Serology, virulence, antimicrobial susceptibility and molecular characteristics of clinical *Vibrio parahaemolyticus* strains circulating in southeastern China from 2009 to 2013

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Abstract

Vibrio parahaemolyticus is a leading cause of food-borne diarrhoea in coastal countries. Although *V. parahaemolyticus* cases have been reported since 1950, they have been poorly documented. From July 2009 to June 2013, we collected 6951 faecal specimens for pathogen detection; *V. parahaemolyticus* strains were isolated from 563 specimens (8.1%). We then analysed the characteristics of the 501 *V. parahaemolyticus* strains that were isolated as the sole pathogen. Twenty-one serotypes were identified among these strains; O3:K6 was the most common serotype (65.1%), followed by O4:K8, O4:K68 and O1:K36. One strain of the O4:K18 serotype was isolated from clinical patients for the first time. Pandemic O3:K6 clones were predominant and accounted for 69.1% of all of the pandemic strains. This is the first report of one strain expressing the O3:K8 serotype with a pandemic genotype. The presence of the haemolysin gene *tdh* (93.0%) was the key characteristic of the virulent strains; however, a few strains carried the *trh* gene. We also confirmed the presence of the type III secretion system 2 (T3SS2) genes in all of the pathogenic strains. Subsequent multilocus sequence typing split the isolates into 16 sequence types (STs), with ST3 and ST88 as the most prevalent in southeastern China. Most isolates were sensitive to common antimicrobial agents, apart from ampicillin. However, the resistance rate to ampicillin has apparently increased in this area. In conclusion, our results indicate that pandemic O3:K6 *V. parahaemolyticus* isolates are predominant in southeastern China, and additional surveillance should be conducted to facilitate control of the transmission of this pathogen.

Keywords: Acute diarrhoea, antimicrobial resistance, multilocus sequence typing, serotypes, *Vibrio parahaemolyticus*, virulence genes
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Introduction

The World Health Organization [1] estimated that 1.4 million people died from diarrhoeal diseases worldwide in 2010, representing 2.7% of all deaths. *Vibrio parahaemolyticus*, which is naturally present in coastal waters, is one of the leading causes of food-borne infections in many Asian countries, including China, where it caused 58.7% of 2041 bacterial outbreaks in 2010 [2], and Japan, where it causes 20–30% of all food

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poisoning cases [3]. Outbreaks are also frequently reported in the Americas [4]. Even in Europe, where the risk of infection with *V. parahaemolyticus* is considered to be quite low, the propagation of pandemic clones has been reported in coastal countries (e.g. Spain, France and Italy) [5–7].

Southeastern China, which is located on the southeastern coast of China, is considered a hotspot from which *V. parahaemolyticus* epidemics often emerge. From March 2010 to May 2012, the prevalence of infections with this bacterium among patients with acute diarrhoea in southeastern China was 8.9%; during this period, *V. parahaemolyticus* ranked as the second most prevalent pathogen, following diarrhoeagenic *Escherichia coli* (DEC) [8]. Despite the high risk of *V. parahaemolyticus* epidemics, little information regarding the prevalence or molecular epidemiology of this organism is available. In this study, we focused on southeastern China as a representative region for *V. parahaemolyticus* surveillance from 2009 to 2013.

Materials and Methods

Study design

Nine hospitals in different areas of southeastern China were selected as surveillance sites, including seven general hospitals, one children's hospital and one community hospital. The subjects were outpatients with acute diarrhoeal disease (defined as three or more watery or loose stools during a 24-h period with a duration $\leq$14 days) and asymptomatic individuals (defined as the absence of diarrhoea in the previous week) with a similar age distribution. The subjects were randomly enrolled each week throughout the year. Each surveillance site administered patient questionnaires, collected faecal specimens, isolated and identified organisms in the specimens, and stored the isolates. The specimens were frozen at −20°C, and the isolates were stored at −80°C in trypticase soy broth containing 20% glycerol. The preserved specimens and isolates were delivered to our laboratory on dry ice for further analysis.

