F. C. Soncini, F. Solomon, E. A. Groisman, *ibid.*, p. 7800.
4. C. Collazo and J. E. Galán, *Mol. Microbiol.* **24**, 747 (1997); Y. Fu and J. E. Galán, *ibid.* **27**, 359 (1998); W.-D. Hardt and J. E. Galán, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9887 (1997).
5. C. Ginocchio, S. B. Olmsted, C. L. Wells, J. E. Galán, *Cell* **76**, 717 (1994).
6. *Salmonella typhimurium* strains were derivatives of SJW1103 [S. Yamaguchi, H. Fujita, A. Ishihara, S. Aizawa, R. M. Macnab, *J. Bacteriol.* **166**, 187 (1986)] or SL1344 [S. K. Hoiseth and B. A. Stocker, *Nature* **291**, 238 (1981)]. The *S. typhimurium* flagellar mutants examined were Δ*flhC*, *flgA*, *flgB*, *flgC*, *flgD*, *flgE*, *flgF*, *flgG*, *flhA*, *flhB*, *flhC*, *fliE*, *fliF*, *fliG*, *fliH*, *fliI*, *fliJ*, *fliM*, *fliN*, Δ*flio-fliR*, *fliP*, Δ*fliP-fliR*, *fliR*, and *fliQ*.
7. T. Kubori *et al.*, unpublished results.
8. K. Kaniga, J. C. Bossio, J. E. Galán, *Mol. Microbiol.* **13**, 555 (1994).
9. D. A. Pegues, M. J. Hantman, I. Behlau, S. I. Miller, *ibid.* **17**, 169 (1995).
10. Bacteria were grown in L broth to an absorbance at 600 nm of 0.8 to 1.0, pelleted, and resuspended in 1/10 of the original volume in a solution of 0.5 M sucrose and 0.15 M Trisma base. Lysozyme and EDTA were added to a final concentration of 1 mg/ml and 10 mM, respectively, and cells were incubated at 4°C for 1 hour. Spheroplasts were lysed by the addition of lauryldimethylamine oxide (LDAO) to a final concentration of 1%, and the samples were further incubated at 4°C for 2 hours. $MgSO_4$ was added to a final concentration of 10 mM, and samples were centrifuged at 5000*g* for 20 min. The clarified sample was adjusted to pH 11 with NaOH, incubated for 1 hour at 4°C, and centrifuged at 60,000*g* for 1 hour. The pellet was resuspended in a solution containing 0.1 M KCl-KOH (pH 11), 0.5 M sucrose, and 0.1% LDAO and centrifuged at 60,000*g* for 1 hour. The pellet was resuspended in TET buffer [10 mM tris-HCl (pH 8.0), 5 mM EDTA, and 0.1% LDAO] and loaded onto a 30% (w/v) CsCl density gradient. Gradient fractions were centrifuged at 60,000*g* for 1 hour, and the pellets were washed with TET buffer.
11. S. I. Aizawa, *Mol. Microbiol.* **19**, 1 (1996).
12. _____, G. E. Dean, C. J. Jones, R. M. Macnab, S. Yamaguchi, *J. Bacteriol.* **161**, 836 (1985).
13. T. Ueno, K. Oosawa, S. Aizawa, *J. Mol. Biol.* **236**, 546 (1994).
14. Polypeptides separated on standard SDS-polyacrylamide gels were electroblotted onto polyvinylidene difluoride membranes, and the bands of interest were applied to a Beckman LF3000 protein sequencer.
15. The amino acid sequences obtained from the different polypeptide species were as follows: 62 kD, SEKIPVTGSG; 52 kD, METSKEKTI; and 31 kD, CKDKD (*19*).
16. N. A. Linderoth, M. N. Simon, M. Russel, *Science* **278**, 1635 (1997); M. Koster *et al.*, *Mol. Microbiol.* **26**, 789 (1997).
17. The *prgH* and *prgK* genes were cloned from the wild-type strain of *S. typhimurium* SL1344 by polymerase change reaction, and derivative strains carrying nonpolar mutations in these genes were constructed by allele replacement (*8*). *Salmonella typhimurium* strains expressing a functional M45 epitope–tagged PrgH protein were constructed as described elsewhere [C. Collazo and J. E. Galán, *Infect. Immun.* **64**, 3524 (1996)]. The M45 epitope tag consisted of 18 residues from the E4-6/7 protein of adenovirus (MDRSRDRLPPFETETRIL) (*19*) [S. Obert, R. J. O'Connor, S. Schmid, P. Hearing, *Mol. Cell. Biol.* **14**, 1333 (1994)].
18. E. Roine *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 3459 (1997).
19. Single-letter abbreviations for the amino acid residues are as follows: C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; R, Arg; S, Ser; T, Thr; and V, Val.
20. T. Kubori, N. Shimamoto, S. Yamaguchi, K. Namba, S.-I. Aizawa, *J. Mol. Biol.* **226**, 433 (1992).
21. We thank S. Yamaguchi for *Salmonella* flagellar mutant strains; M. Iwakura for amino acid sequence analysis; S. Makishima, T. Kubo, and N. Kobayashi for assistance in the needle preparation; and J. Bliska, R. Donis, and members of the Galán laboratory for critical reading of this manuscript. Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture (S.-I.A.), the American Heart Association, and Public Health Service Grants from the NIH (J.E.G.).

