ANTIBIOGRAM PROFILES AND VIRULENCE CHARACTERISTICS OF PANDEMIC *VIBRIO PARAHAEMOLYTICUS* ISOLATES FROM DIARRHEAL PATIENTS IN HAT YAI HOSPITAL, SOUTHERN THAILAND

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Abstract. Fifty-seven pandemic *Vibrio parahaemolyticus* isolates (*tdh* positive, *trh* negative GS-PCR positive) obtained from diarrheal patients at Hat Yai Hospital, Songkhla, Thailand during 2001-2016 were examined for their antibiogram profiles and virulence characteristics. Resistance to ampicillin, ciprofloxacin and norfloxacin was present in 100%, 12% and 2% of the isolates, respectively, with over half of the isolates showing intermediate resistance to the two fluoroquinolones. Virulence-associated genes encoding type III secretion systems (T3SS1 and T3SS2) and type VI secretion systems (T6SS1 and T6SS2) were widely distributed among the isolates. All isolates were able to produce similar levels of thermostable direct hemolysin. Eighty-nine percent and 44% of the isolates exhibited swarming and twitching motility, respectively. Sixty-eight percent of the isolates were able to use 50 μM hemoglobin as the only iron source and 81% in the presence of 155 μM hemoglobin. This study highlights the antimicrobial resistance and multiple virulence characteristics of clinical *V. parahaemolyticus* isolates, information that will be useful in clinical and epidemiological investigations of this pathogenic organism.

Keywords: *Vibrio parahaemolyticus*, antibiogram, iron acquisition, motility, virulence gene, Thailand

INTRODUCTION

Vibrio parahaemolyticus is one of the major causes of gastroenteritis in humans. Cases are often associated with eating raw or undercooked shellfish or even cooked foods that contaminated with raw shellfish (Yeung and Boor, 2004). Recently, the number of *V. parahaemolyticus* infections in Thailand has been gradually increasing and this is a major cause for concern (Bureau of Epidemiology, 2015). *V. parahaemolyticus* O3:K6 serotype, positive by PCR for group-specific *toxRS* regions and thermostable direct hemolysin gene (*tdh*) but not *tdh*-related hemolysin gene (*trh*)

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was shown to be responsible for pandemic outbreaks of diarrhea in various parts of the world, including Asia, America, Africa and Europe (Okuda *et al*, 1997; Daniels *et al*, 2000, Martinez-Urtaza *et al*, 2004; Ansaruzzaman *et al*, 2005; Bureau of Epidemiology, 2015). *V. parahaemolyticus* serotypes O1:K25, O4:K68, and O1:K untypeable (KUT) were also reported as pandemic serotypes originating from O3:K6 clone (Chowdhury *et al*, 2000; Matsumoto *et al*, 2000; Bhuiyan *et al*, 2002). In Thailand, these serotypes were reported to be continually detected in patients since 2000 (Wootipoom *et al*, 2007; Thongjun *et al*, 2013).

Pathogenicity of *V. parahaemolyticus* depends on multiple factors. TDH and type III (T3SSs) and type VI secretion systems (T6SSs) are recognized as virulence-associated factors of *V. parahaemolyticus* isolates (Ceccarelli *et al*, 2013; Zhang and Orth, 2013).TDH is responsible for *V. parahaemolyticus* cytotoxicity and hemolytic activity (Vuddhakul, 2008, Wang *et al*, 2015), while T3SSs and T6SSs are responsible for translocation of effector proteins [VcrD1(T3SS1), VcrD2 (T3SS2), VipA1 (T6SS1), and VipA2 (T6SS2)] by needle-like bacterial structures (Calder *et al*, 2014). VopC and VopQ of T3SSs (encoded by *vopC* and *vopQ*, respectively) are responsible for host cell cytotoxicity and enterotoxicity (Calder *et al*, 2014). T6SSs play an important role in environment fitness of *V. parahaemolyticus* (Salomon *et al*, 2013; Wang *et al*, 2013b) and are necessary for adhesion to host cells (Wang *et al*, 2015).