Questionnaires covered the demographic characteristics of the participants, the symptoms of their illnesses, the results of routine stool tests and any medications taken before their hospital visits. Each faecal specimen was collected after informed consent was obtained from the patient or, if the patient was a child, from the child's parents.

Microbiological analysis

Stool specimens were cultured on selective media and in enrichment broths to detect *E. coli* (for screening DEC), *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Campylobacter* spp., *Vibrio cholerae*, *V. parahaemolyticus*, *Plesimonas shigelloides* and *Yersinia enterocolitica* [9]. DEC, calicivirus, adenovirus and astrovirus were detected using PCR. Rotavirus was detected using a commercial ELISA kit. Further characterization was performed only on *V. parahaemolyticus* strains that were isolated as the sole pathogen.

Serotyping

O and K antigen determination was performed using an agglutination test with 11 O (lipopolysaccharide) and 65 K (capsule) antisera (Denka Seiken Ltd., Tokyo, Japan) according to the instructions provided with the reagents. One serotype was defined as a unique combination of O and K serogroups.

Detection of virulence-associated genes

Vibrio parahaemolyticus isolates were tested for the presence of a species-specific marker (*tlh*) and haemolysin genes (*tdh*, *trh*) by real-time PCR [10], pandemic markers (*toxRS/new*, *orf8*) by conventional monoplex PCR [11] and the type III secretion system 1 (T3SS1) genes (VP1670 (*vscP*), VP1686 (*putative*), VP1689 (*vscK*) and VP1694 (*vscF*)), the T3SS2α genes (VP1362 (*vopB2*), VP1339 (*vscC2*), VP1335 (*vscS2*) and VP1327 (*vopT*)) and the T3SS2β genes (*vscC2*, *vopB2*, *vopC*, *vscS2*) by conventional four-multiplex PCR, respectively [10]. A monoplex PCR assay was performed if the results of the multiplex PCR were unsatisfactory. The pathogenic group was defined as tdh^+ and/or trh^+; all other isolates were assigned to the non-pathogenic group. In the other analyses, the pandemic group was defined as tdh^+, trh^-, $toxRS/new^+$ and $orf8^{+/-}$, and all other isolates were assigned to the non-pandemic group [11].

Multilocus sequence typing

The multilocus sequence typing (MLST) scheme used internal fragments of the seven housekeeping genes (*recA*, *gyrB*, *dnaE*, *dtdS*, *pntA*, *pyrC* and *tnaA*). The protocols were published on the *V. parahaemolyticus* MLST web site (http://pubmlst.org/vparahaemolyticus/). BioNumerics v6.1 (http://www.appliedmaths.com) was used to generate a minimum spanning tree from the non-concatenated sequences of seven alleles.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on the isolates using the agar dilution method. The results were analysed with WHONET 5.6 software using the CLSI breakpoints [12]. *Escherichia coli* ATCC 25922 was used as a control for the antimicrobial susceptibility testing.

Data management and analysis

Statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of the differences between groups

TABLE 1. Demographic characteristics of the patients with acute diarrhoea from which *Vibrio parahaemolyticus* was isolated in southeastern China from July 2009 to June 2013

Variable and category	No. of samples tested	No. (%) of positive samples	p
Time			<0.001
March to May	1216	17 (1.4%)	
June to August	3379	444 (13.1%)	
September to November	1320	100 (7.6%)	
December to February	1036	2 (0.2%)	
Age (years)			<0.001
≤4	1677	40 (2.4%)	
5–17	851	13 (1.5%)	
18–44	2564	366 (14.3%)	
45–59	1105	86 (7.8%)	
≥60	754	58 (7.7%)	
Gender			0.383
Male	3730	312 (8.4%)	
Female	3221	251 (7.8%)	

was determined using the chi-square test or Fisher's exact test. All p-values were two-sided, and p <0.05 was considered statistically significant.

