A decrease in proportion of infections by pandemic *Vibrio parahaemolyticus* in Hat Yai Hospital, southern Thailand

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ABSTRACT

Since infection by the pandemic clone of *Vibrio parahaemolyticus* has been prevalent in southern Thailand, we have been actively surveying the incidence of *Vibrio parahaemolyticus* infections in this area. A total of 865 isolates of *V. parahaemolyticus* were obtained from patients at Hat Yai Hospital, the main public hospital in Songkhla Province, Thailand during 2000-2005. The isolates were examined by GS-PCR, specific to pandemic clone, and for the presence of two major virulence genes, *tdh* and *trh*, and O:K serotype. Representative isolates were also examined for antibiogram and the DNA fingerprint using an arbitrarily primed PCR method to better understand clonal relationships between isolates. The total number of isolates was less in 2000 and more in 2004 and 2005 than those in the 2001-2003 periods. The increase in the numbers of infections in 2004 and 2005 was not due to the emergence of a particular clone having unique characteristics but rather likely due to climate change. From 2000-2003, the percentages of pandemic strains of *V. parahaemolyticus* defined as GS-PCR-positive tdh^+ trh^- strains was stable at 64.1%, 67.5%, 69.7%, and 67.7% of the total isolates each year, respectively. However, in 2004 and 2005, the percentages decreased to 56.1% and 55.5%, respectively. The O:K serotypes of the pandemic isolates remained unchanged. The proportional decrease in infections caused by the pandemic strains are likely due to the population in this area gradually developing immunity to the pandemic clone while continuing to be susceptible to other strains.

INTRODUCTION

V. parahaemolyticus infections cause acute, self-limited gastroenteritis, typically characterized by diarrhea, abdominal cramps, nausea, vomiting, fever, and chills lasting 1-3 days, the onset usually occurs within 24 hours after eating contaminated food. Cases are most commonly reported during the warmer months and are often associated with eating raw or undercooked shellfish or other cooked foods that have been cross-contaminated with raw shellfish (Yeung and Boor, 2004). Not all strains of *V. parahaemolyticus* are considered pathogenic. Most clinical isolates exhibit the Kanagawa phenomenon (KP) (Nishibuchi and Kaper, 1995). KP-positive strains cause β-hemolysis, which is induced by a thermostable direct hemolysin (TDH) in Wagatsuma agar. TDH is encoded by the *tdh* gene. Some KP-negative clinical isolates carry the *trh* gene, encoding a TDH-related hemolysin (TRH). The *trh* gene sequence varies from strain to strain, and can be clustered into two subgroups, *trh1* and *trh2* (Kishishita *et al.*, 1992). Molecular epidemiological studies have shown that clinical isolates possess the *tdh* gene, *trh* gene, or both genes; but environmental isolates rarely carry these genes (Shirai *et al.*, 1990). Isolates lacking both *tdh* and *trh* genes have also been isolated from clinical specimens and possible explanations for their isolation were presented (Bhoopong *et al.*, 2007). Since 1996, the *V. parahaemolyticus* O3:K6 serotype carrying the *tdh* gene was confirmed as being responsible for infections in many Asian countries, Europe, and the United States (Okuda *et al.*, 1997a, Matsumoto *et al.*, 2000). These strains are now considered to be pandemic strains. A molecular typing technique named Group-specific PCR (GS-PCR) can detect nucleotide variations within the 1364-bp *toxRS* region that are unique to the pandemic clone (Matsumoto *et al.*, 2000). The use of GS-PCR on recent clinical isolates has shown that some GS-PCR-

positive isolates belong to non-O3:K6 serotype: O4:K68, O1:KUT, O1:K25, and others It has been reported that these serotypes probably originate from the same clone as O3:K6 (Bhuiyan *et al.*, 2002; Chowdhury *et al.*, 2000; Chowdhury *et al.*, 2004; Matsumoto *et al.*, 2000).

Since the emergence of the pandemic strains, a surveillance program on *V. parahaemolyticus* has been operating in the southern part of Thailand. In 1998, 87% of 23 isolates from Hat Yai Hospital, the main public hospital located in Songkhla Province, southern Thailand, were pandemic strains (Vuddhakul *et al.*, 2000). In 1999, 76% of 317 isolates from Hat Yai Hospital and Songklanagarind Hospital, a university hospital in Songkhla Province, were pandemic strains (Laohaprertthisan *et al.*, 2003). In this study an investigation of *V. parahaemolyticus* isolates was carried out at Hat Yai Hospital from 2000-2005. We examined isolated strains for GS-PCR reaction, toxin gene profiles, the O:K serotype, antibiogram, and other features. A noteworthy finding is a significant decrease in the percentage of pandemic strains in 2004 and 2005. We discuss a possible reason based on the characteristics of the isolated strains.