In addition to these factors, *V. parahaemolyticus* exhibits swarming and twitching forms of motility. Swarming motility enables bacteria to survive in the environment by enhancing colonization (Fraser and Hughes, 1999) and twitching motility is involved in attachment, biofilm formation and pathogenesis (Ottemann and Miller, 1997; McCarter, 2001). Iron acquisition was reported to correlate with the infection ability of *V. parahaemolyticus* isolates (León-Sicairos *et al*, 2015). Under iron-limited conditions, *V. parahaemolyticus* produces a siderophore, vibrioferrin, considered one of the importance factors for pathogenicity of *V. parahaemolyticus* due to its ability to chelate host cell iron and association with iron transportation (Vuddhakul, 2008; León-Sicairos *et al*, 2015).

In order to extend our understanding of pandemic *V. parahaemolyticus* characteristics in this region, this study determined antibiogram profiles and virulence properties of clinical *V. parahaemolyticus* isolates from Hat Yai Province, southern Thailand.

MATERIALS AND METHODS

Collection and identification of pandemic *V. parahaemolyticus*

V. parahaemolyticus isolates were obtained from clinical samples at Hat Yai Hospital, Songkhla, Hat Yai Province during 2001-2016 as part of routine microbiological diagnosis. Species classification was confirmed by PCR amplification of *toxR* (Kim *et al*, 1999), and identification of pandemic clone-specific characteristics (*tdh* and *toxRS* positive and *trh* negative) was performed by PCR as described previously (Tada *et al*, 1992; Matsumoto *et al*, 2000) using primers listed in Table 1. In brief, reaction mixture (20 μl) containing 2 μl of template DNA for *toxR, tdh* and *trh* or 2.5 μl of template DNA for *toxRS*, 0.2 mM dNTPs for *toxR, tdh* and *trh* or 0.125 mM dNTPs for *toxRS*, 0.2 mM of each primer or 1 mM of vopQ primers, 0.5 U *Taq* DNA polymerase (NEB, Ipswich,

Table 1
Primers used in this study.

Target gene	Encoded protein	Primer name	Sequence (5′-3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
toxR	ToxR regulatory protein	tox R-F toxR-R	GTCTTCTGACGCAATCGTTG ATACGAGTGGTTGCTGTCATG	368	63	Kim *et al* (1999)
toxRS	ToxRS regulatory protein	GS-VP.1 GS-VP.2	TAATGAGGTAGAAACA ACGTAACGGGCCTACA	651	45	Matsumoto *et al* (2000)
tdh	Thermostable direct hemolysin	tdh 1 tdh 2	GGTACTAAATGGCTGACATC CCACTACCACTCTCATATGC	251	55	Tada *et al* (1992)
trh	TDH-related hemolysin	trh-F trh-R	GGCTCAAAATGGTTAAGCG CATTTCCGCTCTCATATGC	250	55	Tada *et al* (1992)
*vcrD*1	T3SS1-structure VcrD1	vcrD1F vcrD1R	CTGCTGGTCTTGTTCGCTCT TCTGGTCGCTTCCTTCTGTG	493	58	Yu *et al* (2003)
vopQ	T3SS1-effector VopQ	VP1680F VP1680R	GCCGAAGCGTATCATCATCAACTC CACAGAGCTTACACCAAACGTACC	183	65	Makino *et al* (2003)
*vcrD*2	T3SS2-structure VcrD2	vcrD2(F) vcrD2(R)	GGTAACACTGCCTGGTGTGGTCATCG GTCTCTCAAAGTCTTCAAACTCACCTGC	196	55	Okada *et al* (2009)
vopC	T3SS2-effector VopC	VPA1321F VPA1321R	GGTTAGTGAATCCAACCAAACCGC TTGCCGTGCATGTCATACAACCAG	485	55	Makino *et al* (2003)
*vipA*1	T6SS1-structure VipA1	vipA1F vipA1R	CACGTGACGGCTCGGTGG CTCTTCTTTCGCGTCTTGGTCG	500	60	Salomon *et al* (2013)
*vipA*2	T6SS2-structure VipA2	vipA2F vipA2R	CGAGTATCCACTCGAAACTTTC TTCTGCTCCCTCAGTACTTTCTG	524	58	Salomon *et al* (2013)