30 December 1997; accepted 2 March 1998

A Distinctive Class of Integron in the *Vibrio cholerae* Genome

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The ability of bacteria to acquire and disseminate heterologous genes has been a major factor in the development of multiple drug resistance. A gene, *intI4*, was identified that encodes a previously unknown integrase that is associated with a "gene-VCR" organization (VCRs are *Vibrio cholerae* repeated sequences), similar to that of the well-characterized antibiotic resistance integrons. The similarity was confirmed by IntI1-mediated recombination of a gene-VCR cassette into a class 1 integron. VCR cassettes are found in a number of *Vibrio* species including a strain of *V. metschnikovii* isolated in 1888, suggesting that this mechanism of heterologous gene acquisition predated the antibiotic era.

Integrons are gene expression elements that acquire open reading frames (gene cassettes) and convert them to functional genes. More than 40 different antibiotic resistance cassettes have been characterized in these structures, permitting their bacterial hosts to become resistant to a broad spectrum of antimicrobial compounds (*1*). The insertion of a gene cassette takes place by site-specific recombination between the circularized cassette and the recipient integron; the essential components are an integrase gene (*intI*) and a linked attachment site (*attI*) required for the efficient site-specific integration of the gene cassettes into the integron structure (*2*). To date, three classes of integron have been defined on the basis of the sequence of their associated integrase (*1*); the integrases have 43 to 58% amino acid sequence identity and are related to the integrases of temperate bacteriophages. The similarity between the three integrases suggests that their evolutionary divergence extended beyond the half century of the antibiotic era, and probably much longer according to the substitution rates calculated by Ochman and Wilson (*3*).

All integron-inserted cassettes identified share specific structural characteristics (Fig. 1A); the boundaries of each integrated cassette are defined by two GTTRRRY (core-site) (*4*) sequences in the same orientation, which are the targets of the recombination process. The integrated cassettes include a gene and an imperfect inverted repeat located at the 3′ end of the gene called a 59-base element, a diverse family of sequences that function as recognition sites for the site-specific integrase. The organization of these elements starts with an inverse core-site, which is complementary to the core-site located upstream from the gene cassette, followed by a sequence of imperfect dyad symmetry, and ending with the downstream core site sequence (Fig. 1). The 59-base elements vary in size from 60 to 141 base pairs (bp); their nucleotide sequence similarities are primarily restricted to the inverse core-site and the core-site. Both the source of gene cassettes and the mechanism of their genesis are unknown, although they are presumed to be bacterial in provenance.

