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Characterization of Extended-Spectrum-β-Lactamase-Producing *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is a major causative agent of gastroenteritis, particularly in areas with high levels of seafood consumption, and it has recently become pandemic due to the emergence of the O3:K6 serotype (12). Tdh and Trh have been implicated as major virulence factors in the strains of *V. parahaemolyticus* that caused most of the clinical infections (17). An estimated 45,000 (90% confidence interval [CI], 23,000 to 75,000) cases of *V. parahaemolyticus* infections occur every year in the United States (16). In Hong Kong, *V. parahaemolyticus* is the leading cause of food-borne illness, although the exact number of cases is not known due to a lack of relevant surveillance systems in Hong Kong (4, 7). *V. parahaemolyticus* gastroenteritis is self-limiting, but the infections can be fatal to the elderly, immunocompromised patients, or those with medical conditions such as liver disease or diabetes where antibiotic treatment is necessary (9, 17).

In this study, *V. parahaemolyticus* isolates were collected from raw shrimp samples purchased in markets in four different locations (Hong Kong Island, Hung Hom, Tsuen Wan, and Sai Kung) in Hong Kong from January to April 2010. A total of 128 shrimp samples were collected during the isolation period. Two typical blue-green colonies were acquired from thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates for each sample and subjected to further confirmation by a PCR assay targeting the *tl* and *atp* genes (10, 19). Of 128 shrimp samples, 119 (93%) were *V. parahaemolyticus* positive (with one or two positive isolates), and a total of 208 *V. parahaemolyticus* isolates were obtained for further characterization (19). The isolation rates for the four locations and 4 months were between 91% and 95% and between 90% and 96%, respectively. Virulence genes *thd* and *trh* were detected in 4 and 2 *V. parahaemolyticus* isolates, respectively, using PCR assays (15), and none of the isolates contained both genes.

The 208 *V. parahaemolyticus* isolates from shrimp samples were assayed for their antimicrobial susceptibilities by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) using 13 antibiotics as shown in Table 1 (5). *V. parahaemolyticus* isolates were resistant to ampicillin (85%), amikacin (62%), tetracycline (53%), and chloramphenicol (35%). Surprisingly, about 6% (12 of 208) of the *V. parahaemolyticus* isolates also showed resistance to cefotaxime, and 6% of them were resistant to ciprofloxacin. No isolate showed concurrent resistance to both cefotaxime and ciprofloxacin. The resistance to most of the antibiotics observed in this study is consistent with other reports in Hong Kong and around the world except for the emergence of resistance to new front-line antibiotics such as fluoroquinolones and extended-spectrum cephalospo-

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TABLE 1 MICs of the ESBL-producing *V. parahaemolyticus* strains and their transconjugants[a]

Strain	MIC (μg/ml)												
	AMP	CRO	CTX	AZT	MER	CIP	NAL	KAN	GEN	AMI	CHL	TET	STR
J53	16	<1	<1	2	<0.5	<0.05	8	<0.5	<4	<8	<4	<4	<4
V15	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V15TC	>128	>64	>64	>64	<0.5	0.125	16	<0.5	<4	<8	<4	<4	<4
V26	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V26TC	>128	>64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V36	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V36TC	>128	>64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V39	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V39TC	>128	>64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V40	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V40TC	>128	>64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V41	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V85	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V85TC	>128	64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V137	>128	32	64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V137TC	>128	64	>64	>64	<0.5	0.5	>64	<0.5	<4	<8	<4	<4	<4
V138	>128	32	64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V138TC	>128	64	>64	>64	<0.5	0.5	>64	<0.5	<4	<8	<4	<4	<4
V189	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V189TC	>128	>64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4
V191	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V201	>128	64	>64	>64	<0.5	<0.05	<4	<0.5	<4	<8	<4	<4	<4
V201TC	>128	64	>64	>64	<0.5	0.125	32	<0.5	<4	<8	<4	<4	<4

[a] TC, transconjugant; AMP, ampicillin; CRO, ceftriaxone; CTX, cefotaxime; AZT, aztreonam; MER, meropenem; CIP, ciprofloxacin; NAL, nalidixic acid; KAN, kanamycin; GEN, gentamicin; AMI, amikacin; CHL, chloramphenicol; TET, tetracycline; STR, streptomycin.