Results

Patients

A total of 6951 faecal specimens from outpatients with acute diarrhoea were collected from July 2009 to June 2013. Table 1 lists the demographic characteristics of the patients with acute diarrhoea. Simultaneously, 521 asymptomatic individuals, including 285 men and 236 women, were enrolled in this study.

Prevalence of *V. parahaemolyticus*

A total of 563 (8.1%) of the 6951 faecal specimens were positive for *V. parahaemolyticus* strains; this pathogen was the second most prevalent bacterial pathogen after DEC (Table 2).

TABLE 2. Frequency of isolated enteric pathogens from 6951 faecal specimens from outpatients with acute diarrhoea in southeastern China from July 2009 to June 2013

Pathogens	No. of samples tested	No. (%) of positive samples
Diarrhoeagenic *Escherichia coli*	6951	1140 (16.4)
Vibrio parahaemolyticus	6951	563 (8.1)
Aeromonas	6951	279 (4.0)
Plesiomonas shigelloides	6951	157 (2.3)
Shigella spp.	6951	74 (1.1)
Salmonella spp.	6951	55 (0.8)
Vibrio cholera (non-O1/non-O139)	6951	35 (0.5)
Campylobacter spp.	6951	35 (0.5)
Yersinia enterocolitica	6951	23 (0.3)
Rotavirus	5810	1464 (25.2)
Calicivirus	5810	848 (14.6)
Astrovirus	5810	93 (1.6)
Adenovirus	5810	52 (0.9)

Vibrio parahaemolyticus was isolated throughout the year, but the peak season was from June to August (Table 1). In the majority of *V. parahaemolyticus*-positive cases (89.0%, 501/563), this organism was reported as the sole pathogen detected; mixed isolates containing more than one pathogen (virus and/or bacteria) were obtained in the remaining 62 cases. Table 3 shows the numbers of cases of single and mixed isolations of *V. parahaemolyticus*.

Only two strains of *V. parahaemolyticus* were isolated from the asymptomatic individuals group, and the incidence of *V. parahaemolyticus* in this group of study subjects was significantly different from that in the patients with acute diarrhoea (p <0.001).

Serotypes

These 501 strains spanned 6 O and 14 K serogroups, including O3 (67.9%, 340/501), O4 (17.6%, 88/501) and O1 (8.8%, 44/501) as the top three O serogroups. Twenty-one serotypes were recovered, and one could not be typed for either O or K antigens (4.0%, 20/501). Of the 501 strains, O3:K6 was the most common (65.1%, 326/501), followed by O4:K8 (8.4%, 42/501), O4:K68 (7.4%, 37/501) and O1:K36 (5.2%, 26/501).

Distribution of virulence-associated genes

The 501 strains were positive for the *tlh* gene. The distribution of the haemolysin genes (*tdh*, *trh*) and the pandemic markers (*toxRS/new*, *orf8*) of *V. parahaemolyticus* is shown in Table 4. The haemolysin gene *tdh* was observed in most of the isolates (93.0%, 466/501), whereas the *trh* gene was detected in only five strains (1.0%, 5/501). In addition, 33 strains (6.6%, 33/501) from the patients with diarrhoea contained neither the *tdh* nor the *trh* gene. The proportions of samples that were positive for the pandemic markers *toxRS/new* and *orf8*, regardless of the results of the presence of the haemolysin gene, were 64.7%

TABLE 3. Distribution of pathogenic strains in faecal samples from patients with acute diarrhoea

Pathogen	No. of cases
Single isolation	
Vibrio parahaemolyticus	501
Mixed isolation	
V. parahaemolyticus + DEC	26
V. parahaemolyticus + Calicivirus	12
V. parahaemolyticus + *Aeromonas*	5
V. parahaemolyticus + *Vibrio cholerae*	4
V. parahaemolyticus + Rotavirus	4
V. parahaemolyticus + *Plesiomonas shigelloides*	2
V. parahaemolyticus + *Campylobacter coli*	1
V. parahaemolyticus + *Yersinia enterocolitica*	1
V. parahaemolyticus + DEC + *Shigella* spp.	2
V. parahaemolyticus + *P. shigelloides* + Calicivirus	1
V. parahaemolyticus + *P. shigelloides* + Astrovirus	1
V. parahaemolyticus + Rotavirus + Adenovirus	1
V. parahaemolyticus + DEC + *P. shigelloides*	1
V. parahaemolyticus + DEC + *Aeromonas*	1

