INTRODUCTION

Vibrio parahaemolyticus is a marine bacterium that causes seafood-borne gastroenteritis after consumption of contaminated raw, or partially cooked, fish or shellfish (29). The disease is often associated with abdominal cramps, nausea, vomiting, headache, occasional diarrhea, mild fever, and chills. The incubation period ranges from 4 to 96 h (3). Most clinical isolates of *V. parahaemolyticus* produce a major virulence factor, known as the thermostable direct hemolysin (TDH) (25), which is responsible for the hemolytic activity, called the Kanagawa phenomenon (KP), displayed on special blood agar (Wagatsuma agar). Another virulence factor, the TDH-related hemolysin (TRH), which is generally associated with some KP-negative strains of *V. parahaemolyticus*, is also involved in food-poisoning outbreaks (8, 10). TDH and TRH are encoded by the *tdh* and *trh* genes, respectively. The *trh* gene sequence can vary from strain to strain to some extent but *trh* gene sequences could be clustered into 2 subgroups, represented by *trh*1 and *trh*2 (12). Almost all clinical isolates carry either *tdh*, *trh*, or both genes, whereas only 1–2% of environmental isolates possess these genes (12, 23). Therefore, the strain carrying either *tdh*, *trh*, or both genes is considered virulent strains (17).

Since 1996, an increased incidence of gastroenteritis due to *V. parahaemolyticus* O3:K6 has been reported in many Asian countries (15), the United States (15), Europe (14), South America (7), and Africa (1). These O3:K6 strains carry the *tdh* gene, but not the *trh* gene, and show identical DNA fingerprints when examined by an arbitrarily primed-polymerase chain reaction (AP-PCR) method (15). Therefore, these strains are considered to be a pandemic clone. A molecular-typing method, known as group-specific PCR (GS-PCR), has detected nucleotide variations within the 1,364-bp *toxRS* region that are unique to the pandemic clone. Examination of recent clinical strains using this

method led to the finding of pandemic clones belonging to non-O3:K6 serotypes e.g., O1:K25, O4:K68 (2, 4, 15). These serotypes might have diverged from the O3:K6 clone (2, 15). In addition, one of the open reading frames (ORFs) of the f237 phage genome, ORF8, was proposed as a marker that might be useful for detection of the pandemic clone (9, 16). Many GS-PCR-positive non-O3:K6 strains carry ORF8, but ORF8 is considered to be less specific to the pandemic clone than the GS-PCR-positive reaction (2, 5). We define pandemic strains as those carrying the *tdh* gene, lacking the *trh* gene, and giving GS-PCR-positive reactions in this work.

Since the emergence of the pandemic strains, a surveillance program for *V. parahaemolyticus* has been established in the southern part of Thailand. Analysis of *V. parahaemolyticus* isolates collected from the Songklanagarind and Hat Yai hospitals during the period from December 1998 to December 1999 showed that 76–87% were pandemic strains, some of which belonged to non-O3:K6 serotypes (O1:K25, O1:K41, and O4:K12) (13, 27). The pandemic strains accounted for 64%, 68%, and 69% of the *V. parahaemolyticus* strains isolated at Hat Yai hospital in the years 2000, 2001, and 2002, respectively. During this 3-year surveillance period, we found that 10.9%, 6.5%, and 9.8% of *V. parahaemolyticus* isolates from this hospital carried neither the *tdh* nor the *trh* gene, respectively (V. Vuddhakul, unpublished data).

In routine clinical investigations of *V. parahaemolyticus* infection, one non-sucrose fermenting colony is picked from one thiosulfate-citrate–bile-salts–sucrose (TCBS) agar plate inoculated with a specimen from a diseased individual. However, analysis of a single isolate per patient may lead to misdiagnosis of the etiological agent due to sampling error if the infecting microbial population is not homogeneous. We hypothesized that the isolation of tdh^- and trh^- strains from individuals exhibiting symptoms of *V. parahaemolyticus* illness might be most likely due to simultaneous

consumption and proliferation of virulent and avirulent strains in a human host followed by isolation of an avirulent *tdh*$^-$ *trh*$^-$ strain by chance, by deletion of the virulence genes (*tdh* and *trh*) from virulent strains *in vivo,* or by mediation of *V. parahaemolyticus* illness by a novel *tdh*$^-$ and *trh*$^-$ independent mechanism. We also hypothesized that *in vivo* serotype conversion may contribute to the diversity of serotypes currently recognized in the pandemic strain. Variability of the serotype, toxin gene profile, and markers for the pandemic clone among the isolates from a single patient, if any, affects epidemiological investigation of *V. parahaemolyticus* infection. To examine these possibilities, we investigated the variability of *V. parahaemolyticus* isolates from single patients in southern Thailand.