MA), and 1X Thermopol buffer (NEB) was subjected to thermocycling using a PCR T100 thermocycler (BioRad, Hercules, CA) as follows: 96°C for 5 minutes; for *toxR*: 35 cycles of 94°C for 1 minute, 63°C for 1.5 minutes and 72°C for 1.5 minutes; for *toxRS*: 25 cycles of 96°C for 1 minute, 45°C for 2 minutes and 72°C for 3 minutes; for *tdh* and *trh*: 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute; all followed by a final heating at 72°C for 7 minutes. Amplicons were analyzed by 1% agarose gel-electrophoresis and ethidium bromide staining.

O (somatic) and K (capsular) serotypes were determined by a slide agglutination test using commercial anti-O and anti-K antibodies (Denka Seiken, Tokyo, Japan). *V. parahaemoltyicus* isolates were stored at -80°C for further analysis.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out using standard disk diffusion method on Mueller-Hilton agar (Difco, Sparks, MD) plate according to guidelines of the Clinical Laboratory and Standards Institute (CLSI, 2010; CLSI, 2017). Antimicrobial disks (Oxoid, Hampshire, UK) contained ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), norfloxacin (10 μg), and tetracyclin (30 μg). The data were interpreted as sensitive (S), intermediate (I) or resistant (R) following CLSI (2017) criteria.

Detection of *V. parahaemolyticus* secretion genes

Chromosomal DNA of pandemic *V. parahaemolyticus* was extracted by a boiling method (Thaithongnum *et al*, 2006). Detection of T3SSs and T6SSs genes (*vcrD1*, *vcrD2*, *vopC*, *vopQ*, *vipA1*, and *vipA2*) was performed by a PCR method as previously described (Laohaprertthisan *et al*, 2003; Makino *et al*, 2003; Yu *et al*, 2003; Okada *et al*, 2009) using primers listed in Table 1 and amplification conditions as described above.

Detection of *V. parahaemolyticus* TDH

Pandemic *V. parahaemolyticus* isolates were cultured on tryptic soy agar (TSA) (Difco, Detroit, MI) supplemented with 1% NaCl at 37°C for 18 hours. Then, a single colony was spotted onto Wagatsuma blood agar prepared as previously described (Hara-Kudo *et al*, 2003) and incubated at 37°C for 18-35 hours. TDH production was detected by presence of a clear zone around the colony.

Detection of *V. parahaemolyticus* swarming and twitching motilities

Swarming motility assay was performed on a swarming plate prepared by adding 6 g of Bacto agar (Difco) to 1,000 ml of Luria Bertani (LB) medium (Difco). In short, pandemic *V. parahaemolyticus* isolate was cultured in LB broth (Difco) at 37°C for 18 hours, then bacteria concentration was adjusted to 0.5 McFarland unit. A 2 μl aliquot of bacteria culture was added onto the swarming plate and diameter of swarm zone was measured after incubation at 37°C for 4 and 8 hours (Inoue *et al*, 2007).

Twitching motility assay was carried out on LB agar (Difco) supplemented with 1.5% NaCl. In brief, pandemic *V. parahaemolyticus* isolate was cultured on TSA (Difco) supplemented with 1% NaCl at 37°C for 18 hours. Then a single colony was inoculated into an LB agar (Difco) supplemented with 1.5% NaCl and incubation at 37°C for 24 hours. The agar layer was removed and twitching zones were visualized at the agar plate interface by staining the zone with 0.1% (w/v) of crystal violet. The diameter of twitch zone was measured from the point of inocula-

tion (Antunes *et al*, 2011).