TABLE 4. Distribution of virulence genes in *Vibrio parahaemolyticus* strains isolated as the sole pathogen from patients with acute diarrhoea

Group	No.	Haemolysin genes		Pandemic markers		Serotypes
		tdh	*trh*	*toxRS/new*	*orf8*	
Pandemic (63.3%, 317/501)	257	+	—	+	+	O3:K6 (*n* = 219), O4:K68 (*n* = 25), O1:K36 (*n* = 24), O4:K8 (*n* = 23), O1:KUT (*n* = 7), O3:K68 (*n* = 2), O3:K8 (*n* = 1), O4:K1 (*n* = 1), O4:K48 (*n* = 1), O4:KUT (*n* = 1), OUT:K22 (*n* = 2), OUT:KUT (*n* = 11)
	60	+	—	+	—	
Non-pandemic (36.7%, 184/501)	91	+	—	—	—	O3:K6 (*n* = 107), O4:K8 (*n* = 19), O4:K68 (*n* = 12), O3:K29 (*n* = 9), O1:K56 (*n* = 8), O4:K9 (*n* = 5), O8:K21 (*n* = 4), O1:K36 (*n* = 2), O2:K3 (*n* = 2), O1:K6 (*n* = 1), O3:K17 (*n* = 1), O4:K18 (*n* = 1), O1:KUT (*n* = 2), O3:KUT (*n* = 1), O5:KUT (*n* = 1), OUT:KUT (*n* = 9)
	55	+	—	—	+	
	3	+	+	—	—	
	1	—	+	—	—	
	1	—	+	+	+	
	18	—	—	—	—	
	9	—	—	—	+	
	3	—	—	+	+	
	3	—	—	+	—	

The light grey shading indicates the pathogenic strains that were positive for at least one of *tdh* or *trh*; dark grey shading indicates the non-pathogenic strains that were negative for both *tdh* and *trh*.

(324/501) and 64.9% (325/501), respectively. A total of 63.3% (317/501) of the tested isolates were classified as pandemic strains, and 93.4% (468/501) of the isolates were classified as pathogenic strains. The most common genotype observed in the pandemic group was tdh^+, $toxRS^+$, $orf8^+$ and trh^- (81.1%, 257/317). The pandemic strains belonged to 12 serotypes and were mainly represented by O3:K6 (69.1%, 219/317), followed by O4:K68 (7.9%, 25/317).

Two types of T3SS genes are essential in the virulence mechanism of *V. parahaemolyticus*. The distribution of T3SS genes in our study is presented in Table 5. T3SS1 genes were identified in all of the strains, and the majority of the isolates contained all four T3SS1 genes (96.8%). The T3SS2α-associated genes were present in all tdh^+ isolates (100%, 466/466), but the T3SS2β genes were detected only in the trh^+ clinical isolates (100%, 5/5).

TABLE 5. Distribution of T3SS genes among *Vibrio parahaemolyticus* strains isolated as the sole pathogen from patients with acute diarrhoea

Gene	No. of strains (*n* = 501)			
	tdh^+ trh^- (*n* = 463)	tdh^+ trh^+ (*n* = 3)	tdh^- trh^+ (*n* = 2)	tdh^- trh^- (*n* = 33)
T3SS1				
VP1670 (vscP)	462	3	2	27
VP1686 (putative)	463	3	1	33
VP1689 (vscK)	460	3	2	32
VP1694 (vscF)	456	3	2	31
All four genes present	455	3	1	26
T3SS2α				
VP1362 (vopB2)	463	3	0	0
VP1339 (vscC2)	463	3	0	0
VP1335 (vscS2)	460	3	0	0
VP1327 (vopT)	463	3	0	0
All four genes present	460	3	0	0
T3SS2β				
vscC2	0	3	2	0
vopB2	0	3	2	0
vopC	0	3	2	0
vscS2	0	3	2	0
All four genes present	0	3	2	0

