental adaptation, which enhanced its pathogenicity compared to that of the other strains.

Iron metabolism factors are also a crucial class of virulence genes in Gram-negative bacteria. They strictly regulate intracellular iron homeostasis through processes such as iron uptake, efflux, and storage. Previous studies have shown that *S. putrefaciens* forms iron bodies through the fez operon, which may play a role in the adaptation to iron starvation under anaerobic conditions[18]. A recent study reported that in *Shewanella*, the synthesis of siderophores was regulated at multiple levels by multiple factors such as BarA/UvrY, SsoR, and Fur[19]. Different signals are integrated into the regulatory network of siderophore synthesis, providing new insights into the regulatory mechanism of bacterial siderophore synthesis.

The secretion system-related virulence genes *vasA* and *vipB/mglB* were only detected in strains in Cluster 1 (Fig. 3). The *vasA* gene encodes an ATPase that is a key component for the energy supply of T6SS and is essential for the assembly and activation of T6SS. T6SS is found in many Gram-negative bacteria, including *Vibrio cholerae* and *Brucella* spp., and can aid in the competition between bacteria as well as attack on host cells. The *vipB/mglB* gene encodes a regulatory protein that is associated with the type IV secretion system (T4SS). T4SS translocates DNA or proteins across both the inner and outer membranes and participates in the exchange of genetic material between bacteria or the injection of effector molecules into the host cell[20–22]. Therefore, it can be hypothesised that the strains in Cluster 1 had a more thorough capacity to invade the organism, interact with the host, and fight other microorganisms, leading to a higher pathogenic potential than the strains in Cluster 2.

The seven *Shewanella* strains in this study included three phenotypic resistance profiles and four genetic resistance profiles (Fig. 4). Previous studies have reported that β-lactam resistance genes are associated with specific species. For example, $bla_{\text{OXA-48-like}}$ may be associated with *S. xiamenensis*, $bla_{\text{OXA-729}}$ with *S. algae*, and $bla_{\text{OXA-900}}$ with *S. putrefaciens*[23]. Although all seven strains belonged to the algae clade and all carried $bla_{\text{OXA-55}}$, only S5 (*S. algae*) was resistant to AMP (a β-lactamase inhibitor combination) and CT (Fig. 4). Kang et al.[9] reported that *S. algae* isolated from sporadic cases of diarrhea in Beijing also had a high rate of resistance to AMP and CT. Several studies have reported that *Shewanella* spp. isolated from neonatal patients with sepsis and wounds of patients bitten by cobras were resistant to CT[24,25]. The quinolone resistance determinant *qnrA* was prevalent in *Shewanella* spp[23]. in our study four of the seven *Shewanella* strains (S3, S5, S6, and S7) carried *qnrA1*, while the remaining three strains (S1, S2, and S4) carried *qnrA2*(Fig. 4). However, all were sensitive to quinolone antibiotics (nalidixic acid and ciprofloxacin), suggesting that there are some differences in the resistance genes and phenotypic resistance against quinolone antibiotics among *Shewanella* spp. of the algae clade. The phylogenetic tree analysis clearly indicated that the three strains of *S. chilikensis* were of the identical clone (Fig. 2). Notably, both S3 and S6 harbored the resistance gene *floR*, whereas S7 conspicuously lacked this specific gene. And the phenotype of florfenicol resistance was consistent with the situation of the resistance gene (Fig. 4). Researchers have confirmed that the *floR* gene carried by *Klebsiella pneumoniae* and *E. coli* may be located on plasmids[26,27]. If the *floR* gene carried by the isolates of the algae clade in this study was also on the plasmid, the gene fragment on the plasmid may not be available based on whole-genome sequencing, resulting in the failure of detection of *floR* in S7. The *floR* gene mediates bacterial resistance to florfenicol and was identified on a plasmid in the fish pathogen *Photobacterium damselae* subsp. *piscicida* in 1996[28]. All three strains of *S. chilikensis* were found to be resistant to TET and STR. Moreover, these strains were found to possess the resistance genes [*tet(59)*], [*aph(3")-Ib*], and [*aph(6)-Id*], suggesting a clear congruence between the observed phenotypic traits and the underlying genetic determinants (Fig. 4). Furthermore, unlike the *S. algae* strains

isolated from Hainan, China[29]the isolates from Beijing were sensitive to both imipenem and colistin, and there was no relevant resistance genes were detected.