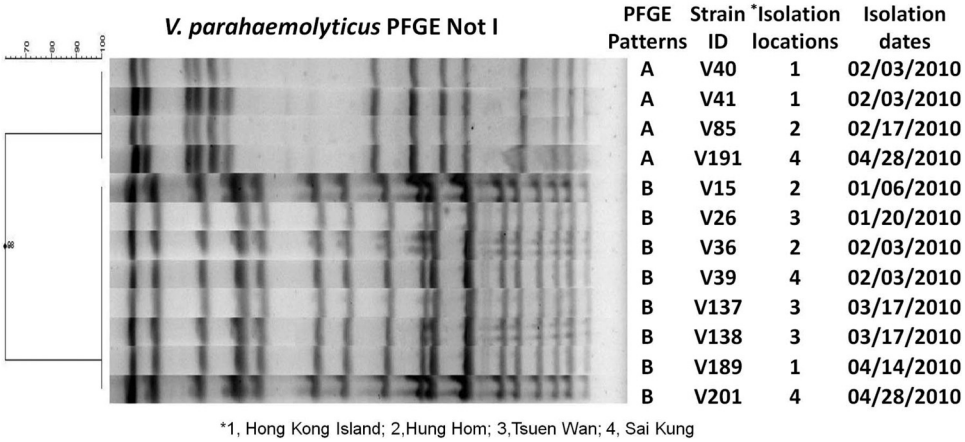

FIG 1 PFGE profile and isolation information for 12 extended-spectrum-β-lactamase (ESBL)-producing *V. parahaemolyticus* strains. Isolation dates are shown as month/day/year.

rins in *V. parahaemolyticus*, which is rare and has been reported only once, in Indonesia in 2003 (1, 3, 8, 13, 14, 18). The 12 cefotaxime-resistant *V. parahaemolyticus* isolates were from 11 independent samples collected from different locations or on difference isolation dates (V137 and V138 were from the same sample), and none of these isolates harbored the virulence gene *tdh* or *trh* (Fig. 1). Pulsed-field gel electrophoresis (PFGE) characterization showed that these 12 isolates belonged to two different PFGE types (Fig. 1). *V. parahaemolyticus* strains of the same PFGE type were isolated from different locations and dates, which suggested that clonal spread of different *V. parahaemolyticus* strains might play an important role in the emergence of extended-spectrum-β-lactam-resistant *V. parahaemolyticus* in Hong Kong.

A conjugation experiment was performed using the cefotaxime-resistant *V. parahaemolyticus* isolates with *Escherichia coli* J53 as the recipient strain as previously described (20) with modifications. Briefly, donor and recipient strains were mixed in a 1:1 ratio, transferred to a filter on an LB agar plate, and incubated for 1 h. The culture on the filter was washed off using saline water, and 100 μl of the washed culture was spread on a selective plate to select for transconjugants. The resistance determinant was found to be encoded on the self-transmissible plasmid and could be transferred to *E. coli*, except for two isolates with identical strains, V41 and V191. The self-transmissible plasmids were found to be ~24 kb in size and to belong to the IncN compatibility group, members of which carry a bla_{PER-1}, a class A extended-spectrum β-lactamase gene, by the use of a PCR assay as previously described (2, 6) (Table 1). PER-1 is mostly associated with Gram-negative clinical pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Salmonella* spp. and has never before been reported in *Vibrio* spp. Surprisingly, transconjugants showed higher (2- to 8-fold) MICs of nalidixic acid than both parental and recipient strains (Table 1). None of the known plasmid-mediated quinolone resistance (PMQR) mechanisms (including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *oqxAB*, *aac*, *and qnrVC*) was detected on the plasmid (data not shown) (11). Further studies are needed to characterize the novel PMQR mechanisms on the conjugative plasmid.

The increasing prevalence of multidrug-resistant *V. parahaemolyticus* in seafood may cause public health problems in Hong Kong, since *V. parahaemolyticus* infections are the leading cause of food-borne illness in Hong Kong (4, 7). More research and surveillance on the multidrug-resistant *V. parahaemolyticus* should be implemented to improve understanding and control of the progress of multidrug resistance in *V. parahaemolyticus*.

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