MLST analysis

The genetic population structure of the *V. parahaemolyticus* isolates was analysed by MLST. A minimum spanning tree of the sequence types (STs) that was constructed based on subtyping information, including serotypes and virulence-associated genes, is shown in Fig. 1. MLST categorized the isolates into 16 STs, of which five were novel (ST654, ST670, ST671, ST672 and ST675). The most frequently observed STs in this study were ST3 (82.6%, 414/501), ST88 (9.6%, 48/501), ST120 (1.8%, 9/501) and ST8 (1.6%, 8/501). Among the novel STs, ST672 were completely comprised of pandemic strains (Fig. 1b).

Relationships among serotypes, virulence genes and STs

In this study, the most common serotypes were O3:K6 and O4:K8, the majority of which were positive for one or two haemolysin genes (92.0% and 100%, respectively). Moreover, the pandemic O3:K6 clones were positive for the virulence-related *tdh* (100%), *toxRS/new* (100%), *orf8* (88.7%), T3SS1 (98.6%-100%) and T3SS2α (99.1%-100%) genes and negative for the *trh* and T3SS2β genes. Twelve different serotypes were clustered in the largest group (ST3), O3:K6 was the most common (77.8%, 322/414), followed by O4:K68 (8.9%, 37/414) and O1:K36 (6.0%, 25/414) (Fig. 1a). ST88 was comprised of all O4:K8 isolates (87.5%, 42/48), with the remaining isolates being OUT:KUT and O4:KUT isolates. As shown in Fig. 1(b), the pandemic strains were grouped into three STs (ST3 (*n* = 288), ST88 (*n* = 25) and ST672 (*n* = 4)), whereas the non-pandemic strains fell into 15 STs. The 33 non-pathogenic strains (tdh^-/trh^-) were clearly assigned to ST3, ST88, ST12 and ST637 (Fig. 1c).