MATERIALS AND METHODS

Isolation and identification of bacterial strains. Stool samples were collected from patients presenting with diarrhea at Hat Yai Hospital between 2000 and 2005. The samples were plated on MacConkey, Salmonella-Shigella and thiosulfate-citrate-bile salt-sucrose agar (TCBS). After overnight incubation at 37°C, samples showing growth predominantly on TCBS were selected. Non-sucrose fermenting colonies were examined by standard biochemical tests for identification as *V. parahaemolyticus*. In addition, the identification was confirmed by PCR targeted to the *toxR* gene (Kim *et al.*, 1999). Boiled broth cultures of *V. parahaemolyticus* were used as the source of DNA template for all PCR assays described below.

Detection of *tdh* and *trh* genes. The presence of *tdh* and *trh* in each isolate was determined by PCR. Primer pairs D3 and D5, R2 and R6 were used to investigate *tdh* and *trh*, respectively, as described previously (Tada *et al.*, 1992).

GS-PCR. GS-PCR to identify pandemic strains was carried out using the technique described by Matsumoto *et al.* (2000).

Determination of O:K serotypes. The O (somatic) and K (capsular) serotypes of isolated strains were determined by agglutination using commercial anti-O and anti-K antisera (Denka Seiken) according to the instructions described by the manufacturer.

Antibiotic susceptibility tests. Susceptibility to antibiotics was examined using the disk diffusion method (National Committee for Clinical Laboratory Standards, 2000). Antibiotic-loaded paper disks were dispensed on Mueller-Hinton agar plates inoculated with a bacterial lawn. After incubation at 37°C for 14 to 18 h, the diameter of the inhibition zone was recorded and interpreted according to the

reference provided by the manufacturer. Seven antibiotic disks were used, ampicillin (10 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (TMP/SMX) (1.25 μg), chloramphenicol (30 μg), tetracycline (30 μg), norfloxacin (10 μg), and azithromycin (15 μg). *Escherichia coli* ATCC 25922 was used as a standard strain.

***trh* subgroup investigation.** Genomic DNA from *V. parahaemolyticus* was digested with *Hind* III restriction enzyme. The *trh* subgroup was detected by Southern blot hybridization. using the digoxigenin-labeled *trh1* and *trh2* probes as described previously (Bhoopong *et al.*, 2007). Hybridization was carried out under high-stringency conditions at 30°C. The hybridized probes were detected using a DNA detection kit (Roche Diagnostics) according to the manufacturer's instructions.

AP-PCR. DNA was extracted using a standard phenol-chloroform extraction method (Sambrook *et al.*, 2001). AP-PCR was carried out using primer 2 (5'-GTTTCGCTCC-3') and primer 4 (5'-AAGAGCCCGT-3') as described previously (Matsumoto *et al.*, 2000).

Statistical analysis. Pearson's chi-square was used to evaluate significant differences in the results.

Toxin gene profiles and GS-PCR results

A total of 865 isolates of *V. parahaemolyticus* were obtained from stool specimens sent to Hat Yai Hospital from 2000 to 2005. The total number of *V. parahaemolyticus* infection was less in 2000 and more in 2004 and 2005 than those in the 2001-2003 periods (Table 1). We classified the isolates into four groups based on the presence or absence of the two virulence genes: *tdh*$^+$ *trh*$^-$, *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$. All isolates were also examined for their GS-PCR reaction. GS-PCR-positive isolates were detected only in the *tdh*$^+$ *trh*$^-$ group. The *tdh*$^+$ *trh*$^-$ group was therefore divided into two subgroups (Table 1). The most prevalent isolates detected in every year belonged to the *tdh*$^+$ *trh*$^-$ group. They totaled 719 isolates in the six years. Within this group, 74.7% (537 isolates) were GS-PCR positive, which we define as pandemic strains in this study (Table 1). Although the highest percentage of pandemic strains was detected in 2002, there was no significant difference in the percentage of pandemic isolates during 2000-2003 (64.1%, 67.5%, 69.7%, and 67.7% of the total isolates during each consecutive year, respectively. Although the total numbers of the GS-PCR positive isolates were higher in 2004 and 2005 than in 2003, the percentage of GS-PCR positive strains significantly decreased by 11.6% and 12.2% in 2004 and 2005, respectively, compared to 2003. On the other hand, the percentage of non-pandemic isolates except for the *tdh*$^-$ *trh*$^+$ group increased. These include the strains belonging to the three groups: GS-PCR-negative *tdh*$^+$ *trh*$^-$, *tdh*$^+$ *trh*$^+$, and *tdh*$^-$ *trh*$^-$ groups (Table 1).