Our study was subject to certain limitations. The sample size was relatively small, only seven out of 354 diarrheal patients tested positive for *Shewanella* spp., which may not comprehensively reflect the true situation of *Shewanella* in diarrheal cases. Additionally, patients' descriptions of suspected contaminated foods are influenced by subjective factors, and the presence of an incubation period can easily lead to misjudgments about the actual contaminated foods, thus limiting the accuracy of source tracing. Future studies should include more cases and optimize the investigation methods to explore the relationship between *Shewanella* and diarrhea. These strains of the algae clade were all identified as *S. algae* based on fully automated bacterial biochemical identification. By contrast, these strains were all identified as *S. putrefaciens* by MALDI-TOF MS, which differed from the results of the ANI analysis to a certain extent (Table 2). This highlights the persistent shortcomings of both the fully automated bacterial biochemical identification and MALDI-TOF MS in the identification of *Shewanella* strains. The above two conventional bacterial identification methods will need to be improved in order to enhance the accurate identification of various species of *Shewanella*.

Conclusions

In this study, *Shewanella* spp. were detected in patients with diarrhea at a certain level during one year of diarrhea surveillance. Moreover, the time of infection may have been clustered due to the presence of dispersed outbreaks. Seafood should be the key food category for monitoring and seafood markets should become a key monitoring site for *Shewanella* spp. The novel ST of the algae clade isolated from diarrhea patients in this study may be potentially helping for tracking circulating strains. Taken together, further in-depth investigations are required to precisely elucidate the correlation between *Shewanella* infections and human diarrhea, as well as the pathogenic characteristics of this infection.

Materials and methods

Sources of diarrhea surveillance samples and investigation of *Shewanella* spp. Positive cases

Sentinel hospitals were set up for the surveillance of diarrhea in Beijing, China. Cases were defined as patients with episodes of diarrhea ≥ 3 times/day and faecal characteristics of watery, loose, mucous, or bloody stools. Surveillance lasted from January 1, 2023 to December 31, 2023. Sentinel hospitals were responsible for collecting the patient information and stool samples. Stool samples were kept in sterile containers and forwarded to the regional centres for disease control (CDC) for pathogenetic testing within 24 h. In the case of a positive detection of *Shewanella* spp., a telephone survey was conducted to obtain further patient details.

Bacterial culture and viral real time polymerase chain reaction of stool samples

The stool samples of diarrhea cases were sent to the laboratory and subjected to isolation and culture of *Shewanella* spp., *Salmonella* spp., *Shigella* spp., diarrheagenic *Escherichia coli*, *Vibrio parahaemolyticus*, *Campylobacter jejuni*, *Campylobacter coli*, and *Yersinia enterocolitica*. The isolation and culture of bacteria in addition to *Shewanella* spp. were performed as previously described[30]. Meanwhile, real time polymerase chain reaction (PCR) was performed for all samples for norovirus, sapovirus, rotavirus, enteric adenovirus, and astrovirus.

The brief description of the isolation of *Shewanella* spp. is as follows: 200 mg of faeces was inoculated in 3% NaCl alkaline peptone water for enrichment culture at 37 °C for 24 h. A 10-μL inoculation ring was used to inoculate the enrichment solution in thiosulfate citrate bile salts sucrose (TCBS) agar and a *Vibrio* chromogenic plate, respectively, to culture at 37 °C for 24 h. The single black colony on TCBS agar or colourless and transparent in *Vibrio* chromogenic plates with dominant growth was transferred to a TSA plate to culture at 37 °C for 24 h. MALDI-TOF MS (Autoflex™ Speed, Bruker) and bacterial biochemical identification (VITEK 2 COMPACT, BioMerieux) were performed to identify the isolated colonies, and those identified as *Shewanella* spp. strains were stored[9,31]. A total of seven *Shewanella* strains were isolated.

Whole genome sequencing and bioinformatic analysis of *Shewanella* strains

Whole genome sequencing of the strains was entrusted to a third-party company. Bacterial DNA extraction was performed using the Promega Wizard™ Genomic DNA Purification Kit. The Illumina Hiseq 2000 platform was used as the sequencing platform, with a sequencing depth of 150×. Paired-end sequencing was performed and reads with a length of 150 bp were used for library construction. The quality control of raw sequencing reads was performed using FastQC. Low quality reads were removed, and genome frame assembly was performed using SOAP *de novo*(version 2.04, https://github.com/aquaskyline/SOAPdenovo2)[32].