A number of species of the *Vibrio* genus, a widely distributed bacterial group found primarily in aqueous environments, are pathogenic for humans and animals. *Vibrio cholerae* has been responsible for major epidemics of human disease, especially since the early 19th century. It is known to have at least two pathogenicity islands (*5*): the locus encoding the cholera toxin, which is carried by a M13-like phage inserted into the chromosome (*6*), and a large cluster of genes encoding the accessory colonization factor and genes for the biogenesis of the toxin–co-regulated pilus, which is harbored in a prophage-like structure (*7*). The *V. cholerae* genome has also been found to contain repeated sequences (VCRs) in clusters that have a similar organization to the integron–gene cassette structures (*8*). They were first identified surrounding

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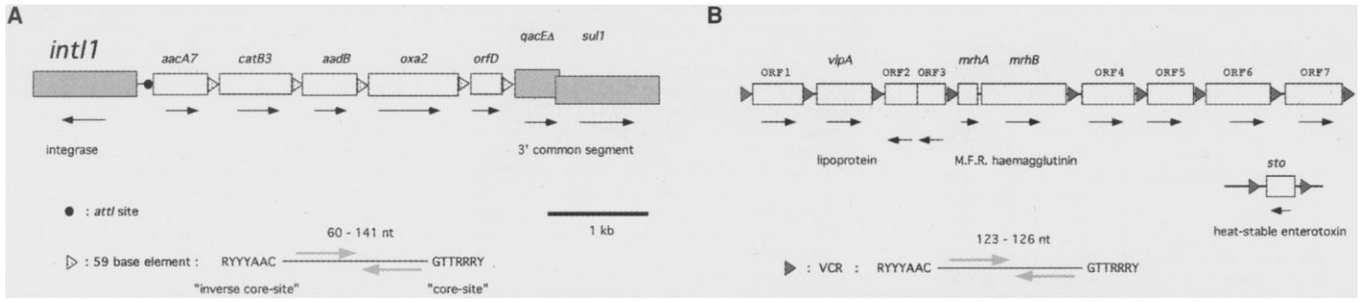

Fig. 1. (**A**) Schematic representation of antibiotic resistance integron In30 (*25*) and (**B**) the VCR clusters characterized in *V. cholerae*. Genes and ORFs are indicated by boxes and labeled when known. M.F.R., mannose-fucose resistant; nt, nucleotide. In (A), the genes belonging to cassettes (white) are distinguished from the nonmobile genes of class 1 integrons (gray). The imperfect dyad symmetries present in the sequences of both the 59-base element and the VCR are symbolized by gray arrows; the range in size of these elements is marked above.

the mannose-fucose–resistant hemagglutinin gene (*mrhA*) (9) and the heat-stable toxin gene (*sto*) (*10*), two pathogenicity genes in *V. cholerae* O:1. VCRs are highly repeated (60 to 100 copies) and situated in a single restriction fragment corresponding to about 10% of the *V. cholerae* genome (*11*). The VCRs are a family of 123- to 126-bp sequences of imperfect dyad symmetry, and the 13 examples sequenced thus far show an overall identity of 92%. Two such sequences flank the heat-stable toxin gene *sto* (Fig. 1B), in both O:1 and non-O:1 *V. cholerae* isolates (*10*), and nine other VCRs have been found in a 6-kb *V. cholerae* fragment encoding the hemagglutinin gene, a lipoprotein gene, and eight other unidentified open reading frames (ORFs) (*11*, *12*). Within these clusters, the VCRs are separated from one another by up to two ORFs (Fig. 1B).

We found a 90% sequence identity between the VCR sequences and the 59-base element associated with *blaP3*, which is an integron-associated antibiotic resistance gene encoding the carbenicillinase CARB-4 isolated from *Pseudomonas* (*13*). Further investigation of the structures of VCR clusters revealed that the gene-VCR organization is essentially identical to that of the resistance gene cassette array typically found in integrons (Fig. 1): (i) the VCRs usually abut a single ORF; (ii) the VCRs have imperfect dyad symmetries starting with an inverse core-site and ending with a core-site identical to the integron cassette consensus GTTRRRY (*4*); (iii) the inverse core-site is always complementary to the upstream VCR core-site; and (iv) all VCRs are in the same orientation to each other.