Detection of *V. parahaemolyticus* iron acquisition

Iron acquisition assay was performed as previously described (Wong *et al*, 1996; Almeida *et al*, 2008) with minor modifications. In short, 2 μl aliquots of hemoglobin (50 μM and 155 μM) were spotted on melted rich medium (1% peptone, 3% NaCl, 0.5% Na_2HPO_4, 0.5% glucose, and 1.5% agar) supplemented with 150 μM 2, 2′-dipyridyl to chelate any exogenous iron in the medium. After incubation for 1 hour, 2 μl aliquot of *V. parahaemolyticus* suspension was placed onto the medium and incubated at 37°C overnight. Growth of bacteria was determined directly by eye and under a light microscope (4x magnification).

RESULTS

Clinical pandemic *V. parahaemolyticus* isolates

During 2001-2016, 57 clinical pandemic *V. parahaemolyticus* isolates were collected from diarrheal patients at Hat Yai Hospital, Songkhla (Table 2).

Antibiogram profiles

Using a disc diffusion assay, *V. parahaemolyticus* isolates showed resistance to ampicillin (100%), followed by ciprofloxacin (12%) and norfloxacin (2%) (Table 3). Intermediate susceptibility to chloramphenicol, ciprofloxacin, cotrimoxazole, and norfloxacin was observed in 2 (4%), 38 (67%), 1 (2%) and 10 (18%) isolates, respectively. Forty (70%) isolates were susceptible to all six antimicrobials tested except ampicillin. Interestingly, one isolate possessed resistance to ampicillin, ciprofloxacin and norfloxacin.

Presence of secretion genes

Gene of T3SS1 effector (*vopQ*) and structural (*vcrD1)* protein was identified in 100% and 93%, respectively of isolates, and that of T3SS2 effector (*vopC*) and structural (*vcrD2*) protein in 96% and 93%, respectively (Table 2). All four T3SS genes were present in 84% of the isolates, both T3SS1 genes in 93% and both T3SS2 genes in 91%. Two T6SS genes were identified in 74% of the isolates.

Virulence-related characteristics

All *V. parahaemolyticus* isolates were positive for the Kanagawa phenomenon, *ie* TDH production and manifested twitching motility (zone diameter ranging from 4 to 52 mm), while 89% possessed swarming motility (diameter of migration zone ranging from 5 to 49 mm) (Table 4). Forty-three (81%) isolates were able to grow on iron-depleted medium supplemented with 155 μM hemoglobin (Fig 1) and 68% in the presence of 50 μM hemoglobin (Table 4). Interestingly, 37 *V. parahaemolyticus* isolates were positive for all tested virulence-related characteristics including Kanagawa phenomenon, motility phenotype and iron-dependent growth.

DISCUSSION

Diarrheal disease remains one of the most important health problems worldwide. Infections by *V. parahaemolyticus* are commonly reported in several areas, especially in Southeast Asia (Ottaviani *et al*, 2010; Bureau of Epidemiology, 2015; Li *et al*, 2015). Treatment with antimicrobial can reduce disease severity and symptom duration.

Our results show that all *V. parahaemolyticus* isolates were resistant to ampicillin, consistent with results reported in China, India, Indonesia, and Mexico where 100%, 87%, 98%, and 94% of the isolates from clinical samples are resistant (Pazhani *et al*, 2014; de Jesús Hernández-Díaz

Table 2
Characteristics of clinical *Vibrio parahaemolyticus* isolates from Hat Yai Hospital, Songkhla, Thailand collected during 2001 - 2016.