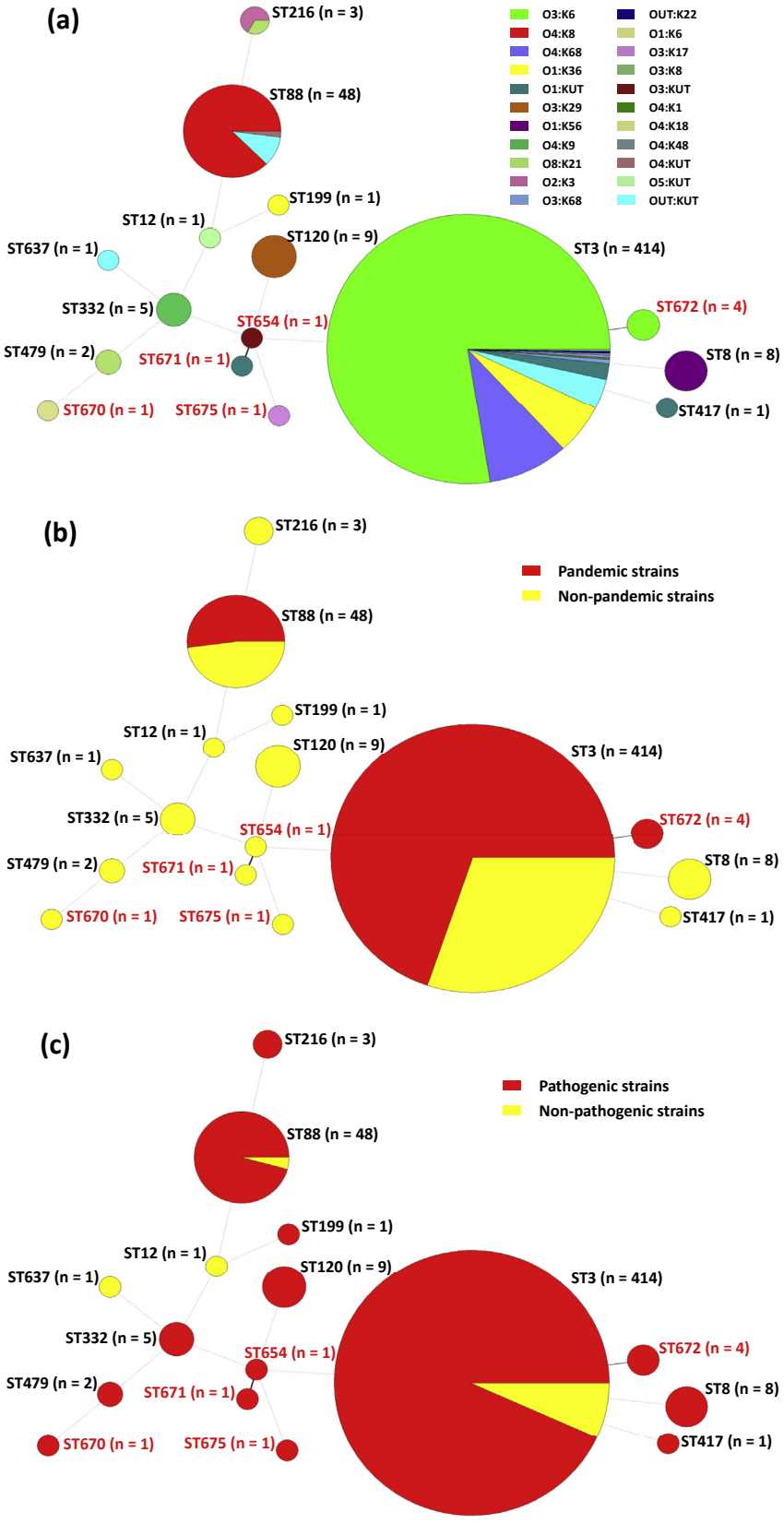

FIG. 1. Minimal spanning tree (MST) analysis of 501 *Vibrio parahaemolyticus* strains based on MLST data. Each circle corresponds to a sequence type (ST), and the size of each circle corresponds to the number of isolates of that particular type. The relationship between strains is indicated by the connections between the isolates and the lengths of the branches linking them. Black lines connecting pairs of STs indicate that they differ in one allele (thick lines, ST654 and ST671), two alleles (thin lines, ST3 and ST672) or three to seven alleles (dashed lines). Novel STs from this study are marked in red, and *N* represents the number of isolates in each ST. Three MST figures have been generated separately based on the available subtyping information. (a) ST versus serotypes; (b) ST versus pandemic/non-pandemic strains; (c) ST versus pathogenic/non-pathogenic strains.

TABLE 6. Antimicrobial susceptibility testing of 501 *Vibrio parahaemolyticus* strains isolated as the sole pathogen from patients with acute diarrhoea

Antimicrobial agent	Susceptible (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	98.5	0.4	1.1
Levofloxacin	99.8	0.2	0.0
Ampicillin	4.0	8.9	87.1
Piperacillin	84.7	12.4	2.9
Ampicillin/Sulbactam	98.3	1.3	0.4
Amoxicillin/Clavulanic acid	97.5	1.9	0.6
Piperacillin/Tazobactam	99.2	0.8	0.0
Cefazolin	5.9	43.7	50.4
Cefuroxime	36.3	56.9	6.8
Cefotaxime	95.6	3.6	0.8
Ceftazidime	99.6	0.0	0.4
Cefepime	99.6	0.2	0.2
Cefoxitin	86.5	12.9	0.6
Meropenem	100.0	0.0	0.0
Imipenem	100.0	0.0	0.0
Amikacin	98.0	1.4	0.6
Gentamicin	97.5	1.9	0.6
Chloramphenicol	98.7	0.4	0.9
Tetracycline	99.6	0.2	0.2
Trimethoprim/Sulphamethoxazole	98.3	0.5	1.2

