

First Detection of AmpC β -Lactamase *bla*_{CMY-2} on a Conjugative IncA/C Plasmid in a *Vibrio parahaemolyticus* Isolate of Food Origin

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Vibrio parahaemolyticus is an important causative agent of gastroenteritis, with the consumption of contaminated seafood being the major transmission route. Resistance to penicillin is common among *V. parahaemolyticus* strains, whereas cephalosporin resistance remains rare. In an attempt to assess the current prevalence and characteristics of antibiotic resistance of this pathogen in common food samples, a total of 54 (17% of the total samples) *V. parahaemolyticus* strains were isolated from 318 meat and seafood samples purchased from supermarkets and wet markets in Shenzhen, China, in 2013. These isolates exhibited high-level resistance to ampicillin, yet they were mostly susceptible to other antimicrobials, except for two that were resistant to extended-spectrum cephalosporins. The β -lactamase gene *bla*_{PER-1} was detectable in one strain, *V. parahaemolyticus* V43, which was resistant to both third- and fourth-generation cephalosporins. Compared to other *bla*_{PER-1}-positive *V. parahaemolyticus* strains reported in our previous studies, strain V43 was found to harbor an \sim 200-kb conjugative plasmid carrying genes that were different from the antimicrobial resistance genes reported from the previous studies. The β -lactamase gene *bla*_{CMY-2} was detectable for the first time in another *V. parahaemolyticus* isolate, V4, which was resistant to third-generation cephalosporins. This *bla*_{CMY-2} gene was shown to be located in an \sim 150-kb IncA/C-type conjugative plasmid with a genetic structure consisting of *traB-traV-traA-ISEcp1-bla*_{CMY-2}-*blc-sugE-encR-orf1-orf2-orf3-orf4-dsbC-traC*, which is identical to that of other IncA/C conjugative plasmids in *Enterobacteriaceae*, albeit with a different size. These findings indicate that the transmission of extended-spectrum- β -lactamase (ESBL) and AmpC β -lactamase genes via conjugative plasmids can mediate the development of extended-spectrum cephalosporin resistance in *V. parahaemolyticus*, thereby posing a potential threat to public health.

Vibrio parahaemolyticus is a halophilic Gram-negative bacterium and one of the most important seafood-borne pathogens worldwide. It is widely distributed in estuarine-marine environments and seafood products, often causing infections via the consumption of raw or undercooked seafood (1, 2). The most common symptoms of *V. parahaemolyticus* infections include gastroenteritis, diarrhea, headache, nausea, and vomiting. For children, the elderly, and people with suppressed immune systems, *V. parahaemolyticus* intestinal infections can be fatal (3). The pathogenicity of *V. parahaemolyticus* is always associated with the presence of the toxin genes *tdh* (encoding the thermostable direct hemolysin [TDH]) and its homolog *trh* (encoding the thermostable direct hemolysin [TDH]-related hemolysin) (4). The U.S. Food and Drug Administration estimates that approximately 4,500 infections occur per year in the United States, and over the past 15 years, outbreaks have been increasing in terms of scale and frequency (3). In recent years, the number of *V. parahaemolyticus* infections in China has increased significantly (1).

For life-threatening infections caused by *V. parahaemolyticus*, antibiotic therapy (mainly including tetracycline, quinolones, and third-generation cephalosporins) is necessary (5). The increasing prevalence of antimicrobial resistance in *V. parahaemolyticus*, presumably due to the extensive use of antimicrobials in clinical treatment and aquaculture systems, was recently reported (6–9). To date, resistance to ampicillin, streptomycin, kanamycin, tetracycline, chloramphenicol, and ciprofloxacin has been reported (9). Recent studies also reported resistance to third-generation cephalosporins in *V. parahaemolyticus* strains due to carriage of an extended-spectrum- β -lactamase (ESBL) gene, *bla*_{PER-1} (10). How-

ever, other ESBL and AmpC-type genes are not detectable in *V. parahaemolyticus*.