O:K serotype

In all years, the pandemic isolates (*tdh*$^+$ *trh*$^-$, GS-PCR positive) were predominantly of the O3:K6 serotype (72.8% overall), followed by the O1:K25 and O4:K68 except that O4:K68 was not detected among the pandemic isolates in year

2002 (Table 2). The serotypes of the isolates belonging to the GS-PCR-negative *tdh*$^+$ *trh*$^-$, *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$ groups varied considerably (Table 3). Many of the isolates belonged to serotypes O4:K8, O3:K29, O4:K45 and O11:KUT. The isolates belonging to serotypes O3:K6 and O1:KUT were also encountered in this non-pandemic group but their AP-PCR profiles were different from those of O3:K6 and O1:KUT isolates in the pandemic group (data not shown). Of interest, was that the K untypeable strains accounted for only 4.5% and 11.0% of the total isolates in the pandemic isolates and GS-PCR-negative *tdh*$^+$ *trh*$^-$ isolates, respectively, whereas K untypeable strains were detected in 58.2%, 73.3%, and 68.7% of the isolates belonging to the *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$ groups, respectively (Fig. 1).

Antibiogram

One hundread and eighty nine isolates randomly selected from five genotype groups were tested for sensitivity to seven commonly used antibiotics. All isolates were susceptible to four of the antibiotics tested: chloramphenicol, tetracycline, norfloxacin and azithromycin (data not shown). Almost all isolates except for two isolates in the *tdh*$^-$ *trh*$^-$ group were resistant to ampicillin (Table 4). The antibiograms of the isolates in the *tdh*$^+$ *trh*$^-$ group were similar regardless of the GS-PCR result but were different from those of the *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$ groups regarding resistance to ciprofloxacin and TMP/SMX. Thirty four to 43.8% of the isolates of the *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$ groups were resistant to ciprofloxacin whereas approximately 81% of *tdh*$^+$ *trh*$^-$ isolates were resistant to this antibiotic. Significantly more isolates in the *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$, and *tdh*$^-$ *trh*$^+$ groups were susceptible to TMP/SMX than from the *tdh*$^+$ *trh*$^-$ isolates.

The *trh* subgroups

To examine if the *trh* subgroups differed between the *tdh*$^+$ *trh*$^+$ and *tdh*$^-$ *trh*$^+$ groups, the *trh* subgroup was determined for 20 of the 55 *tdh*$^+$ *trh*$^+$ isolates and 12 of the 16 *tdh*$^-$ *trh*$^+$ isolates. The *trh1* gene predominated among the *tdh*$^+$ *trh*$^+$ isolates (90.0%), whereas the *trh2* gene predominated among the *tdh*$^-$ *trh*$^+$ isolates (66.7%).

AP-PCR analysis

To investigate whether infections due to the non-pandemic isolates belonging to GS-PCR-negative *tdh*$^+$ *trh*$^-$, *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$ and *tdh*$^-$ *trh*$^+$ isolates were caused by a specific clone in each group, DNA fingerprints of 139 randomly selected isolates obtained during 2000-2005 were examined using the AP-PCR technique. Except for the *tdh*$^-$ *trh*$^+$ isolates in which all those tested displayed non-identical AP-PCR profiles (data not shown), isolates from any year with the same serotype within each group mostly produced identical AP-PCR profiles; two patterns were obtained for *tdh*$^+$ *trh*$^-$ O4:K8, GS-PCR-negative, the first pattern contained 9 isolates which gave identical patterns for both primers (Fig. 2, Panels A1 and A2, lanes 2 -10), the second pattern contained 2 isolates which gave identical patterns for both primers (Fig. 2, Panels A1 and A2, lanes 13 -14). For the 10 O1:KUT isolates from the *tdh*$^+$ *trh*$^+$ group 8 gave identical patterns (Fig. 2, Panels B1 and B2, lanes 3 -8, 10, and 11), and 3 of the 12 O11:KUT isolates in the *tdh*$^-$ *trh*$^-$ group gave identical patterns (Fig. 2, Panels C1 and C2, lanes 9 -11).