The ANI of strains was analysed using fastANI[33]. The six *Shewanella* model bacteria selected in this study were *S. algae* JCM21037^T, *S. carassii* 08MAS2251^T, *S. chilikensis* KCTC22540^T, *S. indica* KCTC23171^T, *S. putrefaciens* ATCC8071^T, and *S. xiamenensis* JCM16212^T. Two strains were determined to be the same species when the ANI value > 95%.

Genome annotation was performed using Prokka (version 1.12)[34] Prodigal (version 2.6.3)[35]and RAST (https://rast.nmpdr.org/). Based on seven housekeeping genes (16SrRNA, *gyrA*, *gyrB*, *infB*, *recN*, *rpoA*, and *topA*), the allele numbers of the housekeeping genes were obtained from the PubMLST (https://pubmlst.org/organisms/shewanella-spp/) online analyses platform to determine the ST. SNP and insertion deletion (InDel) analyses were performed with the *S. algae* JCM 21,037^T genome as the reference sequence using Snippy (version 4.3.6)[36]. Moreover, a phylogenetic tree was constructed for the strains using the maximum likelihood method based on cgSNP using iqtree (version 2.0.6)[37].

The virulence factor database (VFDB, http://www.mgc.ac.cn/VFs/) and the PathogenFinder 1.1 database (https://cge.cbs.dtu.dk//services/PathogenFinder/) were used in combination to predict the potential virul

ence-related genes in the genome of *Shewanella* spp.. The amino acid sequences of the whole genome were searched against the database using BLASTp, with the cutoff values set to identity ≥ 60%, coverage ≥ 60%, and an E value of 1e-5. Potential resistance genes were predicted by BLASTp homology search using the ResFinder database and the comprehensive antibiotic resistance database (CARD), with parameters set to identity ≥ 90%, coverage ≥ 80%, and an E-value of 1e-5.

Antibiotic susceptibility testing of *Shewanella* strains

Antibiotic susceptibility testing was performed using the broth microdilution method to obtain the minimum inhibitory concentration (MIC). Susceptibility was determined as sensitive (S), intermediate (I), or resistant (R) according to the CLSI M100 *Performance Standards for Antimicrobial Susceptibility Testing*(florfenicol with reference to *Campylobacter* and others test antibiotics with reference to *Vibrio*). The test antibiotics and the cut-off points for determining resistance were: ampicillin (AMP, > 32 μg/mL), tetracycline (TET, > 16 μg/mL), colistin (CT, > 4 μg/mL), ciprofloxacin (CIP, > 1 μg/mL), azithromycin (AZI, > 32 μg/mL), chloramphenicol (CHL, > 32 μg/mL), nalidixic acid (NAL, > 32 μg/mL), streptomycin (STR, > 32 μg/mL), sulfamethoxazole tablets (SXT, > 4/76 μg/mL), amikacin (AMK, > 64 μg/mL) and florfenicol (FLO, > 8 μg/mL).

Data availability

The datasets of newly sequenced Shewanella isolates analyzed in the current study are available in the GenBank repository in the BioProject PRJNA1208244.

Received: 1 March 2025; Accepted: 11 August 2025
Published online: 19 August 2025