With the rapid development of the aquaculture industry, infections associated with *V. parahaemolyticus* in seafood have become increasingly common in China and other Asian countries. In China, research on the epidemiology and antimicrobial resistance of *V. parahaemolyticus* from food products, and meat products in particular, is very rare. This study reports the surveillance of the prevalence and antimicrobial resistance of *V. parahaemolyticus* collected from different food products, including shrimps, pork, beef, and chicken, in Shenzhen, China. The data will provide valuable information for the future control of *V. parahaemolyticus* infections in China and neighboring countries.

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MATERIALS AND METHODS

Isolation and identification of *V. parahaemolyticus*. A total of 318 food samples, including those from shrimps, chicken, pork, and beef, were collected from supermarkets and wet markets in Shenzhen, China, from June to September 2013. The isolation of *V. parahaemolyticus* was conducted as previously described, with some modifications (11). Briefly, food samples were purchased and transferred to a laboratory within 3 h under low-temperature conditions (stored in insulated bags containing ice cubes). Five grams of food samples was homogenized in 45 ml of alkaline peptone water (APW) and incubated overnight at 37°C. A loopful of culture was then streaked on thiosulfate citrate-bile salts-sucrose (TCBS) agar. After 16 h of incubation at 37°C, suspicious colonies (blue or green) were picked and streaked on CHROMagar plates for identification. The mauve colonies on CHROMagar plates, presumptively identified as *V. parahaemolyticus*, were transferred into brain heart infusion (BHI) broth with 3% NaCl and incubated overnight at 37°C. The bacterial culture was stored in 15% glycerol at -80°C for further characterization.

Isolates with typical phenotypes on the TCBS and CHROMagar plates were confirmed by PCR detection of the thermolabile hemolysin (*tlh*) gene and DNA sequencing. Detection of virulence genes (*tdh* and *trh* genes) was also performed using the PCR approach, as previously described (12).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on *V. parahaemolyticus* isolates using the standard agar dilution method, as described by the Clinical and Laboratory Standards Institute (13). Fourteen antimicrobials were tested, including nalidixic acid, ciprofloxacin, ofloxacin, amikacin, gentamicin, tetracycline, ampicillin, amoxicillin-clavulanic acid, ceftazidime, meropenem, imipenem, trimethoprim-sulfamethoxazole, and chloramphenicol. The resistance breakpoints published by the Clinical and Laboratory Standards Institute were used (14). *Escherichia coli* strain ATCC 25922 was included as the quality control strain.

PFGE subtyping. Pulsed-field gel electrophoresis (PFGE) was conducted to investigate the genetic relatedness of the identified isolates according to the PulseNet protocol (<http://www.pulsenetinternational.org/protocols/>), with minor modifications. In brief, the cell suspensions (at an optical density at 610 nm [OD₆₁₀] of 0.9) with proteinase K (1 mg/ml) were immobilized into 2% low-melting-point agarose (Amersham, United Kingdom) at a ratio of 1:1 before incubation in a lysis buffer with proteinase K (0.1 mg/ml). After five consecutive steps of washing with Tris-EDTA buffer (pH 8.0) at 50°C in a water bath shaker, the total DNA was digested with 40 U of NotI or SfiI (New England BioLabs) for 4 h at 37°C or 50°C, respectively. Chromosomal DNA of *Salmonella enterica* serotype Braenderup (strain H9812) digested with XbaI was used as a reference marker. The DNA bands were separated using a Chef Mapper XA pulsed-field gel electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) for 19 h, with an initial switch time of 10 s and final switch time of 35 s at 6 V/cm, at 14°C in 0.5× Tris-boric acid-EDTA buffer. The gels were stained with GoldView dye and photographed using a Bio-Rad Gel Doc 1000 system. A PFGE dendrogram was generated using the BioNumerics 7.1 software.

β-Lactamase gene screening and analysis of genetic environment. The prevalence of β-lactamase genes among the test strains was determined by PCR assays that covered most of the known β-lactamase genes, as previously described (15). Screening of class I integrons was also performed as described previously (5). The entire sequence of the *bla*_{CMY-2} gene was amplified using primers CMY2_F and CMY2_R (Table 1). The amplified product was cloned into a TOPO pCR2.1 vector (Invitrogen) and sequenced with universal primers. Primer walking and PCR mapping were performed to investigate the *bla*_{CMY-2} gene environment. A set of primers (Table 1) was designed based on the published flanking sequence of the *bla*_{CMY-2} gene (16). The PCR products were sequenced and subjected to sequence alignment analysis using the BioEdit software.