Year of collection	Isolate	Serotype	Virulence gene			Secretion gene						Antibiotic resistance
						T3SS1		T3SS2		T6SS1	T6SS2	
			toxRS	*tdh*	*trh*	*vcrD1*	*vopQ*	*vcrD2*	*vopC*	*vipA1*	*vipA2*	
2001	PSU 1	O1:KUT	+	+	-	+	+	+	+	+	-	AMP, CIP
2002	PSU 2	O1:KUT	+	+	-	-	+	+	+	+	+	AMP
	PSU 3	O1:KUT	+	+	-	-	+	+	+	+	+	AMP
	PSU 4	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 5	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 6	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
2003	PSU 7	O1:KUT	+	+	-	+	+	+	+	+	+	AMP, CIP
	PSU 8	O1:KUT	+	+	-	+	+	-	+	+	+	AMP
	PSU 33	O1:K25	+	+	-	+	+	-	+	+	-	AMP, CIP
	PSU 34	O1:K25	+	+	-	+	+	+	+	+	+	AMP, CIP
2004	PSU 9	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 10	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 11	O1:KUT	+	+	-	+	+	+	+	-	-	AMP
	PSU 12	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
2005	PSU 13	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 14	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
	PSU 15	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
2006	PSU 16	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
	PSU 17	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
	PSU 35	O1:K25	+	+	-	+	+	+	+	+	-	AMP
2008	PSU 18	O1:KUT	+	+	-	+	+	+	+	+	-	AMP
	PSU 19	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 36	O1:K25	+	+	-	+	+	-	+	-	-	AMP

Table 2 (Continued)

Year of collection	Isolate	Serotype	Virulence gene			Secretion gene						Antibiotic resistance
						T3SS1		T3SS2		T6SS1	T6SS2	
			toxRS	*tdh*	*trh*	*vcrD1*	*vopQ*	*vcrD2*	*vopC*	*vipA1*	*vipA2*	
	PSU 37	O1:K25	+	+	-	+	+	+	+	+	+	AMP
	PSU 38	O1:K25	+	+	-	+	+	+	+	+	+	AMP
	PSU 39	O1:K25	+	+	-	+	+	+	+	+	+	AMP, CIP, NOR
	PSU 40	O1:K25	+	+	-	+	+	+	+	+	+	AMP
	PSU 41	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 42	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 43	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 44	O3:K6	+	+	-	+	+	+	+	+	+	AMP, CIP
	PSU 45	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 46	O3:K6	+	+	-	+	+	+	+	+	+	AMP, CIP
2009	PSU 20	O1:KUT	+	+	-	+	+	+	+	-	+	AMP
	PSU 21	O1:KUT	+	+	-	+	+	+	-	+	+	AMP
	PSU 22	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 23	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 24	O1:KUT	+	+	-	+	+	-	-	-	+	AMP
	PSU 25	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 26	O1:KUT	+	+	-	+	+	+	+	-	+	AMP
2011	PSU 27	O1:KUT	+	+	-	-	+	+	+	+	+	AMP
	PSU 28	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 29	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
	PSU 30	O1:KUT	+	+	-	-	+	+	+	+	-	AMP
	PSU 31	O1:KUT	+	+	-	+	+	+	+	+	+	AMP

Table 2 (Continued)

Year of collection	Isolate	Serotype	Virulence gene			Secretion gene						Antibiotic resistance
						T3SS1		T3SS2		T6SS1	T6SS2	
			toxRS	*tdh*	*trh*	*vcrD1*	*vopQ*	*vcrD2*	*vopC*	*vipA1*	*vipA2*	
2012	PSU 32	O1:KUT	+	+	-	+	+	+	+	+	+	AMP
2013	PSU 47	O3:K6	+	+	-	+	+	+	+	+	+	AMP
2014	PSU 48	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 49	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 50	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 51	O3:K6	+	+	-	+	+	+	+	+	+	AMP
2016	PSU 52	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 53	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 54	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 55	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 56	O3:K6	+	+	-	+	+	+	+	+	+	AMP
	PSU 57	O3:K6	+	+	-	+	+	+	+	+	+	AMP
		Prevalence (%)				93	100	93	96	91	79	

+, present; -, absent. AMP, ampicillin; CIP, ciprofloxacin; NOR, norfloxacin.