DISCUSSION

In this study, isolates of *V. parahaemolyticus* from the same hospital were investigated continuously for 6 years. The total number of *V. parahaemolyticus* infections was higher in years 2004 and 2005 than in other years. We do not know the exact reason for this increase. The number of isolates belonging to four genotype groups increased except those belonging to the *tdh*$^-$ *trh*$^+$ group (Table 1). Based on the following results the increase in each of the four groups is not accounted by the emergence of a new dominant clone. Analysis of the antibiogram (Table 4) did not distinguish between isolates, but the use of serotypes and DNA fingerprints proved to be very useful. The serotypes of the GS-PCR-positive *tdh*$^+$ *trh*$^-$ isolates remained unchanged at predominantly O3:K6 over the six year period (Table 2). For any of the other groups serotypes varied considerably (Table 3) and not all the isolates with the same serotypes had the same DNA fingerprints (Fig. 2). In each of the GS-PCR-negative *tdh*$^+$ *trh*$^-$, *tdh*$^+$ *trh*$^+$, and *tdh*$^-$ *trh*$^-$ groups; isolates with the same DNA fingerprint patterns and serotypes persisted over the study period but dominance in only 2004 and 2005 was not observed. The increase in the number of the isolates is rather likely to be related with climate change. The bacteria belonging to the genus *Vibrio* are expected to propagate more at a higher temperature in their natural habitat, marine and estuarine environments. Increase in ambient temperature and the sea surface temperature of the coastal water is associated with increase in the infection by *Vibrio cholerae* (Colwell, 1996; Pascual *et al.*, 2002). It is probably true with *V. parahaemolyticus*. The average highest ambient temperatures around Songkhla during 2000-2005 periods were 36.1, 37.0, 36.6, 36.5, 37.3 and 36.8 °C respectively (WWW.songkhlamet.org). The relatively small number of infections in 2000 (Table 1) may be explained by the lowest temperature in this year. We speculate increases in the number of infections in 2001

and 2004 from previous years (Table 1) may have been stimulated by high temperatures (37.0 or more) in these years and the number of infections may have not decreased after increase since temperatures did not drop drastically after its increase. This change in the number of infections may have been mediated by the change in the number of *V. parahaemolyticus* in the environment, but there is no data to support this hypothesis.

The percentage of pandemic isolates (*tdh*$^+$ *trh*$^-$, GS-PCR positive) decreased considerably and that of non-pandemic isolates increased during the 2004-2005 period. The majority of the serotypes detected among the pandemic isolates were O3:K6, O1:K25, O1:KUT, and O4:K68 and this remained unchanged over the entire 6-year period (Table 2). On the other hand, the serotypes of the increased isolates of the non-pandemic isolates varied considerably (Table 3). This may be related to the immunity possibly acquired by the local people. The pandemic clone emerged around 1995 (Matsumoto *et al.*, 2000). Infection by *V. parahaemolyticus* is prevalent in southern Thailand where seafood is a very popular food, and previous studies (Vuddhakul *et al.*, 2000; Laohaprertthisan *et al.*, 2003; Bhoopong *et al.*, 2007) and this study has revealed that infection has been caused mostly by the pandemic clone at least since 1998 in this area. Infection by *V. parahaemolyticus* can induce a lipopolysaccharide (the O antigen)-specific immune response in patients (Qadri *et al.*, 2003). Frequent infections by the pandemic strains with limited O:K serotypes would induce immunity more frequently and specifically than do infrequent infections by non-pandemic strains. Individuals previously exposed to pandemic strains may develop immunity to them but continue to be susceptible to non-pandemic clones with different serotypes. Such populations may have gradually increased in the last decade. This phenomenon has been described for *V. cholerae*. In Thailand, people infected by *V. cholerae* O1 serotype Ogawa became infected by serotype Inaba after 7-8 years (Supawat and Huttayananont, 1997). The