Conjugation and plasmid characterization. A filter mating experiment was carried out to study the transferability of resistance phenotypes.

Overnight cultures of donors (*V. parahaemolyticus*) and recipients (sodium-azide-resistant *E. coli* strain J53) were mixed together at a donor-to-recipient ratio of 2/1, plated on a filter membrane, and selected on MacConkey agar with ceftazidime (4 μg/ml) and sodium azide (100 μg/ml) to select transconjugants.

The major incompatibility (Inc) groups of plasmids from transconjugants and parental strains were identified by a PCR-based replicon typing (PBRT) method (16). S1 nuclease PFGE (S1-PFGE) was used to analyze the plasmids that played a role in conferring resistance to β-lactams, according to the standard PFGE protocol described above, except for the use of S1 nuclease instead in the digestion step. After washing with Tris-EDTA buffer (pH 8.0), total DNA was digested with 50 U of S1 nuclease (Thermal Scientific) for 30 min at 37°C. The linear plasmids were separated from the chromosomal DNA by electrophoresis for 20 h, with an initial switch time of 2.16 s and final switch time of 63.8 s at 6 V/cm at 14°C in 0.5× Tris-boric acid-EDTA buffer, using a Chef Mapper XA pulsed-field electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). Southern blot hybridization was carried out in accordance with the manufacturer's instructions for digoxigenin (DIG)-High Prime DNA labeling and detection starter kit II (Roche Diagnostics), using the *bla*_{CMY-2} and *bla*_{PER-1} digoxigenin-labeled probes.

For *bla*_{PER-1}-positive strains, a comparison was conducted between a strain from this study and another two *bla*_{PER-1}-positive *V. parahaemolyticus* strains identified in our previous studies (5, 10). The primers used to amplify different β-lactamase genes, the *qnrVC* gene, and integrons are listed in Table 1.

RESULTS AND DISCUSSION

Prevalence of *V. parahaemolyticus* in different food samples in Shenzhen. A total of 54 (17% of the total samples) *V. parahaemolyticus* isolates from different food samples were recovered from 318 food samples in wet markets and supermarkets. Of these isolates, 20/76 (26%), 19/84 (23%), 9/85 (11%), and 6/73 (8%) were isolated from shrimps, chicken, pork, and beef, respectively. *V. parahaemolyticus* was considered the most important pathogen causing seafood-borne infections (17). Reports about *V. parahaemolyticus* from other kinds of foods are scarce. In this study, *V. parahaemolyticus* was detected in all types of meat products, such as chicken, pork, and beef. The prevalence of *V. parahaemolyticus* in chicken was similar to that in shrimps, whereas its prevalence in pork and beef was slightly lower than that in shrimps. It appears that the chicken, pork, and beef food samples might be cross-contaminated during the process of handling and storage. More in-depth investigations of the origin of *V. parahaemolyticus* in foods other than seafood should be performed.

In order to determine whether these isolates were virulent, the prevalence of the *tdh* and *trh* genes was screened by PCR. Although the *tdh* and *trh* genes are known to occur in 99% of clinical isolates (1), none of the 54 food isolates contained these two virulence genes. This finding was consistent with those of previous studies indicating a low prevalence of *tdh* and *trh* genes in *V. parahaemolyticus* from food samples (7, 9). The relationship between clinical and environmental strains should be investigated further in the future.