et al, 2015; Chen *et al*, 2016). As resistance to ampicillin has become widespread, fluoroquinolones, such as ciprofloxacin and norfloxacin, have been the first-line drug for initial treatment of acute diarrhea in adult patients (Riddle *et al*, 2016). In Thailand, fluoroquinolone is the most commonly prescribed antibiotic for treatment of acute diarrhea in adults (Supcharassaeng and Suankratay, 2011). In our study, intermediate resistance of clinical *V. parahaemolyticus* isolates to the fluoroquinolones were present in over 50% and (of concern) complete resistance in 12% of the samples. Ciprofloxacin-resistant *V. parahaemolyticus* isolates (11%) have been reported in Indonesia since 2001 (Tjaniadi *et al*, 2003). Not only in *V. parahaemolyticus* but resistance to ciprofloxacin and other fluoroquinolones was present in *Campylobacter* spp isolated from patients with diarrhea in Thailand and Indonesia (Kuschner *et al*, 1995; Tjaniadi *et al*, 2003). Careful surveillance and appropriate use of antimicrobial

Table 3
Antibiogram profile of clinical *Vibrio parahaemolyticus* isolates from Hat Yai Hospital, Songkhla, Thailand collected during 2001 - 2016.

Antimicrobial agent	*V. parahaemolyticus* isolates (*n* = 57)		
	Resistant[a] *n* (%)	Intermediate resistant[a] *n* (%)	Sensitive[a] *n* (%)
Ampicillin	57 (100)	-	-
Chloramphenicol	-	2 (4)	55 (96)
Ciprofloxacin	7 (12)	38 (67)	12 (21)
Cotrimoxazole	-	2 (4)	55 (96)
Norfloxacin	1 (2)	10 (18)	46 (80)
Tetracycline	-	-	57 (100)

[a]Based on CLSI (2017).

agents are required to preventing emergence of drug-resistant bacteria. The increase in resistance among pathogens might be due to overuse of antibiotics.

T3SSs and T6SSs are believed to be responsible for pathogenicity of *V. parahaemolyticus* (Ceccarelli *et al*, 2013; Zhang and Orth, 2013). All *V. parahaemolyticus* isolates in this study carried at least one gene encoding for T3SS. T3SS1 and T3SS2 genes are detected by microarray hybridization in all pandemic *V. parahaemolyticus* isolates (Meador *et al*, 2007). In this study, the prevalence of T3SS genes *vcrD1*, *vcrD2*, *vopC*, and *vopQ* were >90%. T6SS genes were detected at lower frequency than those of T3SS. An earlier study described all clinical isolates possess T3SS1, whereas 84% and 92% of clinical isolates were positive for T6SS1 and T6SS2, respectively, but no information regarding the pandemic genetic background (GS-PCR, tdh^+, trh^-) is provided (Kongrueng *et al*, 2015). Further studies are needed to examine the prevalence of other genes in the T6SS group.

Production of TDH (Kanagawa phenomenon-positive) among clinical *V. parahaemolyticus* isolates and its relationship with *V. parahaemolyticus* pathogenicity has been reported (Ceccarelli *et al*, 2013). This phenomenon was previously shown to be strongly associated with tdh^+ trh^- *V. parahaemolyticus* isolates (Suzuki *et al*, 1997). In the current study all tdh^+ trh^- *V. parahaemolyticus* isolates were TDH producers of similar levels.

V. parahaemolyticus isolates demonstrated variations in swarming and twitching motilities, but all isolates possessed at least a swarming or twitching motility. *V. parahaemolyticus* can be motile by several ways, depending on the environment (McCarter, 1999; Kim and McCarter, 2000; Broberg *et al*, 2011) and also on the presence of number of genetic regulatory mechanisms, motility proteins, and surface sensors (Kim and McCarter, 2000; Mattick, 2002; Wang *et al*, 2013a). The inability to swarm observed in six isolates might be due to defects in cell division during the swarm cell cycle, lateral flagella formation or ability to produce auto

Table 4
Clinical *Vibrio parahaemolyticus* isolates from Hat Yai Hospital, Songkhla, Thailand collected during 2001 - 2016 grouped according to virulence-related phenotypes.