same phenomenon occurred in India, *V. cholerae* O1 serotype Inaba was predominant until 1989 and was replaced by Ogawa. It reappeared again in almost 10 years later (Garg *et al.*, 2000). This is thought to be due to the development of the host immune response to the lipopolysaccharide O antigen (Sack and Miller, 1969; Gangarosa *et al.*, 1967; Sheehy *et al.*, 1966). It is likely that human infection with *V. parahaemolyticus* has similar features. Another possible explanation for the decrease in the percentage of pandemic isolate would be a decrease of the proportion of pandemic strains in their natural habitat caused by environmental changes such as climate change. Pandemic strains carry several unique DNA regions in the genome (Williams *et al.*, 2004; Okura *et al.*, 2005; Hurley *et al.*, 2006; Wang *et al.*, 2006). If any of these DNA regions are associated with survival or propagation of pandemic strains in their natural habitat, distribution of pandemic strains relative to non-pandemic strains may be influence by environmental changes. Surveys on specific immunity among local people and on the distribution of pandemic vs. non-pandemic strains in the environment are needed to examine the above possibilities.

The susceptibilities of our isolates to some of the antibiotics were partly different from the reports by other workers. Serichantalergs *et al.* (2007) reported that all *V. parahaemolyticus* collected from patients in Bangkok during 2001-2002 were susceptible to TMP/SMX and 52% of isolates were resistant to ampicillin. However, we found that 11.6% (22/189) of isolates we examined were resistant to TMP/SMX and most isolates were resistant to ampicillin. Our results are similar to those reported for pandemic and non-pandemic strains of *V. parahaemolyticus* by Wong *et al.* (2000). They characterized pandemic strains from Asia including Thailand and showed 97.4% and 100% of pandemic strains and non-pandemic strains respectively were resistant to ampicillin. Okuda *et al.* (1997b) reported that their pandemic and non-pandemic strains isolated between 1994 and 1996 in India

were sensitive to ciprofloxacin. However, 81.6% of the pandemic isolates were resistant to this antibiotic in our study. In this study, the *tdh*$^+$ *trh*$^+$ group was more sensitive to antibiotics than the *tdh*$^+$ *trh*$^-$ group; its antibiotic response pattern was more closely related to the *tdh*$^-$ *trh*$^-$ and *tdh*$^-$ *trh*$^+$ groups (Table 4). Although this may indicate that *tdh*$^+$ *trh*$^+$ and *tdh*$^-$ *trh*$^+$ groups have existed in similar ecological niches recently, the two groups seem to have different origins as the *tdh*$^+$ *trh*$^+$ strains carried predominantly the *trh1* gene (90.0% of total isolates) and the majority of the *tdh*$^-$ *trh*$^+$ isolates had the *trh2* gene (66.7% of total isolates). *V. parahaemolyticus* is distributed in marine and estuarine environments. It is important to study how this bacterium, particularly the *tdh*$^+$ *trh*$^-$ group including the pandemic strains, acquires resistance to antibiotics.

Since infections by strains belonging to the *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^+$, and *tdh*$^-$ *trh*$^-$ groups are much less frequent compared to the infections caused by *tdh*$^+$ *trh*$^-$ strains, there is not much information available on the properties of the strains of the former groups. We noticed that many of isolates of these groups could not be typed to existing K serogroups (KUT isolates in Fig. 1). A possible reason for detecting KUT isolates at high frequencies is that the O:K typing scheme was established by examining KP-positive strains that produce large amounts of TDH and were mostly clinical specimens isolated in Japan. Although *tdh*$^+$ *trh*$^+$ strains produce TDH, the amounts of TDH are small and these strains exhibit a KP-negative phenotype (Okitsu *et al.*, 1997). It is only recently that clinical strains possessing the *trh* gene have been included in the serotyping scheme. Therefore, not enough clinical strains belonging to *tdh*$^+$ *trh*$^+$ and *tdh*$^-$ *trh*$^+$ groups have so far been evaluated for adding to the serotyping scheme. The isolates belonging to *tdh*$^-$ *trh*$^-$ groups have been left outside the serotyping scheme even if they were isolated from clinical specimens.

In conclusion, a decrease in the percentage of *V. parahaemolyticus* infections by the pandemic isolates was observed towards the end of the 6-year

observance period. This is probably due to an acquirement of immunityof local people by continued exposure to pandemic strains. This phenomenon has not been previously reported for *V. parahaemolyticus* although it is known to occur with *V. cholerae*. Continued surveillance would confirm this hypothesis and it will be useful for the future control of infections by this pathogen.