Antimicrobial susceptibility profile and PFGE typing. Antimicrobial susceptibility testing of the 54 *V. parahaemolyticus* isolates showed that relatively high rates of resistance to ampicillin (100%) and tetracycline (11%) were observed. In addition, five isolates (9%) were found to be resistant to sulfamethoxazole-trimethoprim, and one was resistant to chloramphenicol. Importantly, two isolates exhibited resistance to ceftazidime and ceftazidime. Consistent with a previous study (9), multiple antimicrobial

TABLE 1 PCR primers used to assess the genetic context of β -lactamase genes

Primer name	Sequence	Length (bp)	Target	Reference or source
CMY2_F	GCTGAGAGCTCATGATGAAAAAATCG	1,146	<i>bla</i> _{CMY-2}	This study
CMY2_R	GGTACGGATCCTTATTGCAGC			
Primers for <i>bla</i> _{CMY-2} genetic environment determination				
TraA-F	ATCAGTTGGCGAATGCCTCA	1,417	<i>traA</i> - <i>ISEcp1</i>	This study
ISEcp1-R	AACACGGCTTCATTTCGCCCAA			This study
ISEcp1-F	ATAAAGACCATGCTCTGCGG	2,537	<i>ISEcp1</i> - <i>bla</i> _{CMY-2}	This study
CMY-2R	ACGGACAGGGTTAGGATA			15
ISEcp1-F	ATAAAGACCATGCTCTGCGG	2,800	<i>ISEcp1</i> - <i>bla</i> _{CMY-2} - <i>blc</i>	This study
blc-R	TACCAGTTCCAGATAGCG			This study
ISEcp1-F(B)	ATAAAGACCATGCTCTGCGG	3,465	<i>ISEcp1</i> - <i>bla</i> _{CMY-2} - <i>blc</i> - <i>sugE</i>	This study
sugE-R(B)	TTGGCCTGAAATACACCCAC			This study
A-F	CGTAGAGGATCTCAGTTCAG	3,700	<i>traB</i> - <i>traV</i> - <i>traA</i> - <i>ISEcp1</i> - <i>bla</i> _{CMY-2}	22
A-R	CCTGCCACTGTTTGCCTGTC			
G-F	CGCCTGCTAAAAGCAAGAAT	2,700	<i>sugE</i> - <i>ecrR</i> - <i>orf1</i>	22
G-R	TGCCATCTGCATGAACAAAT			
H-F	AAGGCTGTTGGCTTCGAGTA	2,500	<i>orf1</i> - <i>orf2</i> - <i>orf3</i>	22
H-R	TCAGCAGTATCACCCCTGCAC			
I-F	ATGTGTTCAAAGCCGAAACC	2,500	<i>orf3</i> - <i>orf4</i> - <i>dsbC</i> - <i>traC</i>	22
I-R	GGTGCCGTAATCGAAGATTT			
Primers for <i>bla</i> _{PER-1} positive plasmid characterization				
PER1-F	GCTCCGATAATGAAAGCGT	520	<i>bla</i> _{PER-1}	15
PER1-R	TTCGGCTTGACTCGGCTGA			
TEM1-F	CATTTCCGTGTCGCCCTTATTC	800	<i>bla</i> _{TEM-1}	15
TEM1-R	CGTTCATCCATAGTTGCCTGAC			
ISCR1-F	AGACGCCGTGGAAGCGTGTG	509	<i>ISCR1</i>	This study
ISCR1-R	GCTCGCTCACACCCTCAGCC			
QnrVC6-F	ATGGAAAAATCAAAGCAATT	657	<i>qnrVC6</i>	5
QnrVC6-R	TTAGTCAGGAACAATGAT			
Int1-F	GGCATCCAAGCAGCAAGC	Various	Gene cassette (class I integron)	25
Int1-R	AAGCAGACTTGACCTGAT			

resistance *V. parahaemolyticus* was not common. The detection of *V. parahaemolyticus* isolates that are resistant to third-generation cephalosporins in mainland China has not been reported. Our susceptibility testing results indicated that front-line drugs, including cefoxitin, ceftazidime, and fluoroquinolones, remained highly effective against *V. parahaemolyticus* infections. However, our finding of emerging *V. parahaemolyticus* resistant to third-generation cephalosporins has raised a concern for public health, despite being nonpathogenic, as the transfer of resistance determinants from food isolates to other pathogenic *Vibrio* strains would have serious consequences.

Among the 54 isolates, a total of 49 PFGE patterns were identified, and 5 strains were untypeable. The PFGE dendrogram (see Fig. S1 in the supplemental material) showed that most isolates did not exhibit significant genetic relatedness, and no extensive clonal dissemination features were observed. However, there were several clusters in which >90% genetic similarity was observed between isolates from different sources. This finding provides evidence of cross-contamination between different types of food in supermarkets.

