RESULTS

Ten colonies were randomly selected from the non-sucrose-fermenting colonies on TCBS agar derived from each of the 62 patients, and all of the isolates were identified as *V. parahaemolyticus*. A total of 629 *V. parahaemolyticus* isolates acquired from the 63 patients were examined for the presence of the *tdh* gene, *trh* gene, and ORF8; the GS-PCR reaction; the O:K serotypes; and the AP-PCR profile. The results are summarized in Table 1.

We classified the patients into 24 groups based on the characteristics of the isolates, as shown in Table 1. Patient groups 1–8 yielded 10 isolates per patient for which all characteristics were identical. We designated these as the homogeneous patient groups, and they accounted for 42 of the 63 patients (66.7%). All of the isolates from 39 patients among the homogeneous patient groups possessed the *tdh* gene. However, those from three patients (patient group 5) lacked the *tdh* and *trh* genes, and all these isolates belonged to the same serotype, O11:K untypeable (UT).

We designated the other patient groups (9–24) as the heterogeneous patient groups, in which at least one of the 10 isolates from a single patient differed in at least one of the characteristics examined. Interestingly, although all 10 isolates from patient groups 9–12 had identical AP-PCR profiles in each group, they differed in one of the other characteristics. The isolates from patient group 9 appeared to belong to the pandemic clone because they were tdh^+, and GS-PCR-positive, had the O3:K6 serotype, and had identical AP-PCR and PFGE genomic fingerprints (data not shown). However, the occurrence of ORF8 was variable as it was only detected in one of the 10 isolates from one patient and from nine of 10 isolates from the second patient in the group. Similarly, one out of the 10 isolates from patient group 10 lacked the *tdh* gene. All of the isolates from this patient group had identical AP-PCR fingerprints and similar PFGE profiles

(data not shown). These results indicate the possibility that ORF8 (patient group 9) and the *tdh* gene (patient group 10) might have been lost during the infection. Patient groups 11 and 12 consisted of one and nine isolates that differed slightly in their serotypes: O1:KUT and O1:K25 in patient group 11, and OR:K56 and O3:K56 in patient group 12.

The remaining heterogeneous patient groups (13–24) harbored isolates that exhibited non-identical AP-PCR patterns within each group. The isolates from these 16 patients also differed in their serotypes within the groups, with between two and four different serotypes detected in each. More importantly, the isolates with differing serotypes exhibited different virulence gene patterns in four of the patient groups: tdh^{-} trh^{+} and $tdh^{+}trh^{+}$ in patient group 17; $tdh^{+}trh^{-}$ and $tdh^{+}trh^{+}$ in patient group 23; and $tdh^{+}trh^{-}$ and tdh^{-} trh^{-} in patient groups 18 and 24. In the last two patient groups, the tdh^{+} genotype was correlated with the GS-PCR-positive genotype. Patient group 16 contained isolates that differed in both the GS-PCR genotype and the serotype. Within this group, the GS-PCR genotype correlated with the AP-PCR profile (Fig.1) and the PFGE pattern (Fig.2) regardless of the serotype. In addition, the DNA fingerprints of the GS-PCR-positive isolates in patient group 16 (three O3:K6 isolates and two O4:K8 isolates) and those of the GS-PCR-positive isolates in patient group 22 (five O1:K25 isolates and one O3:K6 isolate) were indistinguishable (data not shown). These DNA fingerprints were different from those of the GS-PCR-negative isolates from patient group 22 (two O3:K5 isolates and two O3:KUT isolates, data not shown).

Altogether, 87.4%, 0.5%, and 7% of the 629 isolates from the 63 patients possessed the *tdh* gene alone, the *trh* gene alone, or both genes, respectively. The remaining 5.1% (32 isolates) lacked both genes. In total, 70% of the *V. parahaemolyticus* isolates (440 out of 629) were GS-PCR positive; these isolates belonged to serotypes

O3:K6 (43.7%), O1:K25 (16.4%), O1:KUT (5.0%), O4:K68 (4.6%), and O4:K8 (0.3%). A total of 62% of the GS-PCR-positive isolates carried ORF8.

In vitro and *in vivo* stability of serotype and toxin gene profile in selected isolates was investigated (Table 2). All six isolates listed in Table 2 were fed to each of fasted rabbits. Rabbits fed with PSU1683 from patient group16 and two rabbits fed with recent isolates PSU2056 and PSU2490 at the concentration of 10^{12} cfu/ml induced *V. parahaemolyticus*-positive stool, while rabbits fed with three other isolates did not (Table 2). Fifty to eighty isolates from each of the *V. parahamolyticus*-positive stool samples were examined for their serotypes and toxin gene profiles. Serotype change, but no change in the toxin gene profile, was observed in one of the three *V. parahaemolyticus*-positive cases. Three of the 50 isolates from the rabbit fed with PSU2056 (serotype O11:KUT) had undergone serotype change to O11:K15. These three isolates showed the same AP-PCR fingerprints as those of the isolates that had not undergone serotype change (data not shown).

V. parahaemolyticus isolates PSU1681, PSU1690, PSU1683, and PSU2056 (Table 2) were used in the *in vitro* stability test. None of the tested isolate showed change in their serotype or toxin gene profile during continuous sub-culture for fifty passages.