The ability of an integrase to recognize potential recombination sites can be assayed by measuring the integration of a single cassette or the co-integration of a plasmid carrying the cassette into a target integron (*14*). Using such an assay we demonstrated that the *blaP3* cassette (pSU38::CARB4) (*15*) and the ORF1 cassette (pSU38::ORF1-cat) (*16*) found in the VCR locus containing the hemagglutinin gene (Fig. 1B) can be directed to the insertion sites of integrons. To track the ORF1 cassette, we tagged it by inserting a *cat* gene [for chloramphenicol resistance (Cm^R)]. As shown in Table 1, in both cases, integration of the cassette into the integron was observed. The precise location of the cassette insertion events was established by polymerase chain reaction (PCR). In the 48 transconjugants studied, the cassettes were inserted at the *attI* site of In3, with recombination occurring at the core-sites of the VCRs (*17*). For both *blaP3* and ORF1::*cat*, the frequencies of transfer by recombination (conduction) were about 10^{-2} and were strictly dependent on IntI1 activity. These frequencies are comparable to those found in studies of integration of several different 59-base elements of known integron cassettes (*14*, *18*).

To investigate the relationship between VCR clusters and integrons, we used nucleic acid hybridization to isolate a gene for an integron integrase in *V. cholerae* (*19*) and identified the *intI4* gene, the product of which has 45 to 50% identity with the three known integrases (Fig. 2). An array of four gene-VCR cassettes is found upstream of *intI4* in the characterized *V. cholerae* fragment. This organization is identical to that of antibiotic resistance integrons; the location of the putative *attI* site is at the proximal boundary of the first cassette, 225 bp upstream from the *intI4* start codon. A cluster of ribosomal protein genes is located downstream from *intI4*. Specific signals were detected in both *V. mimicus* and *V. metschnikovii* by Southern (DNA) hybrid-

Table 1. Recombination frequencies of the *blaP3* and ORF1::*cat* cassettes. The indicated donor strains were mated with *Escherichia coli* UB5201 (*14*) and transconjugants separately selected for trimethoprim resistance (Tp^r) and ampicillin resistance (Amp^r) for ω3 and ω5 or chloramphenicol resistance (Cm^r) for ω8 and ω9.

Donor strain*	Plasmids†	Conduction frequency‡
ω3	R388, pSU38::CARB4	$<10^{-6}$
ω5	R388, pSU38::CARB4, pSU2056	1.1×10^{-2} (0.65)
ω8	R388, pSU38::ORF1-cat	$<10^{-6}$
ω9	R388, pSU38::ORF1-cat, pSU2056	2.0×10^{-2} (0.13)

*These donor strains are derivatives of *E. coli* UB1637 (*14*) containing the plasmids listed in the second column. †R388 is a conjugative plasmid harboring the integron In3, whose cassette *dfrB2* confers Tp^r; pSU2056 overexpresses the *intI1* integrase gene (*14*); pSU38::CARB4 harbors the *blaP3* cassette (*15*); and pSU38::ORF1-cat harbors ORF1::*cat* cassette (*16*). ‡The conduction frequency is the number of Amp^r (ω3 and ω5) or Cm^r (ω8 and ω9) transconjugants divided by the number of Tp^r transconjugants. The frequencies of the integration of the cassette alone versus the total conduction frequencies were measured by the frequency of kanamycin-sensitive clones among the Amp^r (ω5) or Cm^r (ω9) colonies and are given in parentheses.

Table 2. *Vibrio* strains screened for the presence of VCR cassettes. Abbreviations: *V. chol.*, *V. cholerae*; *V. met.*, *V. metschnikovii*; *V. par.*, *V. parahaemolyticus*; *V. mim.*, *V. mimicus*; *V. fis.*, *V. fischeri*; Path., pathogenicity; cass., cassettes. Symbols: +, more than two different amplification products (*24*); −, no product. PCR primer sequences are given in (*17*).