β -Lactamase gene screening. β -Lactamase gene screening was performed on two *V. parahaemolyticus* isolates that were resistant to third-generation cephalosporins. For strain V43, which was resistant to all tested β -lactams, including cefepime, but not imi-

penem or meropenem, *bla*_{TEM-1} and *bla*_{PER-1} were detectable. Since the β -lactamase gene *bla*_{PER-1} was previously detectable in *V. parahaemolyticus* isolated from shrimps in Hong Kong in our laboratory (5, 10), this *bla*_{PER-1}-positive strain was selected for further characterization in this study. On the other hand, the β -lactamase gene *bla*_{CMY-2} was detected for the first time in strain V4, which was resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, and ceftriaxone, and had reduced susceptibility to ceftazidime but not cefepime or carbapenems (Table 2).

Characterization of *bla*_{PER-1}-bearing plasmids in *V. parahaemolyticus*. Two genetically unrelated *bla*_{PER-1}-positive isolates, *V. parahaemolyticus* 2010V36 and 2011V1 (see Fig. S2 in the supplemental material), which were isolated from our previous studies, were selected for further characterization, along with strain V43 recovered in this study (5, 10). Conjugation experiments showed that plasmids carrying *bla*_{PER-1} in these three *V. parahaemolyticus* strains were transferrable to *E. coli* J53. The transconjugants V43-J53, 2010V36-J53, and 2011V1-J53 were found to have acquired phenotypic resistance to most of the β -lactam antibiotics tested, including resistance/intermediate resistance to fourth-generation cephalosporins, such as cefepime, but not carbapenems (Table 2). Understanding the molecular mechanisms by which *V. parahaemolyticus* exhibits resistance or intermediate resistance to fourth-generation cephalosporins, especially those in-

TABLE 2 Antimicrobial susceptibility profiles of extended-spectrum-cephalosporin-resistant *V. parahaemolyticus* strains (V4, V43, 2010V36, and 2011V1) and the corresponding transconjugants

Strain	β -Lactamase gene	MIC (mg/liter) of ^a :											
		AMP (32)	CTX (4)	CRO (4)	CAZ (16)	CEP (16)	TET (16)	GEN (16)	NAL (32)	CIP (4)	CHL (32)	KAN (64)	TRI (16)
J53 ^b		1	≤0.125	≤0.125	≤0.125	≤0.125	2	1	2	≤0.125	2	0.25	≤0.125
V4	<i>bla</i> _{CMY-2}	128	8	32	8	0.5	32	1	0.5	≤0.125	2	8	16
V4-J53	<i>bla</i> _{CMY-2}	>128	16	64	64	0.5	64	1	2	≤0.125	64	1	>64
V43	<i>bla</i> _{PER-1}	>128	>128	>128	>128	32	32	8	1	≤0.125	2	8	32
V43-J53	<i>bla</i> _{PER-1}	128	128	128	>128	16	64	8	2	≤0.125	32	0.5	>64
2010V36	<i>bla</i> _{PER-1}	>128	>128	>128	>128	32	1	1	0.25	≤0.125	0.25	2	16
2010V36-J53	<i>bla</i> _{PER-1}	>128	>128	>128	>128	16	2	1	2	≤0.125	2	2	>64
2011V1	<i>bla</i> _{PER-1}	>128	>128	>128	>128	16	1	4	0.5	≤0.125	16	64	>64
2011V1-J53	<i>bla</i> _{PER-1}	>128	>128	>128	>128	32	2	4	4	≤0.125	16	64	>64

^a Numbers in parentheses are the breakpoint for each agent. AMP, ampicillin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; CEP, cefepime; TET, tetracycline; GEN, gentamicin; NAL, nalidixic acid; CIP, ciprofloxacin; CHL, chloramphenicol; KAN, kanamycin; TRI, trimethoprim.