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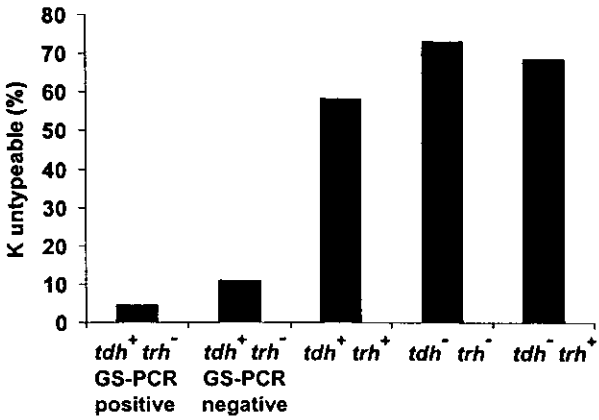

Fig. 1. The percentage of K untypeable strains detected in each group

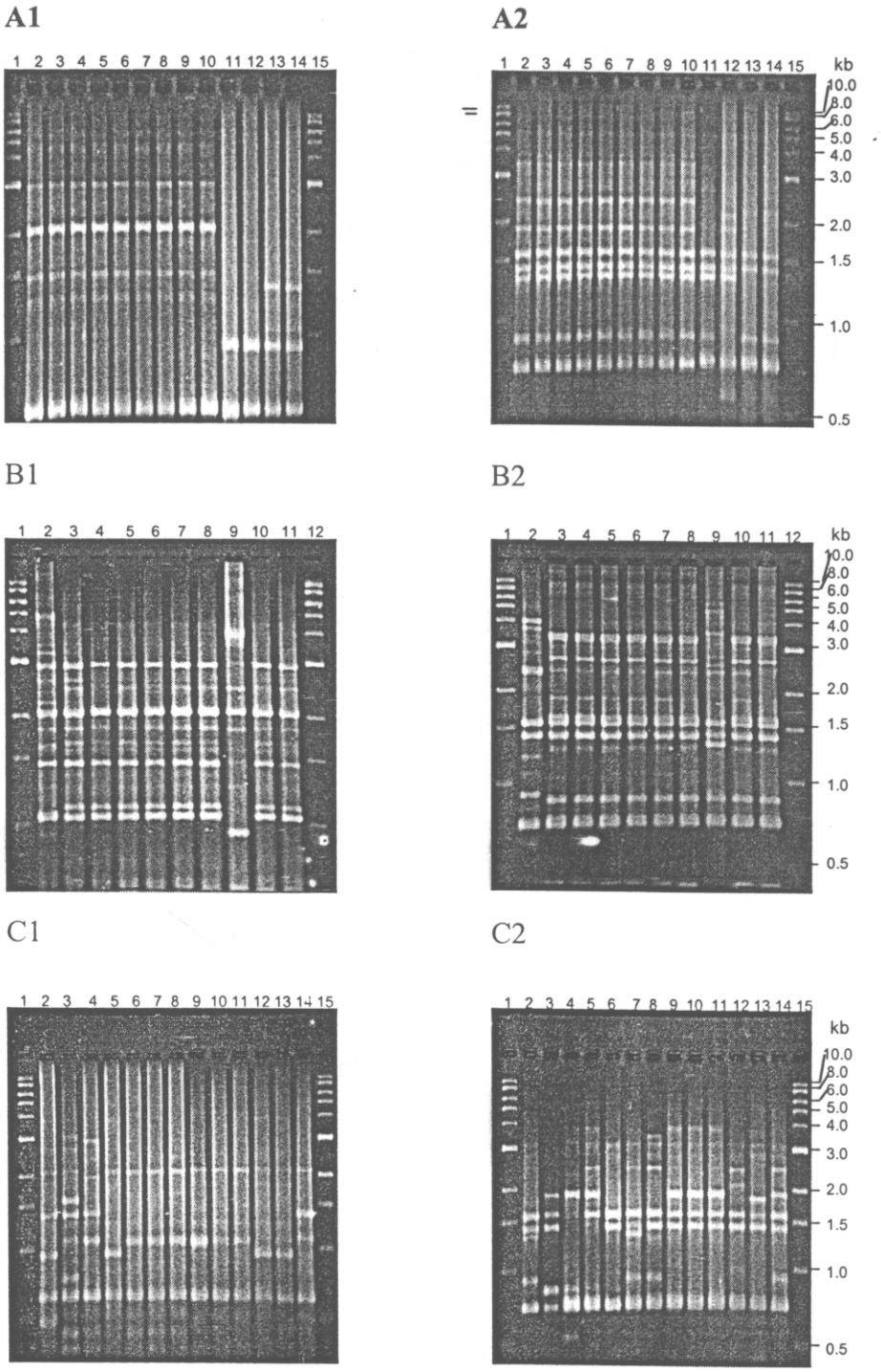

Fig. 2. AP-PCR profiles of *tdh*$^+$ *trh*$^-$ *V. parahaemolyticus* non-pandemic isolates (A1 and A2), *tdh*$^+$ *trh*$^+$ isolates (B1 and B2), and *tdh*$^-$ *trh*$^-$ isolates (C1 and C2) obtained from 2001 to 2005. The results were obtained with primer 2 (A1, B1, and C1) and primer 4 (A2, B2, and C2). For A1 and A2 lanes: 1 and 15, MW markers (1 kp DNA Ladder); lanes 2, 3, 4 (O4:K8), isolates in 2000, 2001, 2002 respectively; lane 5 to 6 (O4:K8), isolates in 2003; 7 to 8 (O4:K8), isolates in 2004; lane 9 to 14 (O4:K8), isolates in 2005. For B1 and B2 lanes: 1 and 12, MW markers; lanes 2, 3 (O1:KUT), isolates in 2001, 2002 respectively; lane 4 to 7 (O1:KUT), isolates in 2003; lane 8 to 11 (O1:KUT), isolates in 2005. For C1 and C2 Lanes: 1 and 15, MW markers; lanes 2, 3, 4, 5 (O11:KUT), isolates in 2000, 2001, 2002, 2003 respectively; lane 6 to 8 (O11:KUT), isolates in 2004; lane 9 to 14 (O11:KUT), isolates in 2005