Antimicrobial profile

The antimicrobial susceptibilities of *V. parahaemolyticus* are listed in Table 6. The largest proportion of *V. parahaemolyticus* strains was resistant to ampicillin (87.1%), followed by cefazolin (50.4%). More than half of the isolates exhibited intermediate levels of susceptibility to cefuroxime (56.9%), followed by cefazolin (43.7%). Additionally, more than 95.0% of the isolates were sensitive to other antibacterials including fluoroquinolones, carbapenems, third-generation and fourth-generation cephalosporins, β-lactamase inhibitor combinations, aminoglycosides, tetracyclines and trimethoprim–sulphamethoxazole.

Discussion

In our study, *V. parahaemolyticus* was found to be the second most prevalent bacterial pathogen detected in patients with acute diarrhoea in southeastern China, with an isolation rate of 8.1%. This rate is significantly higher than both the rate of 1.3% reported for India for 2001–2010 [13] and the rate of 5.1% that was observed in northwest coastal Mexico in 2004–2010 [14]. Moreover, it is also higher than the rate of 6.0% reported for southern coastal China in 2007–2012 [15]. These results indicate that the distribution of *V. parahaemolyticus* varies geographically and that the pathogen is emerging within southern coastal China. Notably, in our study, the *V. parahaemolyticus* isolation rate in patients with acute diarrhoea was much higher than in asymptomatic individuals, implying that the presence of *V. parahaemolyticus* in the stools of patients with diarrhoea may be significant. Moreover, it is worth mentioning that, in our study, in the majority of the *V. parahaemolyticus*-positive cases, this organism was detected as the sole pathogen, indicating the importance of *V. parahaemolyticus* as one of the major aetiological agents of diarrhoea in this region. However, in China, routine pathogen detection in patients with diarrhoea only involves tests for *V. cholerae* (O1 and O139 serogroups), *Salmonella* spp. and *Shigella* spp. in clinical microbiology laboratories at present. Our study found that isolates of *V. cholerae* (O1 and O139 serogroups), *Salmonella* spp. and *Shigella* spp. accounted for only 5.5% of the bacterial pathogens. Hence, we suggest that patients with diarrhoea should be tested for a wider range of pathogens, including *V. parahaemolyticus*, as part of routine clinical care in the future.

In this study, an increase in the number of *V. parahaemolyticus* infections was observed during the summer months. This seasonal variation had also been observed in Thailand [11], and this phenomenon may be associated with the distribution of *V. parahaemolyticus* in the environment, as the bacterium may survive in sediments during the winter and be released into the water column in late spring or early summer when the temperature rises to 15°C or higher [16]. The incidence rate of *V. parahaemolyticus* infections fluctuated with age in this study. Similar results have been reported in India [13] and Vietnam [17]. The source of the *V. parahaemolyticus* infections comes from the consumption of raw or inadequately cooked seafood and foods contaminated by seafood materials. Adults, particularly adults between 18 and 60 years old, have more economic ability to afford high-priced seafood and have more opportunities to have dinner in restaurants. This may explain this fluctuation.

In our study, a high degree of serotypic diversity was observed (21 serotypes), with O3:K6 as the predominant serotype. Since 1996, the O3:K6 serotype of *V. parahaemolyticus* with specific genetic markers (with *tdh* and *toxRS/new* genes and with or without *orf8* genes) has been prevalent in China [15] and other Asian countries, as well as in the Americas, Europe and Africa [18]. Our data showed that 63.3% of *V. parahaemolyticus* isolates exhibited pandemic traits, and these strains were not associated with any local outbreak. In the pandemic group, the O3:K6 serotype (69.1%) was the predominant isolate, followed by 11 other non-O3:K6 serotypes with pandemic traits. Recent studies have shown that since 1996, at least 21 serotypes have emerged that share genetic markers specific for the pandemic serotype O3:K6 [14,18]. It has been reported that these non-O3:K6 serotypes most likely originated from the same clones as O3:K6 [14]. Serovar alteration through mutation or horizontal gene transfer of capsular K and somatic O antigen-encoding genes may be one method by which *V. parahaemolyticus* adapts to

environmental changes and human immune responses [19]. Notably, the O3:K8 serotype that was identified in this study was the first found to have pandemic traits. The non-O3:K6 serotypes with pandemic traits are isolated increasingly worldwide and therefore may have pandemic potential.