^b J53, *E. coli* recipient.

volving acquisition of the ESBL gene *bla*_{PER-1}, will allow us to define the breakpoint of cefepime for *V. parahaemolyticus*, since this is the only report of resistance/intermediate resistance to cefepime in *V. parahaemolyticus*. On the basis of the breakpoints of *E. coli*, as well as a comparison between the drug susceptibility levels of transconjugants and the test *V. parahaemolyticus* strains, our data indicate that a breakpoint of 16 mg/liter or 8 mg/liter is more appropriate. Apart from the *bla*_{PER-1}-associated phenotypes, other phenotypes, such as those showing resistance to tetracycline and trimethoprim in V43, resistance to trimethoprim in 2010V36, and resistance to chloramphenicol, kanamycin, and trimethoprim in 2011V1, could also be transferred to *E. coli* J53 through conjugation. These resistance phenotypes were consistent with those conferred by the integrons carrying different resistance genes that were detectable in the conjugative plasmids (Table 3). S1 nuclease PFGE showed that V43 contained one plasmid with a size of ~200 kb that could be transferred to *E. coli* J53. Southern hybridization confirmed the presence of *bla*_{PER-1} on this plasmid (Fig. 1). Strain 2010V36 was shown to harbor two plasmids, with sizes of ~175 kb and ~110 kb, with the ~175-kb plasmid being transferrable to *E. coli* J53. The plasmid size in 2010V36 here was different from that in the previous study, presumably because the use of the S1-PFGE method offered more accurate plasmid size determination in this study. Southern hybridization also confirmed the presence of a *bla*_{PER-1} gene on the ~175-kb plasmid. Strain 2011V1 was found to contain three plasmids with sizes of ~200 kb, ~70 kb, and ~45 kb. The ~200-kb plasmid was shown to be self-transmissible. Southern hybridization also confirmed the presence of *bla*_{PER-1} in the ~200-kb plasmid (Fig. 1).

In our previous study, the partial genetic environment of *bla*_{PER-1} was determined for strain 2011V1 (5). To check whether *bla*_{PER-1} in V43 and 2010V36 also contained genetic structures similar to that of strain 2011V1, further characterization of plasmids recovered from V43, 2010V36, and 2011V1 was performed using primers to amplify the flanking region of *bla*_{PER-1} with *bla*_{PER-1} of 2011V1 as a template for primer design (Table 1). The results, as summarized in Table 3, showed that the genetic structures of these plasmids were different, despite the fact that the plasmids from strains V43 and 2011V1 were both about 200 kb in size. In addition to *bla*_{PER-1}, a plasmid from V43 also contained a *bla*_{TEM-1} gene, ISCR1, and an integron carrying the *arr-3* and *dfrA27* genes; although the conjugative *bla*_{PER-1}-containing plasmid from strain 2010V36 contained ISCR1 and an integron carrying the *arr-3* and *dfrA27* genes, it was slightly smaller in size (~175 kb) than the other two plasmids. The conjugative plasmid from strain 2011V1 contained ISCR1 and a new integron carrying the *aacA3*, *catB2*, *dfrA1*, and *aadA1* genes. In addition, it carried a novel PMQR gene, *qnrVC6* (5). These data indicate that all three *bla*_{PER-1}-bearing plasmids recoverable from three different *V. parahaemolyticus* strains were genetically different. A further sequencing approach will be applied to understand the evolutionary features of these three plasmids in *V. parahaemolyticus*.

Characterization of plasmids carrying the *bla*_{CMY-2} gene in *V. parahaemolyticus*. Conjugation experiments were also performed on the V4 strain, with the results showing that the plasmid containing the *bla*_{CMY-2} gene in this strain could be transferred to *E. coli* J53. The transconjugant V4J53 exhibited slightly higher MICs of various β -lactam antimicrobials than those of the origi-

TABLE 3 Comparison of genetic contents of *bla*_{PER-1}-bearing plasmids recovered from three *V. parahaemolyticus* strains and the corresponding transconjugants