Strain*	Year isolated	Path.	VCR cass.
V. chol. O:1 569B	1946	+	+
V. met. CIP A267	1888	+	+
V. met. CIP 69.14T	1922	+	+
V. par. CIP 75.2T	1953	+	+
V. mim. ATCC 33653	1982	+	+
V. fis. CIP 101047	1958	−	−

*Strains obtained from the Pasteur Institute Collection (CIP) except *V. cholerae* O:1 strain 569B provided by J.-M. Fournier (Pasteur Institute) and *V. mimicus* American Type Culture Collection (ATCC) 33653 provided by A. Paccagnella (British Columbia Provincial Laboratory).

ization with an *intI4* probe (*20*).

A number of *Vibrio* isolates dating from 1888 to 1982 have been screened by means of oligonucleotide primers (*17*) corresponding to the most conserved regions of the VCR sequences (Table 2). *Vibrio cholerae* O:1 569B exhibited a complex pattern of amplification composed of more than 10 different PCR products, and both *V. metschnikovii* isolates showed a pattern of at least six distinct products (*20*). This result demonstrates that VCR cassettes were found in the *Vibrio* lineage before the emergence of antibiotic resistance integrons. At least three VCR cassettes were found in *V. mimicus* and *V. parahaemolyticus*, but none was present in the well-studied luminescent, nonpathogenic *V. fisheri*. *Vibrio mimicus* is phylogenetically close to *V. cholerae*, whereas *V. metschnikovii*, *V. parahaemolyticus*, and *V. fischeri* belong to other lines of descent in the *Vibrio* genus (*21*). The presence of relatively fewer VCR cassettes in *Vibrio* species other than *V. cholerae* may be real or it may indicate substantial nucleotide sequence variation between the VCR sequences present in these strains. Despite the high variability in 59-base element sequences, it has been established that antibiotic resistance cassettes are all substrates for the integron-encoded integrases; thus, we predict the same to be true for the gene-VCR clusters. These results do not distinguish between the possibilities that *intI4* and its associated cassettes were acquired by a *Vibrio* ancestor before the separation of these species and lost in some (for example, *V. fisheri*), or that this integron invaded only pathogenic *Vibrio* during their evolution.

Of the six different cassettes sequenced from the *V. metschnikovii* PCR products, one showed 67% identity to the previously described ORF5 *V. cholerae* cassette (Fig. 1B), which encodes a 15-kD protein of unknown function, suggesting that *V. cholerae* and *V. metschnikovii* shared the same pool of cassettes and that VCR cassettes are disseminated among *Vibrio* species. The gene encoding the heat-stable toxin (*sto*) is harbored by a VCR cassette in *V. cholerae* (*10*) and is dispersed in *V. cholerae* (both O:1 and non-O:1 serotypes), as well as among *V. mimicus* strains; the *sto* genes found in *V. cholerae* and *V. mimicus* are highly similar (*22*). In the case of *V. mimicus*, it is not known whether the *sto* gene is a component of a VCR cassette, but its variable distribution among different *V. mimicus* isolates suggests the presence of such a structure.

Our studies show that VCR islands are integron-like structures and that their formation likely occurred by typical integrase-mediated processes, suggesting that integrons (widely spread in antibiotic-resistant Gram-negative bacteria) also existed for the purpose of gene capture in *Vibrio* species. The variation observed in codon usage of the gene-VCR cassettes as well as in their GC content (between 33 and 45%, compared with 47% for the *V. cholerae* genome) is consistent with the idea that the VCR-associated genes were recruited from other microbial sources. The observation that there are 60 to 100 VCR copies in the *V. cholerae* genome (*11*) implies that there must be an equivalent number of cassettes, that is, more than 10 times the number found in the largest antibiotic resistance integron. The function of this "super-integron" may extend beyond the clustering of genes for pathogenicity into a generalized system for the entrapment and spread of other biochemical functions, for example, the antibiotic resistance–encoding cassette *blaP3* (which is a VCR cassette). It is well established that integrons had a major role in the recent spread of multidrug resistance among Gram-negative bacteria. Our studies support their function in bacterial genome evolution, through the fixed integration of genes at secondary sites, as has already been observed for antibiotic resistance cassettes (*1*). A striking feature of the VCR integron, compared with the antibiotic resistant integrons, is the conserved sequence of the recombination elements of its endogenous cassettes (the VCR itself). Given the relationship between VCR sequences and the numerous 59-base elements, it is possible that each resistance gene cassette with a different 59-base element represents a single member of a clustered group of cassettes. If this is true, we may expect to find such structures in many bacterial genera.