References

1. Gressier, M. et al. First case of human spondylodiscitis due to Shewanella algae. *Int. J. Infect. Dis.* **14**(Suppl 3), e261–264. https://doi.org/10.1016/j.ijid.2009.11.007 (2010).
2. Huang, Z. et al. Distribution and molecular characterization of Shewanella in China. *Dis. Surveill.* **38**, 384–390. https://doi.org/10.3784/jbjc.202212300556 (2023).
3. Martín-Rodríguez, A. J., Suárez-Mesa, A., Artiles-Campelo, F., Römling, U. & Hernández, M. Multilocus sequence typing of Shewanella algae isolates identifies disease-causing Shewanella chilikensis strain 6I4. *FEMS Microbiol. Ecol.* **95** https://doi.org/10.1093/femsec/fiy210 (2019).
4. Noguchi, T. & Arakawa, O. Tetrodotoxin–distribution and accumulation in aquatic organisms, and cases of human intoxication. *Mar. Drugs.* **6**, 220–242. https://doi.org/10.3390/md20080011 (2008).
5. Khashe, S. & Janda, J. M. Biochemical and pathogenic properties of Shewanella Alga and Shewanella putrefaciens. *J. Clin. Microbiol.* **36**, 783–787. https://doi.org/10.1128/jcm.36.3.783-787.1998 (1998).
6. Wang, D. et al. Identification of tetrodotoxin-producing Shewanella spp. From feces of food poisoning patients and food samples. *Gut Pathog.* **5**, 15. https://doi.org/10.1186/1757-4749-5-15 (2013).
7. Nath, R., Saikia, L., Choudhury, G. & Das, P. P. Isolation of Shewanella algae from rectal swabs of patients with bloody diarrhoea. *Indian J. Med. Microbiol.* **29**, 422–425. https://doi.org/10.4103/0255-0857.90186 (2011).
8. Fang, Y. et al. Shewanella carassii sp. nov., isolated from surface swabs of crucian carp and faeces of a diarrhoea patient. *Int. J. Syst. Evol. Microbiol.* **67**, 5284–5289. https://doi.org/10.1099/ijsem.0.002511 (2017).
9. Kang, Y. et al. Prevalence and molecular characteristics of Shewanella infection in diarrhea patients in beijing, China 2017–2019. *Front. Microbiol.* **15**, 1293577. https://doi.org/10.3389/fmicb.2024.1293577 (2024).
10. Yu, K., Huang, Z., Xiao, Y. & Wang, D. Shewanella infection in humans: epidemiology, clinical features and pathogenicity. *Virulence* **13**, 1515–1532. https://doi.org/10.1080/21505594.2022.2117831 (2022).
11. Ng, W. W., Shum, H. P., To, K. K. & Sridhar, S. Emerging infections due to Shewanella spp.: A case series of 128 cases over 10 years. *Front. Med. (Lausanne).* **9**, 850938. https://doi.org/10.3389/fmed.2022.850938 (2022).
12. Lee, C. K. et al. Evolution of cell size homeostasis and growth rate diversity during initial surface colonization of Shewanella oneidensis. *ACS Nano.* **10**, 9183–9192. https://doi.org/10.1021/acsnano.6b05123 (2016).
13. Martin-Rodriguez, A. J. et al. Reduction of alternative electron acceptors drives biofilm formation in Shewanella algae. *NPJ Biofilms Microbiomes.* **7** https://doi.org/10.1038/s41522-020-00177-1 (2021).
14. Yuan, J. et al. Shrimp shapes a resistance trait against vibriosis by memorizing the colonization resistance of intestinal microbiota. *PLoS Pathog.* **20**, e1012321. https://doi.org/10.1371/journal.ppat.1012321 (2024).
15. De la Cruz, M. A. et al. Gene expression profiling of transcription factors of Helicobacter pylori under different environmental conditions. *Front. Microbiol.* **8**, 615. https://doi.org/10.3389/fmicb.2017.00615 (2017).
16. Liu, X. et al. A cheZ-Like gene in azorhizobium caulinodans is a key gene in the control of chemotaxis and colonization of the host plant. *Appl. Environ. Microbiol.* **84** https://doi.org/10.1128/AEM.01827-17 (2018).
17. Stock, A. M., Mottonen, J. M., Stock, J. B. & Schutt, C. E. Three-dimensional structure of chey, the response regulator of bacterial chemotaxis. *Nature* **337**, 745–749. https://doi.org/10.1038/337745a0 (1989).
18. Grant, C. R. et al. Distinct gene clusters drive formation of ferrosome organelles in bacteria. *Nature* **606**, 160–164. https://doi.org/10.1038/s41586-022-04741-x (2022).
19. Xie, P., Xu, Y., Tang, J., Wu, S. & Gao, H. Multifaceted regulation of siderophore synthesis by multiple regulatory systems in Shewanella oneidensis. *Commun. Biol.* **7**, 498. https://doi.org/10.1038/s42003-024-06193-7 (2024).
20. Christie, P. J. & Type IV secretion: intercellular transfer of macromolecules by systems ancestrally related to conjugation machines. *Mol. Microbiol.* **40**, 294–305. https://doi.org/10.1046/j.1365-2958.2001.02302.x (2001).
21. Mougous, J. D. et al. A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. *Science* **312**, 1526–1530. https://doi.org/10.1126/science.1128393 (2006).
22. Christie, P. J., Atmakuri, K., Krishnamoorthy, V., Jakubowski, S. & Cascales, E. Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu. Rev. Microbiol.* **59**, 451–485. https://doi.org/10.1146/annurev.micro.58.030603.123630 (2005).
23. Cerbino, G. N. et al. Comparative genome analysis of the genus Shewanella unravels the association of key genetic traits with known and potential pathogenic lineages. *Front. Microbiol.* **14**, 1124225. https://doi.org/10.3389/fmicb.2023.1124225 (2023).
24. Charles, M. V., Srirangaraj, S. & Kali, A. Neonatal sepsis caused by Shewanella algae: A case report. *Australas Med. J.* **8**, 64–66. https://doi.org/10.4066/amj.2015.2292 (2015).
25. Liu, P. Y. et al. Cobra bite wound infection caused by Shewanella algae. *Int. J. Infect. Dis.* **20**, 11–12. https://doi.org/10.1016/j.ijid.2013.08.014 (2014).
26. Cloeckaert, A. & Schwarz, S. Molecular characterization, spread and evolution of multidrug resistance in Salmonella enterica typhimurium DT104. *Vet. Res.* **32**, 301–310. https://doi.org/10.1051/vetres:2001126 (2001).