Genetic component	Strain			Transconjugant		
	V43	V43-J53	2010V36	2010V36-J53	2011V1	2011V1-J53
<i>bla</i> _{PER-1}	+	+	+	+	+	+
<i>bla</i> _{TEM-1}	+	+	–	–	–	–
ISCR1	+	+	+	+	+	+
<i>qnrVC6</i>	–	–	–	–	+	+
Class I integrons	<i>arr-3</i> , <i>dfrA27</i>	<i>arr-3</i> , <i>dfrA27</i>	<i>arr-3</i> , <i>dfrA27</i>	<i>arr-3</i> , <i>dfrA27</i>	<i>aacA3</i> , <i>catB2</i> , <i>dfrA1</i> , <i>aadA1</i>	<i>aacA3</i> , <i>catB2</i> , <i>dfrA1</i> , <i>aadA1</i>
No. of plasmids (size [kb])	1 (200)	1 (200)	2 (175, 110)	1 (175)	3 (200, 70, 45)	1 (200)

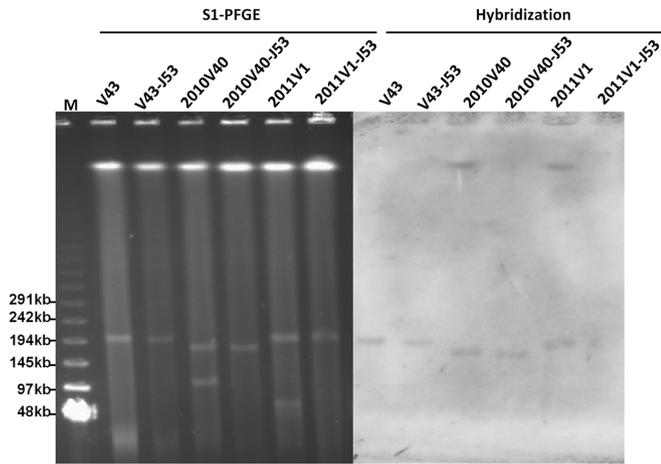


FIG 1 S1-PFGE patterns and Southern blot hybridization of *bla*_{PER-1}-positive strains and the corresponding transconjugants. M, Lambda ladder PFGE marker (New England BioLabs).

nal V4 strain (Table 2). Interestingly, V4 did not exhibit resistance to chloramphenicol, whereas V4J53 became resistant to chloramphenicol, presumably due to the fact that some resistance genes might be better expressed in *E. coli* (Table 2). This phenomenon was also observable in our previous study (10). S1 nuclease PFGE and Southern hybridization analysis showed that the V4 strain harbored 4 plasmids, with sizes ranging from ~45 kb to ~150 kb. The conjugative plasmid detected in transconjugant V4J53 was ~150 kb. Southern hybridization confirmed that the *bla*_{CMY-2} gene was located in this transmissible plasmid in both V4 and V4J53 (Fig. 2). Plasmid typing indicated that the transmissible plasmid belonged to the IncA/C type plasmid. Plasmids harboring *bla*_{CMY-2} have been found to belong to different plasmid types (IncA/C, IncII, etc.) detectable in members of *Enterobacteriaceae* recovered from various sources. The sizes of the plasmids varied

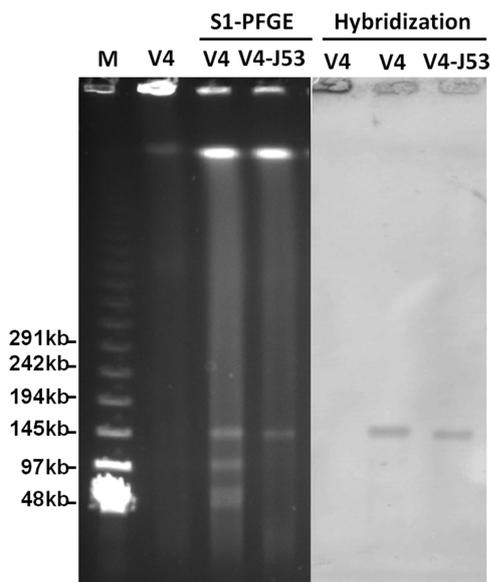


FIG 2 S1-PFGE patterns and Southern blot hybridization of V4 and transconjugant V4J53. M, lambda ladder PFGE marker (New England BioLabs); V4, V4 genomic DNA without S1 nuclease digestion.