Table 1. Characteristics of *V. parahaemolyticus* isolates from Hat Yai Hospital from 2000-2005

Year	No. of total isolates	*tdh*$^+$ *trh*$^-$ (%)		*tdh*$^+$ *trh*$^+$ (%)	*tdh*$^-$ *trh*$^-$ (%)	*tdh*$^-$ *trh*$^+$ (%)
		GS-PCR* positive	GS-PCR negative			
2000	64	41 (64.1)	12 (18.7)	0	8 (12.5)	3 (4.7)
2001	123	83 (67.5)	25 (20.3)	5 (4.1)	8 (6.5)	2 (1.6)
2002	142	99 (69.7)	18 (12.7)	8 (5.6)	14 (9.8)	3 (2.1)
2003	124	84 (67.7)†	25 (20.2)	6 (4.8)	7 (5.6)	2 (1.6)
2004	205	115 (56.1)‡	50 (24.4)	18 (8.8)	19 (9.3)	3 (1.5)
2005	207	115 (55.5)‡	52 (25.1)	17 (8.2)	20 (9.7)	3 (1.4)
Total	865	537	182	54	76	16

*Pandemic strains
†Significantly different from ‡ (P = 0.027)

Table 2. Serotypes of GS-PCR-positive *tdh*$^+$ *trh*$^-$ *V. parahaemolyticus* isolated between 2000 and 2005

Year	No. of isolates*	Serotype†
2000	30	O3:K6
	8	O1:K25
	2	O4:K68
	1	O2:K3
2001	63	O3:K6
	11	O1:K25
	8	O4:K68
	1	O1:KUT
2002	74	O3:K6
	18	O1:K25
	5	O1:KUT
	1	O3:K29, O1:K41
2003	60	O3:K6
	12	O1:K25
	6	O1:KUT
	3	O4:K68
	1	O3:K29, O5:KUT, R:KUT
2004	81	O3:K6
	22	O1:K25
	6	O4:K68
	4	O1:KUT
	1	O3:KUT, O3:K46
2005	83	O3:K6
	17	O1:K25
	9	O4:K68
	5	O1:KUT
	1	O4:K4

* No. of isolates per serotype.
† R, rough.