27. Blickwede, M., Valentin-Weigand, P. & Schwarz, S. Subinhibitory concentrations of florfenicol enhance the adherence of florfenicol-susceptible and florfenicol-resistant Staphylococcus aureus. *J. Antimicrob. Chemother.* **54**, 286–288. https://doi.org/10.1093/jac/dkh273 (2004).
28. Kim, E. & Aoki, T. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, pasteurella piscicida. *Microbiol. Immunol.* **40**, 665–669. https://doi.org/10.1111/j.1348-0421.1996.tb01125.x (1996).
29. Wang, L. et al. Genome characterization of Shewanella algae in Hainan province, China. *Front. Microbiol.* **15**, 1474871. https://doi.org/10.3389/fmicb.2024.1474871 (2024).
30. Li, Y. et al. Prevalence and molecular characterization of Campylobacter spp. Isolated from patients with diarrhea in shunyi, Beijing. *Front. Microbiol.* **9**, 52. https://doi.org/10.3389/fmicb.2018.00052 (2018).
31. Yu, K. et al. Establishment and application of Matrix-Assisted laser desorption/ionization Time-of-Flight mass spectrometry for detection of Shewanella genus. *Front. Microbiol.* **12**, 625821. https://doi.org/10.3389/fmicb.2021.625821 (2021).
32. Luo, R. et al. SOAPdenovo2: an empirically improved memory-efficient short-read de Novo assembler. *Gigascience* **1** https://doi.org/10.1186/2047-217X-1-18 (2012).
33. Thompson, C. C. et al. Microbial genomic taxonomy. *BMC Genom.* **14**, 913. https://doi.org/10.1186/1471-2164-14-913 (2013).
34. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153 (2014).
35. Hyatt, D. et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform.* **11**, 119. https://doi.org/10.1186/1471-2105-11-119 (2010).
36. Bush, S. J. Generalizable characteristics of false-positive bacterial variant calls. *Microb. Genom.* https://doi.org/10.1099/mgen.0.000615 (2021).
37. Nguyen, L. T., Schmidt, H. A., von Haeseler, A. & Minh, B. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**, 268–274. https://doi.org/10.1093/molbev/msu300 (2015).

Author contributions
Conceptualization, Y.L. and Z.H.; methodology, Y.L. and K.Y.and G.Z. and Z.H.; soffware, Yw.L. and Z.H. and A.Y. and M.H.; investigation, Y.L. and Z.H. and A.Y. and M.H.; resources, Yw.L. and A.Y. and M.H.; data curation, Y.L.; writing-original draff preparation, Y.L. and Z.H.; writing-review and editing, Y.L. and Z.H.; visualization, T.P. and Y.L.; supervision, T.P. and Y.L.; project administration, T.P. ; funding acquisition, T.P. and Y.L. All authors have read and agreed to the published version of the manuscript.

Funding
Capital's Funds for Health Improvement and Research (No.2024-2G-7106).

Declarations

Competing interests
The authors declare no competing interests.

Conflict of interest
The authors declare no confficts of interest.

Ethical approval
The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Shunyi District Center for Disease Control and Prevention, Beijing, China.

Informed consent
Statement.

Informed consent
was obtained from all subjects involved in the study.

Additional information
Correspondence and requests for materials should be addressed to Z.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.