Group	Number of isolates	Isolate ID	Serotype	Kanagawa phenomenon[a]	Motility phenotype		Iron-dependent growth[d]	
					Swarming[b]	Twitching[c]	155 μM hemoglobin	50 μM hemoglobin
1	2	PSU 40, 44	O3:K6	+	+	++	+	+
2	3	PSU 45, 46	O3:K6	+	+	++	+	-
		PSU 28	O1:KUT					
3	7	PSU 43, 53	O3:K6	+	+	++	+	+
		PSU 4, 10, 11, 12, 22	O1:KUT					
4	9	PSU 47, 48, 49, 51, 52, 54, 55, 57	O3:K6	+	+	++	-	-
		PSU 21	O1:KUT					
5	28	PSU 41, 42	O3:K6	+	+	+	+	+
		PSU 33, 34,36,37,38 39	O1:K25					
		PSU 3, 5, 6, 7, 8, 9,15,16, 17, 18, 19, 20, 23, 25, 26, 27, 29, 30, 31, 32	O1:KUT					
6	2	PSU 35	O1:K25	+	+	+	+	-
		PSU 1	O1:KUT					
7	2	PSU 2, 14	O1:KUT	+	-	++	+	-
8	2	PSU 50, 56	O3:K6	+	-	++	-	-
9	1	PSU 13	O1:KUT	+	-	++	+	+
10	1	PSU 24	O1:KUT	+	-	+	+	+

[a]+, positive; -, negative. [b]+, swarm zone >5 mm ; -, no swarm zone. [c]+, twitch zone >4 mm (above mean value); ++, twitch zone >13mm (above mean value); -, no twitch zone. [d]+, growth; -, no growth.

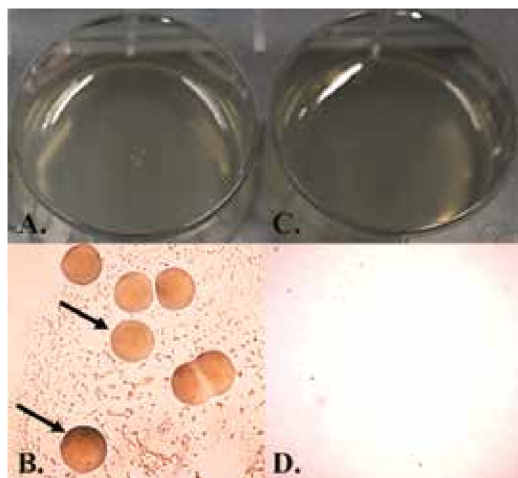

Fig 1-Growth of clinical *Vibrio parahaemolyticus* isolates on melted rich medium supplemented with 150 μM 2, 2′-dipyridyl and 155 μM hemoglobin. A). 1x magnification. B). 4x magnification. C). Negative control, 1x magnification. D). Negative control, 4x magnification. Arrow indicates *V. parahaemolyticus* colony.

inducers (McCarter, 1999).

Iron is essential for almost all bacteria including *V. parahaemolyticus*. During infection, *V. parahaemolyticus* utilizes siderophores for uptake of iron during growth in iron-limited environment (León-Sicairos *et al*, 2015). Isolates were examined for ability to utilize iron from hemoglobin on iron-limited agar plate. A minority of *V. parahaemolyticus* isolates were unable to grow when tested with 50 μM hemoglobin in agreement with a previous report (Wong *et al*, 1996) suggesting these isolates might possess low virulence in humans where "free" iron is limited. In this study, 65% of isolates were able to grow at low iron concentration with swarming and twitching motility. These phenotypes could help *V. parahaemolyticus* to survive under stress condition, especially during infection.

In summary, the study reveals the majority of clinical *V. parahaemolyticus* isolates collected from a hospital in southern Thailand over a 16-year period had pandemic properties; all isolates were resistant to ampicillin and over 50% partially or completely resistant to the first-line drugs, fluoroquinolones; all isolates possessed virulence characteristics, such as secretion, twitching and/or swarming motility and iron acquisition, as well as genes required for hemolytic activity. The antibiogram profiles identified in these clinical isolates are of importance for both clinical and epidemiological purposes. Moreover, data from these investigations should be of assistance in furthering our understanding of virulence properties and underlying mechanisms of this pathogenic organism.

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