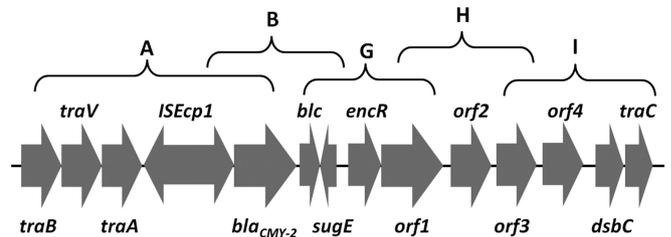


FIG 3 Genetic structure of *bla*_{CMY-2}-bearing IncA/C conjugative plasmid in *V. parahaemolyticus* strain V4. A, B, G, H, and I represent the primer targeting regions. The primer sequences are shown in Table 1.

from ~45 kb to ~200 kb (18–21). IncA/C plasmids carrying *bla*_{CMY-2} with a similar size (~150 kb) were mainly reported in *E. coli* and *S. enterica* (20). It is likely that the *bla*_{CMY-2}-bearing IncA/C plasmid in this strain originated from *Enterobacteriaceae*; alternatively, the formation of this plasmid may be due to mobilization of the *bla*_{CMY-2} gene to different plasmid backbones through *ISEcp1*-mediated transposition activities (22).

The flanking sequence of *bla*_{CMY-2} was amplified by PCR mapping, according to published sequences, and confirmed by sequencing. It was found that the arrangement of the genetic environment of *bla*_{CMY-2} was in the order *traB-traV-traA-ISEcp1-bla*_{CMY-2}-*blc-sugE-encR-orf1-orf2-orf3-orf4-dsbC-traC* (Fig. 3). The β -lactamase gene *bla*_{CMY-2} is the most commonly reported plasmid-carried AmpC β -lactamase gene in *Salmonella* spp., *E. coli*, and other species of *Enterobacteriaceae* worldwide (23). This gene renders the host organism resistant to a variety of β -lactams, including oxyimino-cephalosporins and cephamycins (23). The genetic content in the vicinity of the *bla*_{CMY-2} gene usually comprised the *ISEcp1* and *blc-sugE* elements in its upstream and downstream regions, respectively (22). Therefore, the *V. parahaemolyticus* V4 strain is genetically similar to those strains described in several other reports (22, 24). A comparison with other published sequences in the NCBI database indicated that the genetic segment in which the *bla*_{CMY-2} gene is located has been identified in plasmids of *Salmonella* spp., *E. coli*, *Klebsiella pneumoniae*, *Providencia stuartii*, and *Shigella sonnei* (22–24). However, the sizes of these previously reported plasmids were different from that of the V4 strain.

Conclusion. In this study, we reported the isolation of *V. parahaemolyticus* from meat products other than seafood. These isolates were susceptible to most of the antimicrobials tested, except that two of them were resistant to extended-spectrum cephalosporins. The β -lactamase gene *bla*_{PER-1} was detectable in one *V. parahaemolyticus* isolate, V43, which was resistant to both third- and fourth-generation cephalosporins. The conjugative *bla*_{PER-1}-borne plasmid recovered from this strain was structurally different from those reported previously. On the other hand, the β -lactamase gene *bla*_{CMY-2} was detected for the first time in another *V. parahaemolyticus* isolate, V4, which was resistant to the third-generation cephalosporins. The *bla*_{CMY-2} gene was shown to be located on an ~150-kb IncA/C type conjugative plasmid with the genetic structure *traB-traV-traA-ISEcp1-bla*_{CMY-2}-*blc-sugE-encR-orf1-orf2-orf3-orf4-dsbC-traC*, which has been reported in other IncA/C conjugative plasmids in *Enterobacteriaceae*, albeit with a slightly different size. This finding indicated that the transmission of ESBL and AmpC β -lactamase genes via different conjugative plasmids mediated the development of resistance to extended-

spectrum cephalosporins and other drugs in *V. parahaemolyticus*. Such a plasmid is transferrable to *Enterobacteriaceae* and possibly other pathogenic *Vibrio* spp., thereby posing a significant threat to public health.

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We declare no conflicts of interest.

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