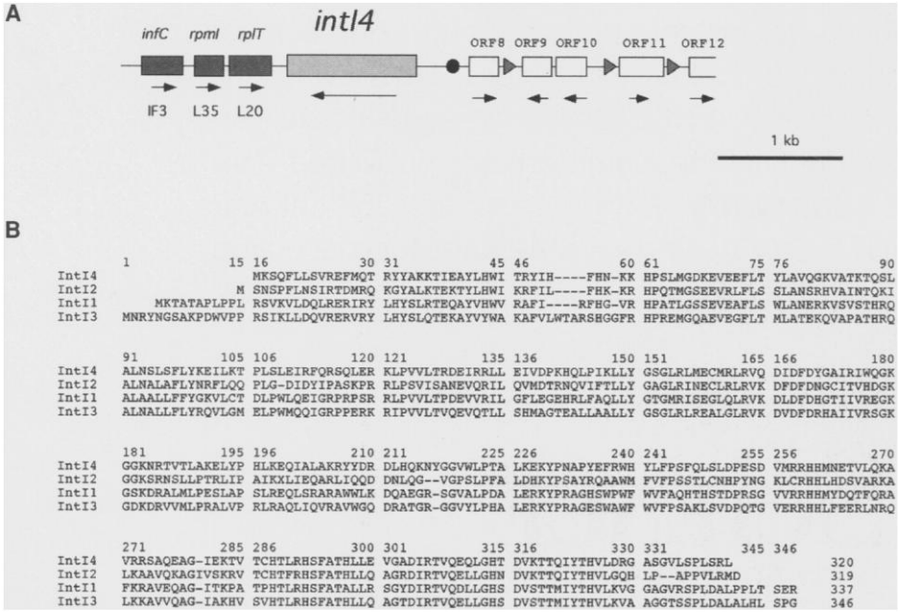

Fig. 2. The *V. cholerae* gene *intI4*. (**A**) Schematic representation of the *intI4* locus, putative *attI* site, and VCR and associated ORFs are as shown in Fig. 1. The light gray box symbolizes the *intI4* gene encoding the integrase, and the dark gray boxes indicate the genes encoding ribosomal proteins and initiation factor 3; arrows show the directions of transcription. (**B**) Alignment of IntI4 (accession number AF 055586) and the three integron integrases (*26*).

REFERENCES AND NOTES

1. G. D. Recchia and R. M. Hall, *Microbiology* **141**, 3015 (1995).
2. R. M. Hall and H. W. Stokes, *Genetica* **90**, 115 (1993).
3. H. Ochman and A. C. Wilson, *J. Mol. Evol.* **26**, 74 (1987).
4. Single-letter abbreviations for DNA nucleotide residues are as follows: A, adenosine; C, cytidine; G, guanosine; T, thymidine; R, any purine nucleoside; and Y, any pyrimidine nucleoside.
5. C. A. Lee, *Infect. Agents Dis.* **5**, 1 (1996).
6. M. K. Waldor and J. J. Mekalanos, *Science* **272**, 1910 (1996).
7. M. E. Kovach, M. D. Shaffer, K. M. Peterson, *Microbiology* **142**, 2165 (1996).
8. G. D. Recchia and R. M. Hall, *Trends Microbiol.* **5**, 389 (1997).
9. W. M. A. M. van Dongen, V. Vlerken, F. K. DeGraaf, *Mol. Gen. (Life Sci. Adv.)* **6**, 85 (1987).
10. A. Ogawa and T. Takeda, *Microbiol. Immunol.* **37**, 607 (1993).
11. A. Barker, C. A. Clark, P. A. Manning, *J. Bacteriol.* **176**, 5450 (1994).
12. A. Barker and P. A. Manning, *Microbiology* **143**, 1805 (1997).
13. GenBank accession number U14749.
14. E. Martinez and F. de la Cruz, *EMBO J.* **9**, 1275 (1990).
15. We constructed pSU38::CARB4 by cloning the *blaP3* cassette into pSU38 (*23*) after amplification from the original integron, using the primers 5′-GCACATAGAGTCCTACAGGCTC-3′ and 5′-TGCTTAGTGCATCTAACGCCGC-3′. The nucleotide sequence of the construction was verified.
16. pSU38::ORF1-cat was constructed in two steps.