In the current study, 93.0% of the strains carried *tdh*, and only five strains carried *trh*. Although *trh* shares approximately 70% homology with *tdh* [20], the two genes confer different advantages on the bacterium, which result in different selection pressures. Previous studies have identified the T3SS1 genes in all *V. parahaemolyticus* strains. The T3SS2α genes have been demonstrated to be co-present and co-regulated with the tdh^+ genes, whereas the T3SS2β genes have generally been identified in trh^+ strains on a pathogenicity island (Vp-PAI) [21,22]. Our results are largely consistent with these previous findings and so confirm the particular relationship between T3SS and the haemolysin genes. In addition, recent investigations in an experimental animal model have shown that T3SS2 is necessary for pathogen colonization and the development of gastroenteritis [23]. Our results are consistent with this finding, as T3SS2 was identified in all pathogenic and pandemic strains. In this study, 33 tdh^- trh^- $T3SS2^-$ strains were isolated from patients with diarrhoea. Recently, similar results were reported by Ottaviani *et al.* [24] and Bhoopong *et al.* [25]. This result suggests that *V. parahaemolyticus* may harbour other virulence factors that are responsible for diarrhoea. However, it is possible that this could be explained by the deletion of the *tdh* and *trh* genes during infection [25].

Better understanding of the genetic relationships among *V. parahaemolyticus* strains has been provided by MLST studies. We found that this pathogen exhibits a high genetic diversity, even within a single geographic region. Notably, ST3 was the most frequent subtype of *V. parahaemolyticus* that was observed in the study. ST3 is widely distributed in Asia and the Americas [4,26] and so poses a significant public health threat worldwide. In addition to ST3, other prevalent STs, including ST88 and ST265 in coastal China and Peru [14,27], and ST36 and ST43 in the USA [4], also vary in their geographical distributions. Earlier observations showed that extensive serotypic diversity occurred among isolates representing the same ST [28]. In our study, the diversity of serotypes was mainly observed among isolates grouped in ST3, ST88 and ST216, including 11, 3 and 2 combinations of O/K serotypes, respectively; in contrast, ST120, ST8, ST332 and ST672 consisted of a single serotype, respectively. These findings suggest that the O- and K-antigen-encoding loci are subject to exceptionally high rates of recombination [27,28].

In cases of severe or prolonged *V. parahaemolyticus* infection, antibiotics can be used. Moreover, the choice of antibiotics should be based on the antimicrobial susceptibilities of the organism. In this study, most isolates were sensitive to common antimicrobial agents except ampicillin (87.1%). Similar results have been observed in Asia [29] and Europe [5]. However, Min Z *et al.* [30] reported that the resistance rate of *V. parahaemolyticus* to ampicillin was less than 50% in this area in 2008. Hence, the resistance rate to ampicillin has unfortunately appeared to increase in southeastern China. It is necessary to understand the trends of drug resistance in pathogens for the prevention and control of disease.

In summary, this study illustrates the importance of *V. parahaemolyticus* infection in patients with diarrhoea in southeastern China. A large number of diverse serotypes were detected in clinical isolates, with pandemic-serotype O3:K6 strains predominating. ST3 and ST88 were the most prevalent STs and included the majority of pandemic strains. Although *V. parahaemolyticus* maintained a high sensitivity to most antimicrobial agents, it will be important to monitor the emergence of drug-resistant strains and strengthen the management of antimicrobial drugs. Therefore, surveillance should be emphasized to monitor epidemic trends and antimicrobial control and to discover more reliable predictors of virulence.

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Transparency Declaration

The authors have declared no conflicts of interest.

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