Table 3. Serotypes of *tdh*$^+$ *trh*$^-$ (non-pandemic), *tdh*$^+$ *trh*$^+$, *tdh*$^-$ *trh*$^-$ and *tdh*$^-$ *trh*$^+$ *V. parahaemolyticus* isolates during 2000-2005

Year	*tdh*$^+$ *trh*$^-$		*tdh*$^+$ *trh*$^+$		*tdh*$^-$ *trh*$^-$		*tdh*$^-$ *trh*$^+$	
	No. of isolates*	Serotype	No. of isolates	Serotype	No. of isolates	Serotype	No. of isolates	Serotype
2000	4	O4:K8	0	-	3	O12:KUT	2	O1:KUT
	3	O4:K9			1	O1:K69, O3:K6, O3:K54, O3:KUT, O11:KUT	1	OUT:KUT
	2	O4:KUT						
	1	O2:K3, O3:K6, O4:K13						
2001	11	O4:K8	2	O3:KUT	1	O1:K46, O1:K56, O1:KUT, O3:K6, O7:KUT, O8:KUT, O11:KUT, OUT:KUT	1	O1:KUT, O4:KUT
	2	O2:K3, O3:K6	1	O1:KUT, O11:K36, O12:KUT				
	1	O1:K56, O1:KUT, O2:K28, O3:K29, O4:K9, O4:KUT, O5:K17, O8:K41, O8:KUT, R:KUT						
2002	9	O3:K29	2	O12:KUT, O8:K74	2	O1:KUT, O10:KUT, O11:KUT, O5:KUT, OR:KUT	1	O1:K58, O1:KUT, O11:KUT
	2	O4:K4, O4:K8	1	O1:K69, O1:KUT, O4:KUT, O5:K15	1	O1:K56, O3:KUT, O8:KUT, O12:KUT		
	1	O1:K38, O3:K6, O3:K7, O5:K17, O8:KUT						
2003	14	O4:K8	4	O1:KUT	1	O1:K56, O3:KUT, O5:KUT, O10:KUT, O11:KUT, O12:KUT, R:KUT	1	O1:K48, O3:KUT
	3	O3:K29, O8:K41	2	O1:K69				
	1	O1:KUT, O3:K6						

		O4:K9, O4:KUT, O8:K21						
2004	17	O4:K8	4	O11:KUT	4	O11:KUT	1	O1:K56, O1:KUT, O3:KUT
	8	O4:K55	2	O3:K56, O5:K15, O5:KUT, O12:KUT	3	O5:KUT		
	7	O3:K29	1	O1:K9, O4:K67, O1:K69, O3:K6, O3:K72, O8:KUT	2	O2:K3, O5:K30, O8:KUT, O12:KUT		
	4	O8:K41			1	1:KUT, O3:KUT, O3:K54, O5:K17		
	2	O1:K56, O1:KUT, O8:K21						
	1	O2:K3, O3:K5, O3:K7, O3:KUT, O4:K9, O4:KUT, O5:K17, O5:KUT						
2005	15	O4:K55	4	O1:KUT, O11:KUT	9	O11:KUT	1	O1:KUT, O4:K49, O4:K55
	12	O4:K8	2	O3:K6, O4:KUT	2	O5:KUT		
	5	O2:K3	1	O1:K9, O4:K4, O4:K13, O5:KUT, O8:K20	1	O1:KUT, O2:KUT, O3:K57, O4:K4, O4:K13, O4:KUT, O10:K71, R:K34, OUT:KUT		
	4	O3:K29						
	3	O1:K25						
	2	O3:K7, O3:KUT, O4:K13						
	1	O1:K58, O1:KUT, O3:K5, O4:KUT, O5:KUT, OUT:KUT, O8:K21						

* No. of isolates per serotype

Table 4. Antibiogram patterns of *V. parahaemolyticus* isolates from 2000-2005

Antibiogram pattern *	*tdh*$^+$ *trh*$^-$ (%)		*tdh*$^+$ *trh*$^+$ (%)	*tdh*$^-$ *trh*$^-$ (%)	*tdh*$^-$ *trh*$^+$ (%)
	GS-PCR† positive	GS-PCR negative			
AMP	9 (18.4)	8 (18.6)	33 (66.0)	18 (58.1)	9 (56.2)
AMP CIP	28 (57.1)	28 (65.1)	14 (28.0)	11 (35.5)	7 (43.8)
AMP CIP TMP/SMX	12 (24.5)	7 (16.3)	2 (4.0)	0	0
AM TMP/SMX	0	0	1 (2.0)	0	0
Total	49 (100)	43 (100)	50 (100)	29‡ (93.6)	16 (100)

* The antibiotics examined were ampicillin (AMP), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (TMP/SMX), chloramphenicol, tetracycline, norfloxacin, and azithromycin.
Both resistant and intermediate reactions are judged as resistant in this study, and only these reactions are listed in these antibiograms.
† Pandemic strains
‡ Two more isolates in the *tdh*$^-$ *trh*$^-$ group were examined. They were susceptible to AMP and are not included in this table.