First, the DNA fragment encompassing VCR1, ORF1, and VCR2 (*11*) was cloned into pSU38 after amplification from *V. cholerae* 569B genomic DNA with primers 5′-CGTGCGTAGAGAGGTTTTACTT-3′ and 5′-AAGATAGCTCTCATGATATCTCC-3′. Then the ORF1 internal Cla I fragment was replaced with the *Tn9 cat* gene that had been amplified by polymerase chain reaction (PCR) from pSU18 (*23*). The nucleotide sequence of the ORF1::*cat* cassette was verified.

17. We precisely mapped the insertion events by using combinations of the following primers: ATT1-5 (5′-TCCAAGCAGCAAGCGCGTTACGCC-3′, located upstream from *attI*); 59-3 (5′-CTGTGAGCAATTATGTGCTTAGTG-3′, located upstream from *qacE*Δ); CARB4-3 (5′-GTTTATTCATCACAATCGCGTTG-3′, located in *blaP3*); CARB4-4 (5′-TACCAGTAAAAAGCTTCATGTAATAA-3′, located in *blaP3*); VCR-1 (5′-GTCCCTCTTGAGGCGTTTGTTA-3′); and VCR-2 (5′-GCCCCTTAGGCGGGCGTTA-3′), corresponding to the first 22 nucleotides and the last 19 nucleotides of the VCR consensus sequence, respectively (*11*). The sequences of the integration junctions for three independent events from both cassettes were determined with primers ATT1-5, CARB4-3, and PORF (5′-GGTAAGTTATGGGATCTGGTGC-3′, located inside ORF1).
18. E. Martinez and F. de la Cruz, *Mol. Gen. Genet.* **211**, 320 (1988).
19. An incomplete ORF having 55% identity with the COOH-terminal half of the integron integrases was identified in the sequence GVCCL68R (*4*) of the *V. cholerae* genome database [The Institute for Genomic Research (TIGR), http://www.tigr.org].
20. D. Mazel, B. Dychinco, V. A. Webb, J. Davies, unpublished data.
21. K. Kita-Tsukamoto, H. Oyaizu, K. Nanba, U. Simidu, *Int. J. Syst. Bacteriol.* **43**, 8 (1993).
22. A. C. P. Vicente, A. M. Coehlo, C. A. Salles, *J. Med. Microbiol.* **46**, 398 (1997).
23. B. Bartolome, Y. Jubete, E. Martinez, F. de la Cruz, *Gene* **102**, 75 (1991).
24. Amplification studies were done using primers VCR-1 and VCR-2 (*17*).
25. K. L. Bunny, R. M. Hall, H. W. Stokes, *Antimicrob. Agents Chemother.* **39**, 686 (1995).
26. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
27. We thank C. Radomski for his assistance in sequencing, R. Levesque for providing the *blaP3* cassette and unpublished sequence data, J. Kaper for providing the *V. cholerae* cosmid library, and J. Shellard, R. Fernandez, and D. Davies for providing helpful comments. Supported by Canadian Bacterial Diseases Network, Natural Sciences and Engineering Research Council of Canada (V.A.W., B.D., and J.D.), Institut Pasteur (D.M.), and North Atlantic Treaty Organization (D.M.).

3 December 1997; accepted 9 March 1998